Journal of Pharcomagnosy and Phytotherapy

Volume 5 Number 8 August 2013
ISSN 2141-2502

ABOUT JPP

The **Journal of Pharmacognosy and Phytotherapy (JPP)** is published monthly (one volume per year) by Academic Journals.

The **Journal of Pharmacognosy and Phytotherapy (JPP)** is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject such as ethnobotany, phytochemistry, ethnopharmacology, zoopharmacognosy, medical anthropology etc.

The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JPP are peer-reviewed.

Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: jpp@academicjournals.org. A manuscript number will be mailed to the corresponding author shortly after submission.

The Journal of Pharmacognosy and Phytotherapy (JPP) will only accept manuscripts submitted as e-mail attachments.

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author.

Editors

Dr. (Mrs) Banasri Hazra
Research Scientist (U.G.C.)
Department of Pharmaceutical Technology
Jadavpur University
Calcutta - 700032
India

Dr. Yuanxiong Deng
Dept of Pharmaceutical Science
School of Medicine
Hunan Normal University
Tongzipo Road 371, Changsha 410013,
Hunan China

Prof. Maha Aboul Ela
Beirut Arab University, Faculty of Pharmacy, Beirut Campus

Dr. S. RAJESWARA REDDY
Assistant Professor, Division of Animal Biotechnology
Department of Biotechnology, School of Herbal Studies and Naturo Sciences,
Dravidian University, Kuppam – 517 425, A.P.
India

Dr. Mekhfi Hassane
University Mohammed the First, Faculty of Sciences,
Department of biology, Oujda, Morocco
Morocco

Dr. Ilkay Erdogan Orhan
Faculty of Pharmacy, Gazi University,
Ankara, Turkey
Turkey

Dr. Arun Kumar Tripathi
Central Insttute of Medicinal and Aromatic Plants
P.O. CIMAP, LUCKNOW-226015,
India

Dr. Wesley Lyeverton Correia Ribeiro
Universidade Estadual do Ceará, Faculdade de Veterinária/Laboratório de Doenças Parasitárias Av. Paranjana, 1700
Itaperi - Fortaleza
60740-903, CE - Brazil

Dr. Maryam Sarwat
C/O A.M. Khan, House No. 195

Dr. Yong-Jiang Xu
Saw Swee Hock School of Public Health,
National University of Singapore, Singapore.

Prof. Dr. Adeolu Alex Adedapo
Department of Veterinary Physiology,
Biochemistry and Pharmacology
University of Ibadan, Nigeria

Dr. Joana S. Amaral
Campus de Sta Apolónia,
Ap. 1134, 5301-857 Bragança,
Portugal

Dr. Asad Ullah Khan
Interdisciplinary Biotechnology UNIT
Aligarh Muslim University,
India

Dr. Sunday Ene-ojo Atawodi
Biochemistry Department
Ahmadu Bello University
Zaria, Nigeria

Prof. Fukai Bao
Department of Microbiology and Immunology,
Kunming Medical College
China

Dr. Bhaskar C Behera
Agharkar Research Institute
Dept. of Secience &Technology,
Plant Science Division
India

Prof. R. Balakrishna Bhat
Walter Sisulu University
Department of Botany
Mthatha, South Africa

Dr. Mohammad Nazrul Islam Bhuiyan
BCSIR Laboratories;
Chittagong cantonment;
Chittagong-4220;
Bangladesh

Dr. Baojun Bruce Xu
Beijing Normal University-Hong Kong Baptist University United International College Zhuhai, Guangdong Province,
China

Dr. Hamad H. Issa
Department of Physical Sciences,
School of natural Sciences,
The University of Dodoma,
Tanzania

Dr. Gagan Deep
Department of Pharmaceutical Sciences
School of Pharmacy,
University of Colorado Denver,
Colorado,
USA

Dr. Fengguo Xu
Dept of Epidemiology and Public Health,
Yong Loo Lin School of Medicine,
National University of Singapore,
Singapore

Dr. Haitao Lv
Medicine and Endocrinology,
Albert Einstein College of Meidicine,
Yeshiva University,
USA

Hassane MEKHFI
University Mohammed the First,
Faculty of Sciences,
Department of biology,
Laboratory of Physiology and Ethnopharmacology,
Morocco

Dr. Subhash C. Mandal
Division of Pharmacognosy
Pharmacognosy and Phytotherapy Research Laboratory,
Department of Pharmaceutical Technology,
Jadavpur University,
India.

Dr. Adibe Maxwell Ogochukwu
Clinical Pharmacy and Pharmacy Management,
Faculty of Pharmaceutical Sciences,
University of Nigeria, Nsukka
Enugu state,
Nigeria.

Dr. Odukoya, Olukemi Abiodun
Department of Pharmacognosy,
Faculty of Pharmacy
University of Lagos.
Nigeria.

Dr. Qinxue Richard Ding
Medical Center at Stanford University,
Palo Alto,
USA

Dr. Sulejman Redžic
Faculty of Science of the University of Sarajevo
33-35 Zmaja od Bosne St.,
Sarajevo,
Bosnia and Herzegovina

Dr. Michal Tomczyk
Medical University of Bialystok,
Faculty of Pharmacy,
Department of Pharmacognosy,
Poland

Dr. Ugur Çakilcioglu
Firat University,
Faculty of Science and Arts,
Department of Biology,
Elazig
Turkey

Prof. Samson Sibanda
National University of Science and Technology
Cnr Gwanda Road/Cecil Avenue,
Ascot, Bulawayo,
Zimbabwe

Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The **cover letter** should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

Article Types
Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process

All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review.
Decisions will be made as rapidly as possible, and the journal strives to return reviewers' comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the JPP to publish manuscripts within weeks after submission.

Regular articles

All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The **Title** should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The **Abstract** should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.
Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard **Abbreviations** should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The **Introduction** should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author's name should be mentioned, followed by 'et al'. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish the works.

Examples:

Cole (2000), Steddy et al. (2003), (Kelebeni, 1983), (Bane and Jake, 1992), (Chege, 1998; Cohen, 1987a,b;Tristan, 1993,1995), (Kumasi et al., 2001)

References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:

Ansell J, Hirsh J, Poller L (2004). The pharmacology and management of the vitamin K antagonists: the Seventh ACCP Conference on Antithrombotic and Thrombolytic. Therapy. 126:204-233

Ansell JE, Buttaro ML, Thomas VO (1997). Consensus guidelines for coordinated outpatient oral anti coagulation therapy management. Ann. Pharmacother. 31:604-615

Charnley AK (1992). Mechanisms of fungal pathogenesis in insects with particular reference to locusts. In: Lomer CJ, Prior C (eds), Pharmaceutical Controls of Locusts and Grasshoppers: Proceedings of an international workshop held at Cotonou, Benin. Oxford: CAB International. pp 181-190.

Jake OO (2002). Pharmaceutical Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria Of *Zea mays*, L. and *Sorghum bicolor* L. Moench for Striga suicidal germination In *Vigna unguiculata*. PhD dissertation, Tehran University, Iran.

Furmaga EM (1993). Pharmacist management of a hyperlipidemia clinic. Am. J. Hosp. Pharm. 50: 91-95

Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences:
(1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.

Fees and Charges: Authors are required to pay a $550 handling fee. Publication of an article in the Journal of Pharmacognosy and Phytotherapy (JPP) is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances.

Copyright: © 2013, Academic Journals.
All rights Reserved. In accessing this journal, you agree that you will access the contents for your own personal use but not for any commercial use. Any use and or copies of this Journal in whole or in part must include the customary bibliographic citation, including author attribution, date and article title.

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

Disclaimer of Warranties

In no event shall Academic Journals be liable for any special, incidental, indirect, or consequential damages of any kind arising out of or in connection with the use of the articles or other material derived from the JPP, whether or not advised of the possibility of damage, and on any theory of liability.
This publication is provided "as is" without warranty of any kind, either expressed or implied, including, but not limited to, the implied warranties of merchantability, fitness for a particular purpose, or non-infringement. Descriptions of, or references to, products or publications does not imply endorsement of that product or publication. While every effort is made by Academic Journals to see that no inaccurate or misleading data, opinion or statements appear in this publication, they wish to make it clear that the data and opinions appearing in the articles and advertisements herein are the responsibility of the contributor or advertiser concerned. Academic Journals makes no warranty of any kind, either express or implied, regarding the quality, accuracy, availability, or validity of the data or information in this publication or of any other publication to which it may be linked.

Journal of Pharmacognosy and Phytotherapy

Table of Contents: Volume 5 Number 8 August 2013

ARTICLES

Research Articles

Drying characteristics of Safed Musli (*Chlorophytum borivilianum*) and its effect on colour and saponin content
Ravi Agrawal, A. Upadhyay, Preeti Sagar Nayak 142

Phytochemical properties and effect of aqueous extract of *Jatropha caucus* root bark on some bacterial isolates
M. S. Auwal, A. L. Fatima, A. Shuaibu, A. M. Ismaila,
A. S. Kyari, A. B. Thaluvwa, A. B. Njobdi 148

Microscopical and physicochemical studies of *Indigofera barberi* (Fabaceae) stem
T. Rajeshwar, T. Yadagiri Rao, K. N. V. Rao, S. Sandhya 153

academicJournals

Vol. 5(8), pp. 142-147, August, 2013
DOI: 10.5897/JPP11.051
ISSN 2141-2502 ©2013 Academic Journals
http://www.academicjournals.org/JPP

Journal of Pharmacognosy and Phytotherapy

Full Length Research Paper

Drying characteristics of Safed Musli (*Chlorophytum borivilianum*) and its effect on colour and saponin content

Ravi Agrawal[1], A. Upadhyay[2] and Preeti Sagar Nayak[2]*

[1]Deparment of Post Harvest Process and Food Engineering, College of Agricultural Engineering, Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur (M.P.) India 482004, India.
[2]Department of Crop and Herbal Physiology, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur (M.P.) India 482004, India.

Accepted 21 September, 2011

An experiment was carried out to adjudge the effect of different drying techniques of post harvest on colour and saponin content of Safed Musli (*Chlorophytum borivilianum*), a medicinal plant belonging to the family Liliaceae, has been traditionally used as adaptogenic drug. The present study was done on drying of fresh Safed Musli root in different condition like: shade, sun, solar and cabinet dryer. The moisture of about 62% is removed in 45 h when dried in shade and the temperature varies from 15 to 18°C, whereas in sun dried, the time taken was about 16.45 h and the temperature range is 25 to 30°C. Further less time that is, 5 h is required to dry the sample in solar cabinet dryer for 54 to 60% moisture loss and the temperature inside the cabinet was observed to be 39°C. But in cabinet drying for the loss of 65 to 70% moisture the time required was 4.45 and 3.30 h and the temperature lies in the range of 67 to 77°C and 64 to 74°C respectively. It was found that the minimum change in colour and saponin content was observed when dried in shade and maximum loss of saponin content was observed when dried in cabinet drier.

Key words: *Chlorophytum borivilianum*, drying, post harvest, saponin.

INTRODUCTION

Medicinal plants constitute a group of industrially important crops, which are of great value for domestic use and for export. Plant based drugs are being increasingly preferred in medicinal science. Forest of Madhya Pradesh are full of medicinal flora and other herbs of commercial importance, due to lack of post harvest knowledge and safe storage practices, a large quantity of produce get spoiled or sold rather in very less prices. Safed Musli is also gaining increasing acceptance as a vitalizer and health-giving tonic, a curative for pre-natal and post-natal problems, a restorative for immunity-improvement and as a remedy for diabetes and arthritis. A feasibility study was conducted for better returns in Safed Musli through processing. According to study, the reason for less processing is lack of knowledge, low quality of Safed Musli and unavailability of processing equipments.

There are about 175 species of *Chlorophytum* that have been reported worldwide. In other parts of the world, *Chlorophytum* is usually grown as an ornamental plant, but in India it has a reputation as a medicinal plant. A total of 13 species of *Chlorophytum* have been reported from India (Oudhia, 2001). In India it is grown in Madhya Pradesh, North Gujrat and Southern Rajasthan.

*Corresponding author. E-mail: preetisagarnayak@rediffmail.com.

In India 7 species viz, *Chlorophytum tuberosum, Chlorophytum arundinaceum, Chlorophytum breviscapum, Chlorophytum attenuatum, Chlorophytum laxum, Chlorophytum borrivillanium* and *Chlorophytum malabaricum* are commonly observed (Manjunatha et al., 2004). Out of these seven cultivated species only *C. borivilianum* is mentioned in Ayurveda as a medicine (Shariff and Chennaveeraiah, 1972).

Tuberous roots of *C. borivilianum* (commonly known as Safed Musli) belonging to the family Liliaceae. It posses immunomodulatory and aptogenic properties and are used to cure impotency, sterility and enhance male potency. Peeled and dried musli roots are considered as the wonder drug in ayurvedic system of medicine due to its aphrodisiac properties. The *Chlorophytum* roots having higher saponin content have high demand in international drug market (Manjunatha et al., 2004). Safed Musli is a rich source of over 25 alkaloids vitamins, minerals, proteins, carbohydrates, steroids, saponins and polysaccharides etc (Seth et al., 1991). Bordia et al. (1990) and Seth et al. (1991) reported that the major constituents of Safed Musli are carbohydrates (42%), protein (8 to 9%), root fibre (3 to 4%) and saponin (2 to 17%), alkaloids 25%, vitamins A, B, D, K and E and minerals 7 to 15%. The main active principles of roots are saponins and are stimulants, metabolic enhancers and have been shown to posses anti – tumour activity (Mimaki et al., 1996; Qiu et al., 2000). The economic part of the herb is root, rich in saponin which is considered to be the potent medicinal compound (Kothari and Singh, 2001). The cultivation of Safed Musli comes across a lot of constraints viz. high input costs, lack of technical guidance and lack of comprehensive package of practice. Due to, the lack of proper cultivation protocol, commercialization techniques and package of practice, the present work was planned to study the influence of different drying technique on Safed Musli roots and its effect on colour and saponin content. The main objective of drying is to reduce the moisture present at the time of harvest and for safe storage.

MATERIALS AND METHODS

Plant materials

The fresh Safed Musli root was procured from the Department of Plant Physiology, JNKVV Jabalpur during the year 2008 to 2009. It was then washed thoroughly with running water so that all dirt is removed. After washing the roots was manually peeled with the help of knife and the peeled root was then dried. The different drying conditions used for this study were shade, sun, solar and cabinet drier. Different dryers had variation in configuration; hence they were used to evaluate their suitability for Safed Musli drying. In case of cabinet drying a simple heat convector (Usha Lexus, make) was used as a source of heat and to blow heated air, the heat convector has provision for single and two coil separately and also for supplying of air at varying velocity from fan that is, 1.62 and 1.88 ms^{-1} respectively. During drying of the samples the observation like time and temperature were recorded with respect to moisture loss. Grinding of the Safed Musli was done in the hammer mill to get the powder in the form of fine particle. The various observations were recorded during the experiment given below.

Moisture

Moisture content of fresh samples and after drying for all the samples was determined by using the standard method (AOAC, 2000).

Colour

The colour of dried Safed Musli for all the samples was determined using Hunter Colour Colorimeter at 65%10°C and the L, a, b values were recorded.

Saponin content

Saponin content of Safed Musli dried under different conditions was determined by HPTLC method (Rajpal, 2002). The fine powder of the root sample (5 g) was extracted in the soxhlet reflux extractor with 50 ml acetone for 24 h to remove lipids, pigments etc. Now change the solvent for methanol and continue extraction for at least another 24 h. Repeat the process 2 times with the methanol. Each of the extracts were combined, partially evaporated and concentrated to dryness under vacuum. After that the extracts were re-dissolved in methanol, combined in a 5 ml volumetric flask and adjusted to the final volume with methanol. Prior to use, all the samples were filtered through 0.45 µm filter.

Sample analyzed by HPTLC

Apply 10 µl of the reference and sample solutions on the different tracks on the silica gel plate (10 × 10 cm) of uniform thickness (0.2 mm thickness). Develop the plate in the solvent system up to a distance of 8 cm. Scan the plate using a Camag thin layer chromatography (TLC) Scanner at 366 nm for both reference and test solution tracks. Peak purity tests were carried out by comparing the peak areas and Rf (2.2) with those present in the reference and test solution tracks. Freshly prepared p-anisaldehyde reagent is used. After drying, the plate was heated at 110°C for 10 min to develop the colour of the spots.

Statistical analysis

All the results were statistically analyzed to estimate the significant difference between different drying conditions on the basis of color, moisture and saponin content.

RESULTS AND DISCUSSION

Effect of different drying condition on moisture content of Safed Musli

The moisture content of about 62% was removed in just 45 h and the temperature lies in the range of 15 to 18°C during this period when fresh peeled Safed Musli root was dried in shade. Whereas when the sample was sun dried the time taken was about 16.45 h and the

Table 1. Moisture depletion pattern of Safed Musli in different dried condition.

Dried in shade		Dried in sun		Solar cabinet dryer		
Time (hours)	Loss of moisture (%)	Time (hours)	Loss of moisture (%)	Time (hours)	Loss of moisture (%)	
					Cabinet I	Cabinet II
3.00	4.2	2.0	3.1	0.30	12	10
6.00	13.4	4.30	43.7	1.00	22	20
24.00	42.8	7.55	56.2	1.30	30	28
27.00	47.2	9.15	59.3	2.00	36	36
45.00	62.13	14.15	62.5	2.30	40	40
				3.00	44	48
				3.30	48	52
60.00	66.53	16.45	65.6	4.00	52	56.0
				4.30	52.8	58.0
				5.00	54.0	60.0

Table 2. Moisture depletion pattern of Safed Musli dried in cabinet dryer at different air velocity and temperature.

Time (hours)	Loss of moisture (%)				
	Air velocity 1.62 ms^{-1} and temperature 67 to 77°C		Time (hours)	Air velocity 1.88 ms^{-1} and temperature 64-74°C	
	Cabinet I	Cabinet II		Cabinet I	Cabinet II
1.15	20	20	0.30	05	05
1.45	45	30	1.00	25	20
2.30	50	58	1.30	45	35
3.15	55	65	2.00	55	55
3.45	60	67	2.30	65	58
4.15	65	70	3.00	68	65
4.45	65	70	3.30	70	69
5.15	68	70			

temperature range is 25 to 30°C which is just double as compare to shade drying temperature due to which the moisture is removed faster in sun drying than in the shade drying. Further, less time that is, 5 h is required to dry the sample in solar cabinet dryer for 54 to 60% moisture loss and the temperature inside the cabinet was observed to be 39°C which is furthermore as compared to the shade and sun drying (Table 1). It is worth to mention here that during solar drying the colour of the sample gets darker than shade and sun dried. This may be because in solar drying the temperature inside the cabinet raises up to 39°C result in fast drying and also the colour of the product gets darkened. The drying time was higher in shade as compared to other driers, may be due to the structural design of the other driers. The lesser time taken in solar drier is due to higher air temperature and lesser relative humidity inside the drier.

In case of samples dried in cabinet dryer at an air velocity of 1.62 ms^{-1} and temperature 67 to 77°C, it takes about 4.45 h to reduce moisture content of 65 to 70%, whereas when dried in cabinet dryer at air velocity of 1.88 ms^{-1} and temperature 64 to 74°C, the time required for same moisture loss is 3.30 h. This may be due to the change in air velocity that is, 1.62 and 1.88 ms^{-1} respectively and is given in the Table 2.

In case of samples dried in cabinet dryer at temperature ranging from 75 to 85°C with air velocity 1.62 ms^{-1} and at temperature ranging from 80 to 92°C with air velocity of 1.88 ms^{-1}, the time required for the loss of moisture of about 70% is same that is, 1.45 h in both the air velocity (Table 3). The rate of drying is more in first cabinet as compared to the second cabinet when dried with an air velocity of 1.62 ms^{-1} and at temperature 75 to 85°C. But did not show any significant effect on the moisture loss in first and second cabinet when dried in air velocity of 1.88 ms^{-1} and at temperature 80 to 92°C.

Effect of different drying condition on colour of Safed Musli

The value of lightness is more or less same in shade

Table 3. Moisture depletion pattern of Safed Musli dried in cabinet dryer at different air velocity and temperature.

Time (hours)	Loss of moisture (%)				
	Air velocity 1.62 ms^{-1} and temperature 75 to 85°C		Time (hours)	Air velocity 1.88 ms^{-1} and temperature 80 to 92°C	
	Cabinet I	Cabinet II		Cabinet I	Cabinet II
0.30	20	18	0.30	20	20
1.00	38	35	1.00	50	50
1.15	55	55	1.15	68	65
1.30	60	58	1.30	70	70
1.45	70	70	1.45	70	70

Table 4. Hunter colour value of dried Safed Musli powder at 65/10°.

Different drying technique	Lightness	Yellow to red	Green to blue
Shade	80.26	0.83	10.93
Sun dried	75.31	2.67	13.68
Solar dried	73.99	2.76	13.30
Cabinet dried, one coil and one fan	77.90	1.26	12.45
Cabinet dried, one coil and two fan	79.09	1.06	12.29
Cabinet dried, two coil and one fan	78.42	0.99	10.55
Cabinet dried, two coil and two fan	79.74	0.80	9.93

Table 5. Saponin content in Safed Musli dried in different drying condition.

Drying condition	Saponin (%)
Shade	0.983
Sun	0.824
Solar	0.426
Cabinet 1 coil, air velocity 1.62 ms^{-1}	0.635
Cabinet 1 coil, air velocity 1.88 ms^{-1}	0.325
Cabinet 2 coil, air velocity 1.62 ms^{-1}	0.743
Cabinet 2 coil, air velocity 1.88 ms^{-1}	0.308

dried and cabinet dried sample whereas lightness decreases when dried in sun and solar dried sample as given in the Table 4.

Effect of different drying condition on saponin content of Safed Musli

The variation in saponin content of dried powder of Safed Musli was due to the difference of drying technique. The maximum saponin content was observed in the sample when dried in shade (0.983%) and minimum (0.308%) in cabinet dryer with air velocity of 1.88 ms^{-1} and temperature range 80 to 92°C. The shade drier hinders direct sunlight and gave the best results. This showed that the drying systems affected the saponin content. This observation if further substantiated by the findings as reported by Kumar et al. (2000) that due to size reduction of turmeric and drying system with covering materials gave better results.

Second best results that is, (0.824%) of saponin were observed when the sample was dried in sun. This may be due to the reason that in cabinet drying the temperature inside the cabinet is more that is, 80 to 92°C whereas in case of shade drying the temperature lies in the range of 15 to 18°C. This indicates that the loss of saponin content take place at higher temperatures (Table 5). The identification of saponin was confirmed by superimposing the UV spectra of samples and standards within the same R_f value as shown in the Figures 1 to 3. Fresh root recovery of the medicinal plant of Safed Musli was acceptable when stored at temperatures between 10°C and ambient but the saponin content decreased with the storage duration. Low storage temperature (2 to 5°C)

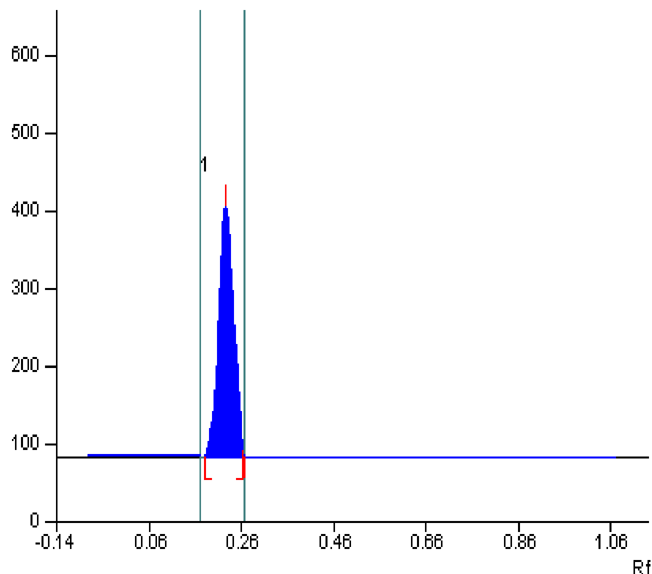

Figure 1. Saponin standard.

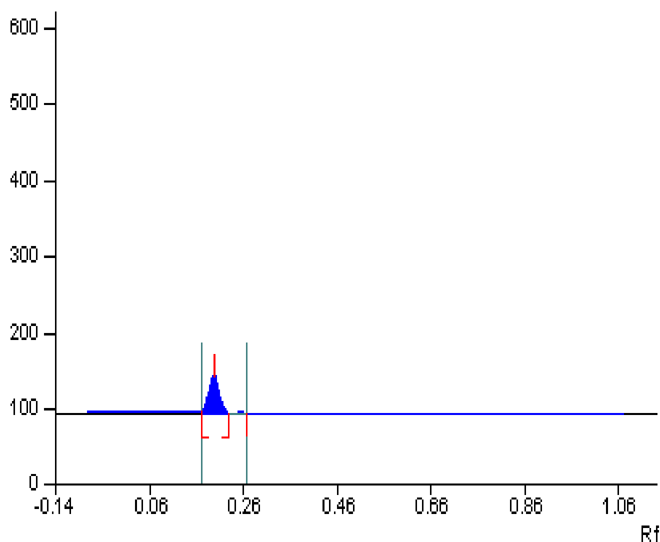

Figure 2. Shade drying.

maintained higher saponin content in roots (DARE / ICAR Annual Report, 2003-2004). Blunt end type roots are better for storage and saponin content (8.9%) than the tapering end types and the first week of December is the best for harvest of roots.

Conclusion

The lightness and saponin content was found to be maximum in shade drying of Safed Musli but drying times takes more when dried in shade than any other condition of drying. Maximum loss of saponin content was observed when dried in cabinet dryer in the temperature

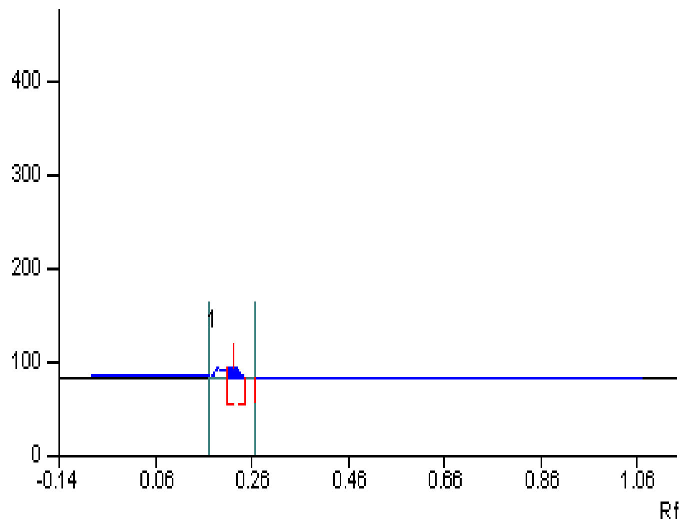

Figure 3. Cabinet drying.

range of 80 to 92°C with the air velocity of 1.88 ms^{-1}. Based on the results, it can be concluded that shelf life of Safed Musli can be enhanced and extended by making it into value added powder by employing traditional processing methods.

REFERENCES

AOAC, (2000). Official methods of analysis. Associations of official analytical chemists Inc. Washington DC.

Bordia PC, Seth P, Simlot MM (1990). Exploration of safed musli (chlorophytum specis) in the Aravali regian and preliminary observations. Paper presented in the National symoposium om conservation and management of living Resources. University of Agricultural Sciences G.K.V.K., Bangalore 10-12 January. Abstract. pp.4

DARE (Department of Agricultural Research and Education) / ICAR (Indian Council of Agricultural Research), Annual Report, (2003-2004). National Agricultural Technology Project 200-201.

Kothari SK, Singh K (2001). Evaluation of Safed musli (*Chlorophytum borivilianum* Santapau & Fernandes) germplasm. J. Spices Arom. Crops 40:147-149.

Kumar VK, Vadiraj BA, Sivadasam CR, Potty SN (2000). Post harvest management of turmeric for higher recovery of curcumin:Spice and aromatic plant: Challenges and opportunities in the new century. Contributing papers, Centennial Conference on Spice and Aromatic Plant, Calicut Kerela, India. 20-30,281-283.

Manjunatha G, Tyagi SK, Shrinivasan K (2004) Safed Musli : A White Gold, Agrobios, Jodhpur, India. pp. 12-14.

Mimaki Y, Kanmato T, Sashida Y, Nishimo A, Setomi Y, Nishino H (1996). Steroidal saponins from the underground parts of *Chlorophytum comosum* and their inhibitory unity on tumor promoter-induced phospholipids metabolism of Hela cells. Photochemistry 41:1405-1410.

Oudhia P (2001). Problems perceived by Safed Musli (*Chlorophytum borivilianum*) growers of Chhattisgarh (India) region: A study. J. Med. Arom. Plant Sci. 22/4A & 23/1A, 396–399.

Qiu SX, Li XC, Xiong Y, Dong Y, Chai H, Fransworth NR, Pezzuto JM, Fong HH (2000). Isolation and characterization of cytotoxic saponin chloromaloside A from *Chlorophytum malayense*. Planta Med. 66(6):587-590.

Rajpal V (2002). Standardization of Botanicals. Testing and Extraction method of medicinal herbs. Vol.1, Eastern Publishers. pp. 223-228.

Seth P, Sharma MK, Simlot MM (1991). Saponins in *Chlorophytum* species. Proc. Indian Soc. Biol. Chem. Biotechnol. Institute of Chem. Biol. Calcutta, India 431-451.
Shariff A, Chennaveeraiah MS (1972). Karyomorphology of four diploid species of *Chlorophytum.* Nucleus 15:39-45.

academicJournals

Vol.5(8), pp. 148-152, August, 2013
DOI: 10.5897/JPP11.078
ISSN 2141-2502 ©2013 Academic Journals
http://www.academicjournals.org/JPP

Journal of Pharmacognosy and Phytotherapy

Full Length Research Paper

Phytochemical properties and effect of aqueous extract of *Jatropha caucus* root bark on some bacterial isolates

M. S. Auwal[1]*, A. L. Fatima[2], A. Shuaibu[3], A. M. Ismaila[4], A. S. Kyari[1], A. B. Thaluvwa[5] and A. B. Njobdi[6]

[1]Department of Veterinary Physiology, Pharmacology and Biochemistry, University of Maiduguri, Borno State, Nigeria.
[2]Department of Veterinary Microbiology and Parasitology, University of Maiduguri, Borno State Nigeria.
[3]Department of Human Anatomy, College of Medical Sciences, University of Maiduguri, Borno State, Nigeria.
[4]Department of Veterinary Medicine, University of Maiduguri, Borno State Nigeria.
[5]Department of Veterinary Public Health and Preventive Medicine, University of Maiduguri, Borno State, Nigeria.
[6]Department of Animal Health, College of Agriculture, Jalingo, Taraba State, Nigeria.

Accepted 25 March, 2013

Phytochemical properties and effect of aqueous extract of *Jatropha caucus* root bark on some bacteria isolates was investigated. The root bark extract revealed the presence of carbohydrates, alkaloids, flavonoids, saponins, cardiac glycosides and terpenes/steroids. The extract applied in graded concentrations of 200, 400 and 600 mg/ml inhibited the multiplication of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Corynebacterium pyogenes*, *Candida albicans*, *Pseudomonas aeroginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Escherichia coli* and *Proteus mirabilis.* Conclusively, the root bark extract of this plant has exhibited good antibacterial activity especially on organisms such as *B. subtilis*, *C. albicans*, *S. pyogenes*, *Proteus* species, *E. coli* and *S. typhi* that are incriminated in causing diseases such as anthrax, reproductive tract infection, skin infection and various gastrointestinal disorders.

Key words: *Jatropha curcas*, phytochemical properties, *in vitro* antibacterial activity, aqueous root bark extract.

INTRODUCTION

Jatropha curcas (Linnaeus) belongs to the family Euphorbiaceae and is closely related to other important cultivated plants like rubber and castor plants. The plant is believed to be a native of South America and Africa but later spread to other continents of the world by the Portuguese settlers (Gubitz et al., 1999).

Various parts of this plant have been documented to have medicinal uses for human and veterinary purposes. The plant has been used in the treatment of infectious and non infectious ailments such as gonorrhea, dropsy, gout, paralysis, scabies, eczema, dermatitis and rheumatoid arthritis (Srinivasan et al., 2001).

Many parts of this plant such as leaves, stem bark and latex have been reported to exhibit antibacterial activity (Oyi et al., 2007; Donlaporn and Suntornsuk, 2010). There are more than 35,000 plant species with various phytochemicals in them being used on various human cultures around the world for medicinal purpose. About 80% of individuals from developed and under developed countries use traditional medicine, which has compound derived from medicinal plants in various form of therapies (Galal et al., 1991).

Phytochemicals are biologically active compounds found in plants such as vegetables and grains in low,

*Corresponding author. E-mail: mshaibu68@yahoo.com. Tel: +2348067682131.

moderate and high amounts; these compounds are not established nutrients, but significantly protect the development of lots of degenerative diseases in animals and humans (Dreosti, 1998; Abo et al., 1991).

Researchers are increasingly turning their attention to natural plant products such as flavonoids, saponins, tannins and others to look for new products to develop better drugs against cancer, as well as mycotic, viral and microbial infections (Hoffmann et al., 1993; Srinivasan et al.,2001).Bacteria have the genetic ability to transmit and acquire resistance to drugs (Cohen, 1992). In the last three decades, numbers of new antibiotics have been produced, but clinical efficacy of these existing antibiotics is being threatened by the emergence of multi drug-resistant pathogens (Bandow et al., 2003). According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs (Santos et al., 1995).

The aim of this study was to evaluate the phytochemical properties and *in vitro* antibacterial activity of the aqueous extract of *J. caucus* root bark on some pathogenic bacterial organisms of medical importance.

MATERIALS AND METHODS

Collection of plant and identification

Fresh roots bark of *J. caucus was* collected from Borgu in Shani Local Government Area of Borno State. The root bark was taken to the Department of Biological Sciences, University of Maiduguri, where it was identified and authenticated by a taxonomist. Voucher specimen was deposited at the University Herbarium for reference. The root bark were kept under a well ventilated shade for several days and allowed to air-dry. The dried root bark were then crushed and pulverized into fine powder and kept in an amber bottle at 4°C.

Preparation of extract

Aqueous root bark extract of the plant was prepared according to the methods of Mittal et al. (1981) and Fernando et al. (1989). 200 g of the powdered root bark was mixed with 1 L of distilled water in a 5 L beaker. The mixture was steamed at 65°C for 1 h, allowed to cool and mixed vigorously. It was filtered using sterile Whatman No.1 filter paper. The aqueous extract filtered was then concentrated by evaporation to dryness at 60°C in a water bath and finally stored at 4°C for use.

Microbial cultures

Pure cultures of some Gram positive and Gram negative bacteria such as *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Corynebacterium pyogenes*, *Candida albicans, Pseudomonas aeroginosa, Klebsiella pneumoniae, Salmonella typhi, Escherichia coli* and *Proteus mirabilis* were obtained from the Department of Veterinary Medicine Laboratory, University of Maiduguri and were used. The isolates were separately cultured on a nutrient plate for 24 h. Twenty milliliters of the culture media was poured into sterile medium sized Petri–dish and allowed to solidify. A colony of each test organism was subcultured in 10 ml nutrient broth and incubated at 37°C for 8 h. One milliliter of the subcultured organisms were inoculated on the agar plates.

Extract concentration and standard drug preparation

Stock solution of the aqueous extract is prepared by dissolving 200, 400 and 600 mg of the extract in 1 ml of distilled water giving the concentration of 200, 400, and 600 mg/ml of the extract, respectively. Amoxicilli as a standard drug (Control) was used at the concentration of 250 mg/ml.

Antibacterial sensitivity testing

Disc diffusion method as described by the National Committee of Clinical Laboratory standards (1993) was used to determine the antimicrobial activity of the aqueous root bark extract. Discs of sterilized Whatman No.1 filter paper (6 mm) in diameter made using a paper puncher were soaked in beakers containing different concentrations of 200, 400 and 600 mg/ml of the extract and dried at 50°C. Each paper disc used for the antibacterial sensitivity test contains 200, 400 and 600 mg of the extract, respectively. Overnight cultures of each bacteria isolate were diluted using sterile normal saline to give an inoculum size of 10^6 CFU/ml. The inoculum was spread on the surface of dried nutrient agar plates with cotton wool swabs, which have been dipped in the diluted suspension of the organisms. The plates are then inoculated at 37°C for 30 min before the discs were applied aseptically. The treated plates were incubated at 37°C for 48 h. Discs of sterilized Whatman No.1 filter papers (6 mm) in diameter made using a paper puncher were soaked in a beaker containing 250 mg/ml of amoxicillin as a positive control. The paper discs used as positive control therefore contain 250 mg of amoxicillin. Plates without the extract or antibiotics were setup as negative control. The zone of inhibition above 6 mm diameter of each isolate was used as a measure of susceptibility of the organisms to the extract and this will be compared to the zone of inhibition of the standard antibiotic (Amoxicillin).

RESULTS

Preliminary phytochemical analysis of the crude extract of *J. curcas* root bark as shown in Table 1 revealed the presence of alkaloids, tannins, flavonoids, saponins, carbohydrates, terpenes, cardiac glycosides and steroids, whereas no anthraquinones were detected in the extract. The antibacterial activity of the aqueous extract of *J. curcas* root bark clearly showed that all the organisms were sensitive to the extract. The extract used in graded concentrations (200, 400 and 600 mg/ml) exhibited graded effect on the microorganisms. At higher concentration of 600 mg/ml, the organisms showed variation in their zones of inhibition as follows, *B. subtilis* (20 mm), *C. albicans* (20 mm), *E. coli* (17 mm), *P. mirabilis* (17 mm), *P. aeroginosa* (15 mm), *S. pyogenes* (15 mm), *S. typhi* (14 mm) with *S. aureus* (12 mm) and *K. pneumoniae* (11 mm) exhibiting less sensitivity. *Corynebacterium pyogenes* is the only microorganism that has exhibited resistance to the extract. Similar trend was shown by the microorganisms to 200 and 400 mg/ml of the extract used. Amoxicillin (control drug) inhibited the growth of all the microorganisms including *C. pyogenes*

Table 1. Phytochemistry of aqueous extract of *J. curcas* root bark.

Phytochemical constituents	Types of test	Inference
Carbohydrates	Molisch's	+
	Barfoed's	-
	Free reducing sugar	+
	Combined reducing sugar	-
	Ketones	+
	Pentoses	-
Tannins	Ferric chloride	+
	Lead ethanoate	+
Anthraquinones	Bournstrager	-
	Free/Combined anthraquinones	-
Saponins	Frothing	+
Glycosides	General test	+
Terpenes and steroids	Lieberman- Buchard's	+
	Salkowski's	+
Flavonoids	Lead acetate	+
	Sodium hydroxide	+
	Ferric chloride	+
	Pew	+
Alkaloids	Dragendorff's	+
	Mayer's	+

-: Not detected; +: Present.

that was resistant to the aqueous extract of *J. caucus* root bark (Table 2). The minimum inhibitory concentration for *S.aureus*, *S. pyogenes* and *S. typhi* is 25 and 50 mg/ml for *Escherichia* (Table 3). The sensitive laboratory bacterial isolates has a minimum bactericidal concentration of 50 mg/ml (Table 4).

DISCUSSION

Plant essential oils and extracts have been used for many thousands of years in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate these plants scientifically, which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens (Ramo-Tejada, 2002).

The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies (Sieradski et al., 1999).

The aqueous extract of *J. caucus* root bark has very important phytochemicals such as alkaloids, tannins, flavonoids, saponins, carbohydrates, terpenes, cardiac glycosides and steroids. Flavonoids are strong antioxidants also found to be effective antimicrobial substance *in vitro* and *in vivo* against wide range of microorganism by inhibiting their membrane bound proteins (Ramo-Tejada, 2002; Cowan, 1999). Tannins have also been reported to posse's antibacterial activity by suppressing certain key enzyme activities involved in metabolic processes in bacteria microorganisms (Narayana et al., 2001; Birk and Petri, 1980).

Plant parts from *Acacia albida, Anchomanes difformis, Boscia senegalensis, Bridelia ferruginea, Ficus ingens, Indigofera pulchra, Moringa oleifera, Mormodica basalmina, Pavetta crassipes, Phyllanthus amarus* and *Vernonia blumeoides* used for antibacterial studies have shown various levels of antibacterial activity (Aliyu et al., 2008).

Parts of *J. caucus* (Stem and Stem bark) used in *in vitro* antibacterial study (Bhaskarwar et al., 2008; Igbinosa et al., 2009) have shown activity, but the root

Table 2. Antibacterial activity of aqueous root bark extract of *J. curcas* on some bacterial organisms.

Extract / antibiotic	Amounts of extract and amoxicillin (mg)	Zone of inhibition diameter(mm)									
		P. aeroginosa	*S. aureus*	*K. pneumoniae*	*S. pyogenes*	*S. typhi*	*B. subtilis*	*E. coli*	*C. pyogenes*	*Proteus* spp.	*C. albicans*
Aqueous extract of *J. caucus*	600	15	12	11	15	14	20	17	R	17	20
	400	10	8	8	12	13	15	14	R	12	14
	200	9	6	6	7	12	13	8	R	11	13
Amoxicillin	250	12	20	15	35	35	30	25	25	30	30

Table 3. Determination of minimum inhibitory concentration (MIC) of *J. curcus* aqueous crude root bark extract.

Organism	Concentration of *Hyphaene thebaica* aqueous pericarp extract (mg/ml)				
	200	100	50	25	12.5
S. aureus	-	-	-	-	+
S. pyogenes	-	-	-	-	+
S. typhi	-	-	-	-	+
E. coli	-	-	-	+	+

+: Growth observed; -: Growth inhibited.

Table 4. Determination of minimum bactericidal concentration (MBC) of *J. curcas* aqueous crude root bark extract.

Organism	Concentration of *Hyphaene thebaica* aqueous pericarp extract (mg/ml)				
	200	100	50	25	12.5
S. aureus	-	-	-	+	+
S. pyogenes	-	-	-	+	+
S. typhi	-	-	-	+	+
E. coli	-	-	-	+	+

+: Growth observed; -: Growth inhibited.

bark extract had better activity. The result of antibacterial activity of the aqueous extract of *J. caucus* root bark exhibited promising activity that could assist lots of people in the sub-Saharan Africa since orthodox drugs are not easily accessible due to financial problem experienced by in the developing countries especially in Africans. All the Gram positive and negative organisms used in this study exhibited sensitivity to this extract except *C. pyogenes*. *S. typhi*, *S. aureus* and *K pneumoniae* that causes serious problems such as typhoid fever, dermal infections and pneumonia in sub-Saharan Africa due to poor primary health care could be effectively controlled by the aqueous extract of *J. caucus* root bark developed in form of suspension, poultices or creams.

The sensitivity shown by *C. albicans to* this

product also indicate the possibility of the extract being used in the management of reproductive tract infection (Candidiasis) that mostly affects female folk.

Conclusion

This research conclusively proved that theaqueous extract of *J. caucus* root bark has antibacterial property principally due to the presence of flavonoids and tannins as active principle, hence its folkloric application in the management of various bacterial disease condition by herbalists and traditionalist in Askira/Uba, Shani and Maiduguri metropolitan in Borno State, Nigeria.

ACKNOWLEDGEMENTS

The authors appreciate the contribution of the members of the Department of Veterinary Physiology, Pharmacology and Biochemistry and Mal. Isa Gulani, (Laboratory Technologist) of the Department of Medicine, Faculty of Veterinary Medicine, University of Maiduguri, Borno State, Nigeria.

REFERENCES

Abo KA, Ogunleye VO, Ashidi JS (1991). Antimicrobial potential of *Spondias mombin, Croton zambesicus* and *Zygotritonia crocea*. J. Pharmacol. Res. 5(13):494-497.

Aliyu AB, Musa AM, Abdullahi MS, Oyewale AO, Gwarzo US (2008). Activity of plant extracts used in Northern Nigerian traditional medicine against methicillin-resistant *Staphylococcus aureus* (MRSA). Nig. J. Pharm. Sci. 7(1):1-8.

Bandow JE, Brötz LIO, Leichert H, Labischinski HM (2003). Proteomic approach to understanding antibiotic action. Antimicrob. Agents Chemother. 47(3):948-955.

Birk Y, Petri I (1980). Saponins: In: Toxic Constituents of Plants Food Stuff, Liener IE (Ed) 2nd Edn, Academic Press, NewYork. 161-182.

Cohen ML (1992). Epidemiology of drug resistance: implications for a post-antimicrobial era. Science 257(5073):1050-1055.

Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. Vol. 12(4):564-582

Dreosti IE (1998). An antioxidant RDI: Feasible and Fancible. Proc Nutr Soc Aust. 22:26-28.

Fernando MR, Wickramasinghe MD, Thabrew MI, Karnayaka EK (1989). A preliminary Investigation of the possible hypoglycemic activity of Asteracanthus longifolia. J. Ethnopharmacol. 27:7-14.

Galal M, Bashir AK, Salih AM, Adam SEI (1991). Activity of water extracts of *Albizia anthelmintica* and *A. lebbek* barks against exprimental Hymenolepis diminuta infection in rats. J. Ethnopharmacol. 31:333-337.

Gubitz GM, Mittelbach M, Trabi M (1999). Exploitation of the tropical oil seed plant Jatropha curcas L. Bioresource Tech. 67:73-82.

Hoffmann JJ, Timmerman N, McLaughlin R, Punnapayak H (1993). Potential antimicrobial activity of plants from the South Western United States. Int. J. Pharmacol. 31:101-115.

Igbinosa OO, Igbinosa EO, Aiyegoro OA (2009). Antimicrobial activity and phytochemical screening of bark extracts from *Jatropha curcas* (Linn). Afri. J. Pharm and Pharmacol. 3(2):58-62.

Mittal GC, Aguwa CN, Eziefe VU, Akabue PL (1981). Preliminary pharmacological studies on antivenom action of *Diodia scandens*. Nig. J. Pharm. 12:32-36.

Narayana KR, Reddy MS, Chaluvadi MR, Krishna DR (2001). Bioflavonoids: Classification, Pharmacology, Biochemical effects and therapeutic potential. Indian J. Pharmacol. 33:2-16.

National Committee of Clinical Laboratory Standards (1993). Performance standards for Antimicrobial Disc Susceptibility Tests: Approved Standard CCLS Document M2 – A5. NCCLS, Wayne, Pennsylvania, USA, pp. 377-379.

Oyi AR, Onaolapo JA, Haruna AK Morah CO (2007). Antimicrobial screening and stability studies of the crude extract of Jatropha caucus Linn latex (EUPHORBIACEAE). Nig. J. Pharm. Sci. 6(2):14-20.

Ramo-Tejada DJ, Ontiveros-Ortega A, Esinosa- Jimnez M, Perea-Carpio R, Chibowski E (2002). Investigation of alumina (+) catechin system properties. Part 1: A study of the system by FTIR-Vis spectrometry. Colliods and surfaces B. Biointerface 24:297-308.

Santos PRV, Oliveira ACX, Tomassini TCB (1995). Controle microbiógico de produtos fitoterápicos. Rev. Farm Bioquím, 31:35-38.

Sieradski K, Roberts RB, Haber SW, Tomasz A (1999). The development of vancomycin resistance in a patient with methicillin-resistant *Staphylococcus aureus* infection. N. Engl. J. Med. 340:517-523.

Srinivasan D, Nathan-Sangeetha T, Suresh P, Lakshmana P (2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J. Ethnopharmacol. 74:217-220.

academicJournals

Vol. 5(8), pp. 153-159, August, 2013
DOI: 10.5897/JPP12.010
ISSN 2141-2502 ©2013 Academic Journals
http://www.academicjournals.org/JPP

Journal of Pharmacognosy and Phytotherapy

Full Length Research Paper

Microscopical and physicochemical studies of *Indigofera barberi* (Fabaceae) stem

T. Rajeshwar[1]*, T. Yadagiri Rao[2], K. N. V. Rao[1] and S. Sandhya[1]

[1]Department of Pharmacognosy, Nalanda College of Pharmacy, Hyderabad Road, Nalgonda, Andhra Pradesh, India.
[2]Bachelor of pharmacy, Nalanda College of pharmacy, Nalgonda, Andhra Pradesh, India.

Accepted 25 March, 2013

The present study mainly focuses on the establishment of pharmacognostical standards of *Indigofera barberi* stem. *I. barberi* is a small diaceous shrub which belongs to the family Fabaceae and is distributed in India. The whole plant is medicinally reported or claimed to cure several diseases in traditional system of medicine folklore in particularl. This study aimed for the pharmacognostic study of the stem has been carried to establish the pharmacognostical standards. The parameters selected were microscopical studies, proximate analysis, fluorescence analysis, behavior of powder drug with different chemical reagents and preliminary phytochemical screening. In physico-chemical evaluation, the ash values and extractive values were studied. Fluorescence analysis performed showed a wide range of fluorescence colours for the crude powder as well as the extracts. Behavior of powder drug with different chemical reagents showed the different colors. The powder of *I. barberi* was successively extracted with petroleum ether, benzene, chloroform, ethylacetate, ethanol and water. In the ethanol was the identification of the best solvent because preliminary phytochemical screening carried out for ethanol extract gave maximum chemical constituents and percentage yield. Phytochemical tests performed identified different chemical constitutions like flavonoids, steroids, cardiac glycosides, phenols and tannins.

Key words: *Indigofera barberi*, proximate analysis, microscopical, fluorescence, ethanolic extract, preliminary phyto chemical screening.

INTRODUCTION

Indian sub-continent is a rich source of plant and animal wealth which is due to its varied geographical and agro-climatic regions. Besides its varied biodiversity, it has a diverse cultural heritage too. Medicinal plants have played an essential role in the development of human culture, for example in religions and different ceremonies. Medicinal plants are resources of new drugs. It is estimated that there are more than 250,000 flower plant species. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. Cultivation and preservation of medicinal plants protect biological diversity (Madhavan et al., 2011).

Many of the modern medicines are produced indirectly from medicinal plants. Plants are directly used as medicines by a majority of cultures around the world. Many food crops have medicinal effects, though at present Indian health care delivery consists of both traditional and modem systems of medicines, both organized traditional systems of medicine like Ayurveda, Siddha and Unani and unorganized systems folk medicine have been flourishing well. Most of the crude are obtained from plant sources, and parameters like phytochemical analysis,

*Corresponding author. E-mail: rajeshwar4826@gmail.com.

pharmacognostic evalution and qualitative analysis are done on them for standardization (Kannan, 2007).

MATERIALS AND METHODS

Collection of plant material and authentication

Plant was collected in the forest regions of Thalakona (Nelakona regions) of Chittoor district, Andhra Pradesh, India in the month of November, 2011. The plant material was taxonomically identified by the taxonomist from Nalgonda. A voucher specimen was certified under Voucher No: NCOP NLG/ph'cog/2010-11/041 which has been preserved in our laboratory for future reference (Madhava Chetty et al., 2008).

Chemicals

All the chemicals and reagents like chloral hydrate, phloroglucinol, glycerin, iodine, petroleum ether, benzene, chloroform, ethyl acetate, ethanol, distilled water and sodium hydroxide used were of laboratory grade and obtained from various other commercial sources.

Equipment

Soxhlet apparatus, rotary vacuum evaporator (Indosati, India), heating mantel (Bio-technics,India), ultra violet (UV) chamber (Secor,India), muffle furnace, silica crucible, stoppered conical flask, microscope were used for this study.

Pharmacognostic study

Transverse section of stem

Dried stems of *Indigofera barberi* were sectioned to obtain a thin transverse section and the micro anatomy was studied.

Preparation of sample for sectioning

This included three simple steps; boiling of the sample, section cutting, mounting and microscopy. Free hand sectioning was done because transverse section (TS) has different parts. A section of the stem was boiled slightly to soften the tissue taken in between the potato. Phloroglucinol, hydrochloric acid and glycerin were used as a stain and mounted on a glass slide and focused under a microscope (Randhawa et al., 2004). The sections were stained with phluroglucinol and hydrochloric acid in the ratio 1:1. Photo micrographs of different magnifications were taken to study the anatomical features (Khandelwal, 2005).

Microscopic Study

Dried stem of *I. barberi* section to obtain a thin transverse section and the micro anatomy was studied (Khandelwal, 2005; Ansari, 2005; Kokate, 2008).

Powder microscopy: Shade dried plant was powdered with the help of an electric grinder till a fine powder was obtained. This fine powder of the plant was subjected to powder microscopy as per standard procedures mentioned (Khandelwal, 2005; Ansari, 2005; Kokate et al., 2009; Iyengar, 1998).

Determination of physico chemical parameters

Total ash, acid insoluble ash, water soluble ash, sulphated ash, crude fiber content, moisture content, foreign organic matter, alcohol soluble extractive value, and water soluble extractive value of stem of *I. barberi* were determined as per standard procedures (Khandelwal, 2005; The Ayurvedic Pharmacopoeia, 2004; Iyengar, 1998; Trease and Evens, 2009; Indian pharmacopoeia, 2007).

Measurement of cell structure and content

The length and width of phloem fibres, calcium oxalate crystals, trichomes and starch grains were measured using stage micrometer and the eye piece micrometer by standard methods (Khandelwal, 2005; Kokate et al., 2009).

UV fluorescence analysis

Powdered whole plant parts of *I. barberi* were subjected to analysis under ultra violet light after treatment with various chemical and organic reagents. Three parameters were taken into account, that is observation under long UV (365 nm), short UV (256nm) and normal day light (Madhavan et al., 2009). Similarly, extracts were also subjected to UV chamber and fluorescence was observed, and consistency was noted as an additional character for identification (Kalaskar et al., 2010; Sama venkatesh et al., 2008).

Behaviour of the powdered drug with different chemical reagents

Small quantity of the powdered drug sample was taken in a watch glass and mixed with different chemical reagents. The change in the color was observed under short UV, long UV and day light (Madhavan et al., 2009; Kalaskar et al., 2010; Sama venkatesh et al., 2008; Kirtikar et al., 2001).

Preliminary phytochemical screening

The extracts obtained from crude drug include petroleum ether, benzene, chloroform, ethyl acetate, ethanol and water. These extracts were subjected to qualitative test for the identification of various plant constituents. The test which was performed gave a broad idea of the organic chemical constituents (Khandelwal, 2005; Pulokk mukherji, 2000). Initially, 25 gm of crude whole plant powder were taken and packed in a packing paper. This packing was placed in a soxhlet extractor for 24 h (approximately) with different solvents (that is petroleum ether, benzene, chloroform, ethyl acetate, ethanol and water) and temperature was adjusted as per the solvent been used in the extraction. After six successive extractions, the extracts were subjected to a vacuum rotary evaporator and concentrated extracts were obtained along with solvent recovery (The Ayurvedic Pharmacopoeia., 2004; Suurabh jain et al., 2010; Wen et al., 2010; Bojaxa et al., 2010; Puratchikody et al., 2011).

RESULTS

Transverse section of stem

The transverase section is represented in Figure 1. A thin transverse section of young dicot stem when examined under the microscope showed the following regions from outside to inside.

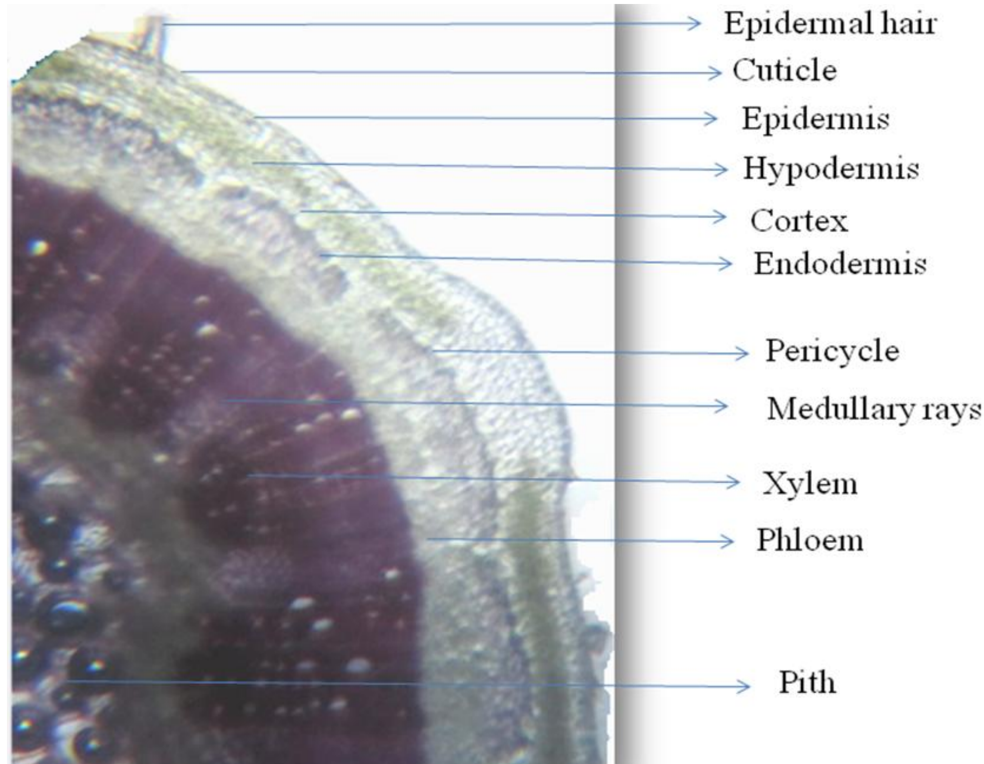

Figure 1. Section stained with phluroglucinol-HCl.

Epidermis: It was the outermost region of the stem and was formed of a single layer of rectangular cells. The outer surface of the epidermis is covered by a layer of cuticle.

Hypodermis: The hypodermis lies just below the epidermis and consist of a few layers of collenchymatous cells. In young stem, the collenchyma contains chloroplast.

Cortex: It is the region next to the hypodermis and is formed of thin walled parenchymatous cells arranged in single layer.

Endodermis: It is a wavy layer of barrel shaped cells end is the innermost layer of the cortex. The cells of endodermis are thickened at their radial walls.

Pericycle: It lies inside the endodermis and is formed of several layers of cells. The pericycle is distinguished in to alternately occurring sclerenchymatous and parenchymatous region, the former situated outside the vascular bundles and the latter in between them. The sclerenchymatous regions of the pericycle provide mechanical support to the vascular region.

Vascular bundles: The vascular bundles in stem are wedge shaped in TS, they are arranged in a ring just inside the pericycle. Each bundle consists of phloem on the outside and xylem on the inner side, both lying on the same radius. Such vascular bundles are called conjoint and collateral. It consists of 4 to 5 layers of cells.

The protoxylem: This is the xylem formed earlier and lies towards the centre, while the metaxylem, that is the later formed xylem, is towards the periphery. This condition is called endarch. Pholem consists of phloem parenchyma and phloem fibres. All elements are lignified. Endarch proto xylem and exarch, together with meta xylem was observered.

Medullary rays: The region between the vascular bundles is called medullary rays. They are formed of radially arranged thin walled parenchymatous cells. The medullary rays are concerned with radial conduction of food and water.

Pith: It constitutes the central region of the stem and is composed of loosely arranged thin walled parenchymatous cells. Pith stores food in its cells. Abundant starch grains were observed.

Table 1. Measurements of different stem cellular components.

Parameter	Length (μm)	Width (μm)
Phloem fibers	125-250-500	12.5-25-37.5
Covering trichomes	50-125-175	12.5-25-37.5
Knee–shaped trichomes	25-50-75	12.5-25-37.5
Calcium oxalate crystals	18.75-37.5-62.5	12.5-25-37.5
Xylem vessels	12.5-37.5-62.5	12.5-25-37.5
Stone cells	12.5-18.75-25	12.5-18.75-25
Starch grains	12.5-18.75-25	12.5-25-37.5

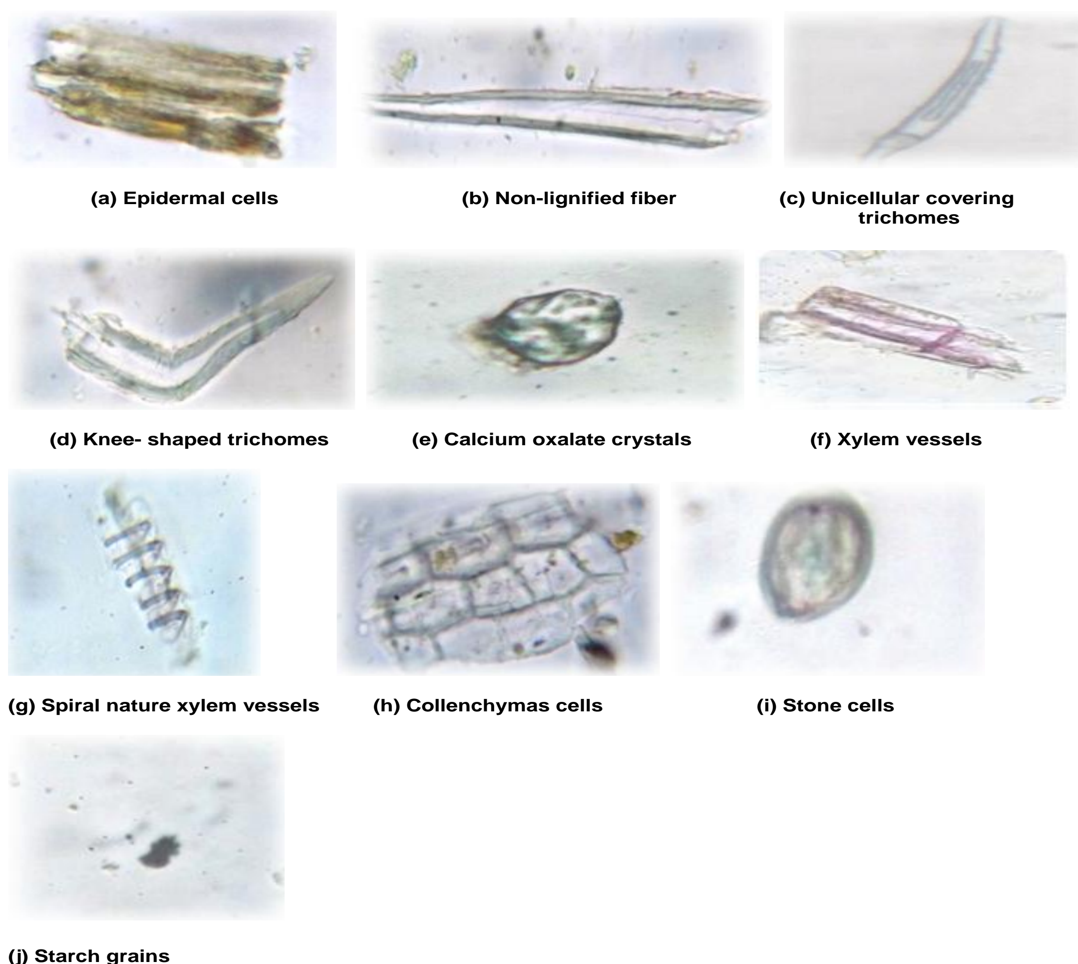

Figure 2. Parts of the stem.

Powder microscopy of stem

Powder microscopy (Table 1) was done according to the standard procedures mentioned. Powder microscopy revealed the presence of the following (Figure 2a to j):

1. Epidermal cells: These cells were present in outer region; the walls may be straight and brownish in colour.
2. Non-lignified fiber: They were present in mid rib region (sclerenchyma region). They are thin walled narrow lumen, and pointed ends.
3. Trichomes: Two types of trichomes were present. (A) Uni cellular covering trichomes: they were found to be long, slender and bent at the base and pointed apex. (B) Knee shaped trichomes: they were present at lamina region, these types of trichome are also present in vasaka leafs.
4. Calcium oxalate crystals: They were found in meophyll

Table 2. Measurements of different proximate values of stem.

S/No	Parameters	Yield (% w/w)
1	Total ash	3
2	Acid insoluble ash	0.1
3	Water soluble ash	1.2
4	Sulphated ash	2.2
5	Loss on drying	0.75
6	Crude fiber content	24
7	Foreign Organic Matter	1.5
8	Water soluble extractive value	8.92
9	Ethanol soluble extractive value	4

Table 3. Fluorescence analysis of stem.

Reagent	Long (365 nm)	Short (256 nm)	Day
50% H_2SO_4	Light yellow	Green	Light yellow
50% HNO_3	Black	Green	Light red
5% NaOH	Black	Green	Dark brown
1 N Me NaOH	Yellow	Light green	Light yellow
1 N KOH	Black	Green	Pale brown
5% KOH	Black	Green	Yellowish black
5% $FeCl_3$	Black	Green	Yellow
Methanol	Light brown	Yellow	Yellow
Conc HCl	Black	Light green	Greenish yellow
Conc H_2SO_4	Black	Light green	Reddish black
Ammonia	Black	Green	Yellow
Conc HNO_3	Black	Green	Wine red

region, they are prismatic and circular in shape.
5. Xylem vessels: The walls were thickened and lignified. They give mechanical support.
6. Spiral vessels: Spiral annular vessels are typical of proto xylem.
7. Collenchyma: Simple, polygonal collenchymas cells were present.
8. Stone cells: Stone cells were strained with green when treated with sulphuric acid. They are single circular in shape. They are heavily lignified with varying lumen (the middle space left over after the lignification). It also varies in shape and size. These are mainly present in stem part and provide valuable diagnostic characters.
9. Starch grains: They were abundant starch grains in hypodermis region which contained chlorophyll containing tissue. They were small and circular; it strained with iodine, showing blue to violet colour.

Proximate analysis of stem

Proximate analysis of *I. barberi stem* were determined by standard method and the results shown in Table 2.

Fluorescence analysis of stem with different chemical reagents

Powdered stem was subjected to analysis under ultra violet light after treatment with various chemical and organic reagents. The findings are shown in Table 3.

Behavior of stem powder with different chemical reagents

Powdered stem was subjected to behavioral analysis with different reagents. The findings are shown in Table 4.

Preliminary phytochemical analysis

Preliminary phytochemical analysis of petroleum ether, benzene, chloroform, ethyl acetate, ethanol and water extracts (Khandelwal, 2005; Tadigoppula et al., 2006; Puratchikody et al., 2011) was performed. Powdered drug was subjected to successive solvent extraction with different solvents. The obtained extracts were subjected to preliminary phytochemical screening according to the standard procedures mentioned. Findings are shown in Table 5.

DISCUSSION

The microscopy of the plant specimens showed valuable information regarding the microanatomy of the *I. barberi*. Section of stem appeared with regions like epidermis, hypoderis, cortex, endodermis, pericycle, vascular bundles, medullary rays, pith which were observed. It consisted of epidermis in the outermost layers covered by cuticle on the surface and unicellular trichomes were also present. Hypodermis consisted of few layers of collenchymatous cells. In the cortex, thin walled parenchymatous cells were arranged in single layer. Endodermis is a wavy layer which is barrel shaped. Pericycle provides mechanical support to vascular region. Vascular bundles were arranged in a ring inside the pericycle. Endarch proto xylem and exarch meta xylem was observed. The medullary rays supply the food and water. Pith is inner part which stored food in its cells and starch grains which were also observed. Powder microscopy of the stem powder showed the presence of epidermal cells, non-lignified fiber, unicellular covering trichomes, knee-shaped trichomes, calcium oxalate crystals, xylem vessels, collenchymas cells, stone cells and starch grains which were present and were considered as tissues of diagnostic importance.

Extractive values play a vital role for the evaluation of the crude alcohol, and water soluble extractive values indicate the presence of the adulterants, faulty processing and poor quality of the drug. Ash values were used to detect the presence of any siliceous contamination and

Table 4. Behaviour of stem powder with different chemical reagents.

Reagent	Observation	Inference
Powder + iodine	Black colour observed	Presence of starch
Powder + $HgCl_2$	No Bule colour observed (black colour)	Absence of Alkaloids
Powder + Ammonia	Light pink colour observed	Presence of cardiac glycosides
Powder + $AgNO_3$	No ppt formed	Absence of protiens
Powder + Picric Acid	No colour change (brown)	Absence of alkaloids
Powder + Water (shaking)	Foam not appeared	Absence of saponins
Powder + Conc H_2SO_4	Black	Presence of starch
Powder + $FeCl_3$	Bluish black	Presence of tannins
Powder + Conc HNO_3	Orange yellow	Presence of tannins

Table 5. Preliminary phytochemical analysis.

Phyto constituents	Petroleum ether	Benzene	Chloroform	Ethyl acetate	Ethanol	Water
Carbohydrates	-	-	-	-	-	-
Amino acids	-	-	-	-	-	-
Proteins	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-
Phenols and Tannins	+	+	+	+	+	+
Steroids	-	-	-	+	+	+
Volatile oils	-	-	-	-	-	-
Flavonoids	+	-	+	+	+	+
Saponins	-	-	-	-	-	-
Cardiac glycosides	+	+	+	+	+	-

presence of any water soluble salts and incorrect preparation. Sulphated ash was used to detect the sulphates and phosphates (how much percent solubility in the ash). Crude fibre content is a useful technique for differentiation of the similar drugs and for the detection of adulteration. Moisture content is an inevitable content in the crude drug, it should be eliminated as much as possible.

While processing, drying of plant material plays crucial role. It helps to fix the constituents, and also aid in preservation. The values obtained aids to establish the suitable monograph of the plant. Fluorescence analysis of the powdered drugs were performed and tabulated, which helps to detect the adulteration, because phytoconstituents exhibits characteristic fluorescence under ultraviolet light when they got mixed with the reagents. The fluorescence exhibited by the mixture was attributed to the chemical constituents present in the crude drug. Prior to the phytochemical screening, a rough estimation of phytoconstituents was done by the behavior of powder drug with different chemical reagents in which powdered drug showed different colours when it got mixed the particular reagents, which reflects the presence of phytochemicals in accordance with the colours obtained.

Phytochemical evaluations like preliminary phytochemical screening were performed according to the standard procedures. The investigation revealed the presence of various active phytoconstituents like flavonoids, steroids, glycosides, phenols and tannins. Based on literature review, successive solvent percentage yield and preliminary phytochemical screening ethanol was taken majorly as a solvent. The preliminary chemical tests confirmed that ethanol was a suitable solvent for extraction of the active principles from the stem of *I. barberi.* The detailed phytochemical investigation strengthens the resourcefulness of the extracts for the further pharmacological evaluations. All these results put together will help in filing a suitable monograph for the stem of *I. barberi*.

Conclusion

The phytochemical screening revealed the presence of flavonoids, cardiac glycosides, steroids, phenols and tannins. All these phytochemicals have potential therapeutic or physiological actions on human system, in that the stem can stand as a potential source of some vital drugs. It may be concluded that *Indigofera barberi*

contains rich amount of phytochemical constituents which may have a variety of pharmacologically activities.

ACKNOWLEDGEMENTS

We would like to thank authorities of Nalanda College of pharmacy, Nalgonda, S.V University, chittoor, Andhra Pradesh, India for providing necessary facility in pharmacognostic work.

REFERENCES

Anonymous, Indian pharmacopeia (2007). Government of India. Ministry of Health, Controller of Publications, New Delhi, India. Vol. I, 78:134.

Ansari SH (2005). Essentials of Pharmacognosy, published by Birla publications Private Limited, Ed. IV, 589-593.

Bojaxa AR, Henry JR (2010). Phytochemical, Pharmacognostical, Antimicrobial activity of *Indigofera aspalathoids* vahl. (Fabaceae) in Int. J. Biol. Technol. 1(1):12-15.

Iyengar MA (2004). Pharmacognosy of powdered crude drugs. Ed. IX, 21-31.

Kailasnath ST, Rama KKN, Sai SRKV, Gourinath A, Satanarayana RP, Editor BB (2003). Intermediate first year botany, Telugu academy, Hyderabad, Andhra Pradesh, Ed. I: 197–242.

Kalaskar MG, Shah DR, Raja NM, Surana SJ, Gond NY (2010). Pharmacognostic and phytochemical investigation of *ficus carica* linn. Ethnobot. leaflets 14:599-609.

Kannan (2007). Thesis entitled Pharmacognostical studies on some selected medicinal plants submitted to Tamil university, Thanjavur, Tamilnadu, India.

Khandelwal KR (2005). Practical Pharmacognosy. Nirali Prakashan, Pune India, Ed. I: 27-35.

Kirtikar KR, Basu BD (2001). Indian medicinal plants, Published by International book distributor's book sellers and publisher, volume - I, 709.

Kokate CK (2008). Practical Pharmacognosy. Vallabh Prakashan, Delhi, Ed. III, 107-121.

Kokate CK, Purohit AP, Gokhale SB (2009). Pharmacognosy. Published by Nirali Prakashan, Ed. XXXXIII, 1.3 - 9.19.

Madhava CK, Sivaji K, Tulasi R (2009). Book of Flowering plants of Chittoor district, Andhra Pradesh, India, Ed. II, 90–95.

Madhavan V, Hema B, Guru DMR, Yoganarsimhan SN (2009). Pharmacognostical evaluation of *Drosera burmannii* Vahl (Droseraceae). Indian J. Trad. Knowledge 8(3):326-333.

Pulokk MK (2008). Quality Control of Herbal Drugs, galaxy printers, Ed. I, 187-190: 357.

Puratchikody A, Swarnalatha (2011). Pharmacognostical, Pharmacological investigation on *Indigofera aspalathoides Vahl.* Rev. J. Pharmaceut. Biol. Med. Sci. 5(23).

Sama V, Reddy YSR, Ramesh M, Swamy MM, Mahadevan NBS (2008). Pharmacognostical studies on *Dodonaea viscose* leaves. Afri. J. Pharm. Pharmacol. 2(4):83-88.

Tadigoppula N, Tanvir K, Anju P, Ramesh C (2006). Reported Antidyslipidemic activity of furano-flavonoids isolated from *Indigofera tinctoria.* Bioorganic. Med. Chem. Letters 16:3411-3414.

The Ayurvedic Pharmacopoeia of India (2004). Published by the controller of publications civil lines, Delhi, Part-1, Volume - IV, Ed. I, 156-160.

Trease EWC, Evans D (2009). Pharmacognosy, International edition, A catalogue record of book available in British library cataloguing in publication data, Library of congress cataloguing in publication data, Ed. XVI, 9.3, 49 :436.

Wen E, Liang H, Zhongguo ZYZZ (2010). Chemical constituents of *Indigofera pseudotinctoria.* School of Pharmaceutical Sciences, Peking University Health Science Center, Beijing, China, 35(20): 2708-2711.

UPCOMING CONFERENCES

International Conference on Biological, Health and Environmental Sciences, London, UK, 19 Jan 2014

International Conference on Psychology, Psychiatry, Neurological, Behavioral and Cognitive Sciences, Barcelona, Spain, 27 Feb 2014

Conferences and Advert

September 2013
61st International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research, Muenster, Germany, 1 Sep 2013

January 2014
International Conference on Medical, Biological and Pharmaceutical Sciences, London, UK, 19 Jan 2014

Journal of Pharcomagnosy and Phytotherapy

Related Journals Published by Academic Journals

- African Journal of Pharmacy and Pharmacology
- Research in Pharmaceutical Biotechnology
- Medical Practice and Reviews
- Journal of Clinical Pathology and Forensic Medicine
- Journal of Medicinal Plant Research
- Journal of Drug Discovery and Development
- Journal of Clinical Virology Research

academicJournals