EDITOR-IN-CHIEF
DR. PAWAN K AGRAWAL
Natural Product Inc.
7963, Anderson Park Lane,
Westerville, Ohio, 43081 USA
agrawal@naturalproduct.us

EDITORS
PROFESSOR GERALD BLUNDEN
The School of Pharmacy & Biomedical Sciences,
University of Portsmouth,
Portsmouth, PO1 2DT U.K.
aza6@dsl.pipex.com

PROFESSOR ALESSANDRA BRACA
Dipartimento di Chimica Bioorganica e Biofarmacia,
Universita di Pisa,
via Bonanno 33, 56126 Pisa, Italy
Email: braca@farm.unipi.it

PROFESSOR DEAN GUO
State Key Laboratory of Natural and Biomimetic Drugs,
School of Pharmaceutical Sciences,
Peking University,
Beijing 100083, China
gdu5858@163.com

PROFESSOR ERNST HASLINGER
Institute of Pharmaceutical Chemistry,
University of Graz,
A-8010 Graz, Austria
Ernst.Haslinger@uni-graz.at

PROFESSOR J. ALBERTO MARCO
Departamento de Quimica Organica,
Universidad de Valencia,
E-46100 Burjasot, Valencia, Spain
alberto.marco@uv.es

PROFESSOR YOSHIHIRO MIMAKI
School of Pharmacy,
Tokyo University of Pharmaceutical Sciences,
Horinouchi 1432-1, Hachioji, Tokyo 192-0392, Japan
mimakiy@ps.toyaku.ac.jp

PROFESSOR STEPHEN G. PYNE
Department of Chemistry,
University of Wollongong,
Wollongong, New South Wales, 2522, Australia
spyne@uow.edu.au

PROFESSOR M. G. REINECKE
Department of Chemistry,
Texas Christian University,
Ft. Worth, TX 76129, USA
m.reinecke@tcu.edu

PROFESSOR YASUHIRO TEZUKA
School of Pharmacy,
Toyoaka Medical and Pharmaceutical University,
Institute of Natural Medicine,
Tokyo, Japan
tezuka@ms.toyama-mpu.ac.jp

ADVISORY BOARD

Prof. Oyvind Andersen
Bergen, Norway

Prof. Yoshinori Asakawa
Tokushima, Japan

Prof. Bruno Botta
Roma, Italy

Prof. Carlos Cerda-Garcia-Rojas
Mexico city, Mexico

Prof. Ioanna Chinou
Athens, Greece

Prof. Josep Coll
Barcelona, Spain

Prof. Geoffrey Cordell
Chicago, IL, USA

Prof. Samuel Danishefsky
New York, NY, USA

Dr. Biswanath Das
Hyderabad, India

Prof. A.A. Leslie Gunatilaka
Tucson, AZ, USA

Prof. Stephen Hanessian
Montreal, Canada

Prof. Michael Heinrich
London, UK

Prof. Kurt Hostettmann
Lausanne, Switzerland

Prof. Martin A. Iglesias Arteaga
Mexico, D. F., Mexico

Prof. Jerzy Jaroszewski
Copenhagen, Denmark

Prof. Teodor Kaufman
Rosario, Argentina

Prof. Norbert De Kimpe
Gent, Belgium

Prof. Hartmut Laatsch
Gottingen, Germany

Prof. Marie Lacaille-Dubois
Dijon, France

Prof. Shoii-Sheng Lee
Taipei, Taiwan

Prof. Chan-Nan Lin
Kaohsiung, China

Prof. Francesco Macias
Cadiz, Spain

Prof. Anita Marsaioli
Campinas, Brazil

Prof. Rachel Mata
México D. F., Mexico

Prof. Imre Mathe
Szeged, Hungary

Prof. Joseph Michael
Johannesburg, South Africa

Prof. Ermino Murano
Trieste, Italy

Prof. Virinder Parmar
Delhi, India

Prof. Luc Pieters
Antwerp, Belgium

Prof. Om Prakash
Manhattan, KS, USA

Prof. Peter Precksh
Düsseldorf, Germany

Prof. William Reynolds
Toronto, Canada

Prof. Raffaele Riccio
Salerno, Italy

Prof. Ricardo Riguer
Santiago de Compostela, Spain

Prof. Satyajit Sarker
Clerdane, UK

Prof. William N. Setzer
Huntsville, AL, USA

Prof. Monique Simmonds
Richmond, UK

Prof. Valentin Stonik
Vladivostok, Russia

Prof. Hermann Stuppner
Innsbruck, Austria

Prof. Apichart Suksamrarn
Bangkok, Thailand

Prof. Hiromitsu Takayama
Tokushima, Japan

Prof. A.A. Leslie Gunatilaka
Huntsville, AL, USA

Prof. William Reynolds
Düsseldorf, Germany

Prof. Peter Proksch
Bergen, Norway

Prof. Oyvind Andersen
Bergen, Norway

Prof. Yoshinori Asakawa
Tokushima, Japan

Prof. Bruno Botta
Roma, Italy

Prof. Carlos Cerda-Garcia-Rojas
Mexico city, Mexico

Prof. Ioanna Chinou
Athens, Greece

Prof. Josep Coll
Barcelona, Spain

Prof. Geoffrey Cordell
Chicago, IL, USA

Prof. Samuel Danishefsky
New York, NY, USA

Dr. Biswanath Das
Hyderabad, India

Prof. A.A. Leslie Gunatilaka
Tucson, AZ, USA

Prof. Stephen Hanessian
Montreal, Canada

Prof. Michael Heinrich
London, UK

Prof. Kurt Hostettmann
Lausanne, Switzerland

Prof. Martin A. Iglesias Arteaga
Mexico, D. F., Mexico

Prof. Jerzy Jaroszewski
Copenhagen, Denmark

Prof. Teodor Kaufman
Rosario, Argentina

Prof. Norbert De Kimpe
Gent, Belgium

Prof. Hartmut Laatsch
Gottingen, Germany

Prof. Marie Lacaille-Dubois
Dijon, France

Prof. Shoii-Sheng Lee
Taipei, Taiwan

Prof. Chan-Nan Lin
Kaohsiung, China

Prof. Francisco Macias
Cadiz, Spain

Prof. Anita Marsaioli
Campinas, Brazil

Prof. Rachel Mata
México D. F., Mexico

Prof. Imre Mathe
Szeged, Hungary

Prof. Joseph Michael
Johannesburg, South Africa

Prof. Ermino Murano
Trieste, Italy

Prof. Virinder Parmar
Delhi, India

Prof. Luc Pieters
Antwerp, Belgium

Prof. Om Prakash
Manhattan, KS, USA

Prof. Peter Precksh
Düsseldorf, Germany

Prof. William Reynolds
Toronto, Canada

Prof. Raffaele Riccio
Salerno, Italy

Prof. Ricardo Riguer
Santiago de Compostela, Spain

Prof. Satyajit Sarker
Clerdane, UK

Prof. William N. Setzer
Huntsville, AL, USA

Prof. Monique Simmonds
Richmond, UK

Prof. Valentin Stonik
Vladivostok, Russia

Prof. Hermann Stuppner
Innsbruck, Austria

Prof. Apichart Suksamrarn
Bangkok, Thailand

Prof. Hiromitsu Takayama
Tokushima, Japan

Prof. A.A. Leslie Gunatilaka
Huntsville, AL, USA

Prof. William Reynolds
Düsseldorf, Germany

Prof. Peter Proksch
Bergen, Norway

Prof. Oyvind Andersen
Bergen, Norway

Prof. Yoshinori Asakawa
Tokushima, Japan

Prof. Bruno Botta
Roma, Italy

Prof. Carlos Cerda-Garcia-Rojas
Mexico city, Mexico

Prof. Ioanna Chinou
Athens, Greece

Prof. Josep Coll
Barcelona, Spain

Prof. Geoffrey Cordell
Chicago, IL, USA

Prof. Samuel Danishefsky
New York, NY, USA

Dr. Biswanath Das
Hyderabad, India

Prof. A.A. Leslie Gunatilaka
Tucson, AZ, USA

Prof. Stephen Hanessian
Montreal, Canada

Prof. Michael Heinrich
London, UK

Prof. Kurt Hostettmann
Lausanne, Switzerland

Prof. Martin A. Iglesias Arteaga
Mexico, D. F., Mexico

Prof. Jerzy Jaroszewski
Copenhagen, Denmark

Prof. Teodor Kaufman
Rosario, Argentina

Prof. Norbert De Kimpe
Gent, Belgium

Prof. Hartmut Laatsch
Gottingen, Germany

Prof. Marie Lacaille-Dubois
Dijon, France

Prof. Shoii-Sheng Lee
Taipei, Taiwan

Prof. Chan-Nan Lin
Kaohsiung, China

Prof. Francisco Macias
Cadiz, Spain

Prof. Anita Marsaioli
Campinas, Brazil

Prof. Rachel Mata
México D. F., Mexico

Prof. Imre Mathe
Szeged, Hungary

Prof. Joseph Michael
Johannesburg, South Africa

Prof. Ermino Murano
Trieste, Italy

Prof. Virinder Parmar
Delhi, India

Prof. Luc Pieters
Antwerp, Belgium

Prof. Om Prakash
Manhattan, KS, USA

Prof. Peter Precksh
Düsseldorf, Germany

Prof. William Reynolds
Toronto, Canada

Prof. Raffaele Riccio
Salerno, Italy

Prof. Ricardo Riguer
Santiago de Compostela, Spain

Prof. Satyajit Sarker
Clerdane, UK

Prof. William N. Setzer
Huntsville, AL, USA

Prof. Monique Simmonds
Richmond, UK

Prof. Valentin Stonik
Vladivostok, Russia

Prof. Hermann Stuppner
Innsbruck, Austria

Prof. Apichart Suksamrarn
Bangkok, Thailand

Prof. Hiromitsu Takayama
Chiba, Japan

Prof. Peter G. Waterman
Lismore, Australia

Prof. Paul Wender
Stanford, USA

INFORMATION FOR AUTHORS

Full details of how to submit a manuscript for publication in Natural Product Communications are given in Information for Authors on our Web site http://www.naturalproduct.us.

Authors may reproduce/republish portions of their published contribution without seeking permission from NPC, provided that any such republication is accompanied by an acknowledgment (original citation)-Reproduced by permission of Natural Product Communications. Any unauthorized reproduction, transmission or storage may result in either civil or criminal liability.

The publication of each of the articles contained herein is protected by copyright. Except as allowed under national “fair use” laws, copying is not permitted by any means or for any purpose, such as for distribution to any third party (whether by sale, loan, gift, or otherwise); as agent (express or implied) of any third party; for purposes of advertising or promotion; or to create collective or derivative works. Such permission requests, or other inquiries, should be addressed to the Natural Product Inc. (NPI). A photocopy license is available from the NPI for institutional subscribers that need to make multiple copies of single articles for internal study or research purposes.

To Subscribe: Natural Product Communications is a journal published monthly. 2007 subscription price: US$1,395 (Print, ISSN# 1934-578X); US$1,095 (Web edition, ISSN# 1555-9475); US$1,795 (Print + single site online). Orders should be addressed to Subscription Department, Natural Product Communications, Natural Product Inc., 7963 Anderson Park Lane, Westerville, Ohio 43081, USA. Subscriptions are renewed on an annual basis. Claims for nonreceipt of issues will be honored if made within three months of publication of the issue. All issues are dispatched by airmail throughout the world, excluding the USA and Canada.
Safed Musli is an important aphrodisiac herb, which forms an essential ingredient of the preparation of more than one hundred Ayurvedic formulations. It has been found to be an ideal aphrodisiac, with none of the negative side effects associated with the chemical-based products. Various plants belonging to the genera *Chlorophytum* and *Asparagus* have been in use as aphrodisiacs under the common name of ‘Safed Musli’ because of their white tuberous roots. An AFLP based experiment was carried out to differentiate the members of the ‘Safed Musli’ complex and resolve the authentication problem prevailing in the herbal drug market.

**Keywords**: Drug adulteration, aphrodisiac, DNA fingerprinting.

‘Safed Musli’ (Liliaceae) is a traditional medicinal plant found in the natural forests of India from the eastern state of Assam to the western state of Gujarat. In spite of the medicinal value of the product and an increase in demand for it, the true identity of the drug is the subject of considerable controversy. Entirely different plant species are referred to as ‘Safed Musli’ in classical and contemporary texts of the Ayurvedic system of medicine and marketed in different part of the country. The various plant species referred to in classical texts are *Asparagus adscendens*, *Chlorophytum arundinaceum* and *C. tuberosum*. However, in recent times another *Chlorophytum* species, *C. borivilianum* has become the most acceptable source of ‘Safed Musli’ in the trade. It is considered as a “wonder drug” in the Indian system of medicine due to its aphrodisiac and natural sex tonic properties, which are responsible for it being referred to as ‘Herbal Viagra’. Because of its high therapeutic importance, ‘Safed Musli’ tubers are the major constituents of more than 100 Ayurvedic preparations [1].

More than 175 species of *Chlorophytum* 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 [2]. All these species are different in their medicinal properties, but due to a lack of correct information, all species are known as ‘Safed Musli’ in the Indian drug market. The ‘Safed Musli’ complex is generally supposed to consist of *Chlorophytum borivilianum*, *C. arundinaceum*, *C. tuberosum* and *Asparagus adscendens*. *C. borivilianum* is believed to have originated in South Africa and been introduced accidentally into India; it propagates through its fleshy roots. Although Indian forests are rich in ‘Safed Musli’, its demand is increasing rapidly in the Indian and international drug markets. Foreign demand has been estimated as 300-700 tones annually [3]. In India, where ‘Safed Musli’ is popularly used for medication, its demand is over 35,000 tons per annum, but the supply is only about 5,000 tons a year. Dried roots of *Chlorophytum* contain 42% carbohydrate, 8–9% protein, 3–4% fiber and 2–17% saponin [3].

A lot of confusion prevails in the herbal drug market regarding the identification of true ‘Safed Musli’ and rampant adulteration of the drug with inferior plant...
material is reported. To resolve the ‘Safed Musli’ complex and to differentiate *Chlorophytm* species in this group and *A. adscendens*, an AFLP-based experiment was carried out with the aim of developing molecular markers for plant authentication purpose.

From a study of the crude drug it is very difficult to identify the species present in the mixture. Although the anatomical features are differentiable between *Chlorophytm* and *Asparagus*, they are not helpful in distinguishing between the various species of *Chlorophytm*. Besides, in the crude drug, it is not possible to study the anatomical features unless intact roots are obtained. So the AFLP based-approach was undertaken to generate a database of unique fragments of DNA. The logic behind this was that the isolated DNA from the crude drug, when subjected to AFLP analysis, would provide an indication of the presence of the different taxa, based on the proximity of fragment-match.

PCR-based markers have been used extensively for assessing genetic variation within a species to measure its genetic diversity [7, 8]. Amplified fragment length polymorphisms (AFLP) are ideally suited to assess germplasm because of their ability to generate and detect numerous polymorphisms that are largely distributed throughout the genome, and the method is highly reproducible [5, 9, 10]. The AFLP method largely detects single nucleotide polymorphisms [11]. The discriminatory power of AFLP has been used to distinguish between closely related inbred species, cultivars and ecotypes, such as *Lactuca* [12,13] and *Arabidopsis thaliana* [14], and was shown to detect mutations in plants arising from *in vitro* clonal propagation of *Arabidopsis* [15].

So, for achieving the objective to resolve the ‘Safed Musli’ complex and differentiating *C. borivilianum* from other species of *Chlorophytm* and *A. adscendens*, an AFLP-based experiment was carried out for developing molecular markers for plant authentication purpose. To study the genetic relatedness of *C. borivilianum* from other species of *Chlorophytm* and *A. adscendens*, an AFLP-based experiment was carried out for developing molecular markers for plant authentication purpose. To study the genetic relatedness of *C. borivilianum* with other species of *Chlorophytm* (*C. arundinaceum*, *C. tuberosum* and *A. adscendens*), molecular characterization of these plants was carried out with a set of 64 AFLP selective primer combinations (MseI/EcoRI). The plant material used for isolation of template DNA for AFLP was generated by pooling leaf samples from a diverse population of plants so as to give proper representation to all the genotypes available in a particular case. Of the 64 primer combinations, only 38 primers responded positively to genomic amplification, producing discrete bands with all the samples. These primers produced a total of 1427 bands. Of these, 17 were monomorphic and 1410 were polymorphic. Among the polymorphic bands, 1128 bands were unique. The analysis revealed ~96% polymorphism among the species. In this case, most of the polymorphic bands were unique. This happened because plants taken in the analysis were either from a different genus or different species of the same genus. As small changes in the genetic composition generate a lot of unique bands in AFLP analysis, this may be the reason for obtaining too many unique bands at interspecific and intergeneric level.

The cluster diagram (Figure 1) generated after cluster analysis showed two major groups. In the first, *A. adscendens* was clustered along with *C. borivilianum*, showing 60% similarity. In the second group, *C. arundinaceum* and *C. tuberosum* were clustered together, showing 40% similarity. The two groups were 25% diverse. Unique bands for all the species were detected and tabulated (Table 1) for identification of adulterants. In the case of *C. borivilianum* 152, of *C. arundinaceum* 431, of *C. tuberosum* 197, and of *A. adscendens* 348 bands were found to be unique. The size of these unique bands ranged between 50 bp and 400 bp. The maximum number of unique bands was detected in the case of *C. arundinaceum*, followed by *A. adscendens*, *C. borivilianum* and *C. tuberosum*. From this analysis we can infer that *C. borivilianum* is closer to *A. adscendens* than to the other species of *Chlorophytm* analyzed in this study. The DNA fingerprint comprised of the unique bands obtained for each of the four pooled populations will provide a reference tool to identify adulterants in the crude
Table 1: Unique/specific AFLP bands identified for differentiating Chlorophytum spp. and Asparagus adscendens populations in adulterated mixtures.

<table>
<thead>
<tr>
<th>Primer combination</th>
<th>Asparagus adscendens (bp)</th>
<th>Chlorophytum borivilianum (bp)</th>
<th>Chlorophytum arundinaceum (bp)</th>
<th>Chlorophytum tuberosum (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAC/AGC</td>
<td>120</td>
<td>180,183,184,185</td>
<td>115,259,309</td>
<td>-</td>
</tr>
<tr>
<td>CAT/ACH</td>
<td>179</td>
<td>108</td>
<td>130,236,279</td>
<td>140,330</td>
</tr>
<tr>
<td>CTA/AGG</td>
<td>109</td>
<td>108</td>
<td>130,236,279</td>
<td>140,330</td>
</tr>
<tr>
<td>CTA/AGG</td>
<td>139</td>
<td>103,371</td>
<td>144,326,377,382</td>
<td>148,171,278,320,321,368</td>
</tr>
<tr>
<td>CTA/ACC</td>
<td>110,179,248,359</td>
<td>105,162,284</td>
<td>101</td>
<td>371</td>
</tr>
<tr>
<td>CTA/AGC</td>
<td>229,366</td>
<td>140,208</td>
<td>103,107,189</td>
<td>-</td>
</tr>
<tr>
<td>CTA/ACA</td>
<td>109,122,134,139,183,204,349</td>
<td>107</td>
<td>120,195,211,304,308,309,334,336</td>
<td>152,165,334</td>
</tr>
<tr>
<td>CTA/ACT</td>
<td>113,135,136,139,144,344,371,383</td>
<td>-</td>
<td>-</td>
<td>106,222,277,247,249,250</td>
</tr>
<tr>
<td>CTA/AGG</td>
<td>181,264,275,370</td>
<td>108,109,170,224,320</td>
<td>107,120,123,168,187,222,260,300,381</td>
<td>106,255</td>
</tr>
<tr>
<td>CTA/ACG</td>
<td>218</td>
<td>109,276</td>
<td>127,189,241,300</td>
<td>146,182,279,318,339,346,372</td>
</tr>
</tbody>
</table>

Asparagus adscendens
Chlorophytum Chlorophytum arundinaceum
Chlorophytum tuberosum

Natural Product Communications Vol. 2 (1) 2007 95
drug supposedly consisting of a particular population. The frequency of the occurrence of these unique bands in the analysis of the DNA isolated from the crude drug preparation could be used as an assay for the presence of a specific population in it. This could help in quantification of the adulteration of the crude drug of different species. This will go a long way in establishing the authenticity and credibility of the ayurvedic drug ‘Safed Musli’, which presently suffers from the problem of adulteration. The principal component of this drug, *A. adscendens*, is mainly responsible for the aforementioned therapeutic properties. However, the presence of other *Chlorophytum* species in the crude drug may alter its efficacy and therapeutic value. Although there has been no study on the deleterious effects of the adulterants on human beings, there is a distinct possibility that they may be harmful for human usage. The importance of the present study stems from the fact that it provides an authentic tool to detect adulterants in the crude drug ‘Safed Musli’ and validates the scientific basis of this drug in Ayurveda.

### Experimental

#### Plant material:
The plant material used in this study consisted of diverse collections of *Asparagus adscendens*, *Chlorophytum borivilianum*, *C. arundinaceum* and *C. tuberosum* taken from the CIMAP gene bank. For each of the four samples, leaf material pooled from the respective populations was used for DNA isolation (20 plants in each species).

#### DNA isolation:
DNA was isolated using the protocol described by Khanuja *et al.* [4] and quantified by fluorimetry using a DyNa Quant 200 fluorimeter.

#### AFLP analyses:
For AFLP analysis, DNA was restricted using two restriction endonucleases, EcoRI and Tru9I (an isoschizomer of MseI), and double-stranded adapters were ligated to the ends of DNA fragments, generating templates for subsequent PCR amplifications (preselective followed by selective). Restriction and ligation reactions were carried out simultaneously in a single reaction [5]. To carry out the reaction, an enzyme master mix for 10 reactions was prepared containing 1μL 10X T4 DNA ligase buffer, 1μL 0.5 M NaCl, 0.5μL 1 mg/mL BSA, 1μL Tru9I (10 U/μL), 4.25μL EcoRI (12 U/μL), 0.5 μL T4 DNA ligase (20 U/μL, high concentration) and 1.75 μL water. The restriction-ligation reaction consisted of 300 ng of DNA (5.5 μL), 1 μL 10X T4 DNA ligase buffer, 1 μL 0.5 M NaCl, 0.5 μL 1 mg/mL BSA, 1 μL MseI adaptors (Applied Biosystems), 1 μL EcoRI adaptors (Applied Biosystems) and 1 μL enzyme master, as described above. The reaction mix was incubated overnight at room temperature and subsequently diluted 20-fold with T10E0.1 (10 mM Tris and 0.1 mM EDTA) buffer. The ligated adaptors served as primer binding sites for a low-level selection in the preselective amplification of restriction fragments. The MseI complementary primer had a 3' -C and the EcoRI complementary primer a 3' -A. Only the genomic fragments having an adaptor on each end amplified exponentially during the PCR. The preselective amplification mix was prepared by adding 4 μL of 20-fold diluted DNA from the restriction-ligation reaction, 0.5 μL AFLP preselective primer (EcoRI, Applied Biosystems), 0.5 μL AFLP preselective primer (MseI, Applied Biosystems), and 15 μL AFLP core mix. The preselective amplification was carried out in a thermal cycler programmed as: 72°C for 2 min; 20 cycles of 94°C for 20 sec, 56°C for 30 sec and 72°C for 2 min; 60°C for 30 min; and 4°C for infinity.

The preamplified DNA was diluted 20-fold with T10E0.1 buffer and selective amplifications were carried out using different MseI and EcoRI primer combinations (Applied Biosystems). Primers chosen for the amplification were from 16 available AFLP combinations (8 fluorescently tagged EcoRI and 8 untagged MseI primers). The EcoRI primers contain 3 selective nucleotides with the sequence 5' [Dye-Primer-Axx]-3', while the MseI primers had the 3 selective nucleotides starting with C with the sequence 5' [Primer-Cxx]-3'. Selective amplification of each sample was done with all the 64 (8x8)-primer combinations (MseI/EcoRI) using multiplex-PCR.

### Table 1 (Continued)

| CTG/AGG | 324,325 | - | 287 | 321,393 |
| CTG/ACG | 106,158,268,302,337 | 398 | 235,263,341 | 223,224,330,331 |
| CTT/ACA | 130,189,388 | 119 | 299,303 | 335 |
| CTT/ACC | - | 328 | 266,317 | 146,330,331 |
| CTT/AGG | 120, 175,347,385 | 367 | 147,148,149,150 | 329,333,335 |
AFLP based detection of adulterants in Safed Musli complex

AFLP reactions. For selective amplification the reactions were set up as follows: 3 μL of 20-fold diluted preselective amplification product, 15 μL AFLP core mix, 1 μL MseI primer 5′-[Primer-Cxx]-3′, 1.5 μL EcoRI primers 5′-[Dye-Primer-Axx]-3′ (0.5 μL of 3 EcoRI primers each were pooled here). Selective amplification was carried out in a thermal cycler programmed as: 94°C for 2 min; 10 cycles of 94°C for 20 sec, 66°C (-1°C/cycle) for 30 sec, 72°C for 2 min; 20 cycles of 94°C for 20 sec, 56°C for 30 sec, 72°C for 2 min; 60°C for 30 min; and 4°C for infinity. The samples were loaded onto a 5% polyacrylamide gel on an ABI Prism 377 DNA Sequencer. For gel electrophoresis, 3 μL of the selective amplification reaction product was mixed with 4 μL of loading buffer {ROX500 size standard (10%), blue dextran (10%), deionised formamid (80%)}, and 1.5 μL of this mix was finally loaded on the gel. For AFLP reactions the AFLP amplification modules and the guidelines supplied by Applied Biosystems, USA were used.

**Data analysis:** For diversity analysis bands were scored as present (1) or absent (0) to form a raw data matrix. A square symmetric matrix of similarity was then obtained using Jaccard similarity coefficient [6] by SPSS v7.5 software. The average similarity matrix was used to generate a tree for cluster analyses by UPGMA (Unweighted Pair Group Method with Arithmetic average) method using NTSys v2.1.

**Acknowledgments** - The authors gratefully acknowledge the financial help provided by ICMR and CSIR, India.

References


Isoquinoline Alkaloids from the Leaves of Dehaasia hainanensis
Chien-Kuang Chen, Su-Chang Chen, Chung-Hsiung Chen and Shoei-Sheng Lee 75

Leaf Essential Oil Composition of Five Species of Beilschmiedia from Monteverde, Costa Rica
William N. Setzer and William A. Haber 79

Antibacterial Activity of the Essential Oil of Lippia oreganoides Against Multiresistant Bacterial Strains of Nosocomial Origin
Judith Velasco, Janne Rojas, Poema Salazar, Mariseg Rodríguez, Tulia Díaz, Antonio Morales and Maria Rondón 85

Essential Oil Composition of the Umbels and Fruit of Prangos uloptera DC
Hossein Nazemiyeh, Seied M. Razavi, Abbas Delazar, Rogaieh Hajiboland, Valiollah Mozaftarian, Lutfun Nahar and Satyajit D. Sarker 89

AFLP-based Detection of Adulterants in Crude Drug Preparations of the ‘Safed Musli’ Complex
Amita Misra, Ajit K Shasany, Ashutosh K. Shukla, V Sundaresan, Seetal P Jain, Guru D. Bagchi, Janardan Singh and Suman P.S. Khanuja 93

Review/Account

Steroidal Saponins and Sapogenins from the Agavaceae Family
Joanne L. Simmons-Boyce and Winston F. Tinto 99

Manuscripts in Press

115
## Contents

**Original paper**

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leishmanicidal Activity of Artemisinin, Deoxoartemisinin, Artemether and Arteether</td>
<td>1</td>
</tr>
<tr>
<td>Claudio M. Lezama-Dávila, Abhay R. Satoskar, Mirna Úc-Encalada, Ricardo Isaac-Márquez and Angélica P. Isaac-Márquez</td>
<td></td>
</tr>
<tr>
<td>A New Irregular Diterpenoid of Biogenetic Interest from the Flowers of <em>Magydaris tomentosa</em> (Desf.) DC. (Apiaceae)</td>
<td>5</td>
</tr>
<tr>
<td>Sergio Rosselli, Antonella Maria Maggio, Gabriella Bellone, Carmen Formisano, Felice Senatore and Maurizio Bruno</td>
<td></td>
</tr>
<tr>
<td>A Bioactive Diterpene from <em>Entada abyssinica</em></td>
<td>9</td>
</tr>
<tr>
<td>Alembert T. Tchinda, Victorine Fuendjiep, Yalemtsehay Mekonnen, Bernadette B. Ngo and Ermias Dagne</td>
<td></td>
</tr>
<tr>
<td>Chemical Variation in the Diterpenes from the Brazilian Brown Alga <em>Dictyota mertensii</em> (Dictyotaceae, Phaeophyta)</td>
<td>13</td>
</tr>
<tr>
<td>Odinéia do Socorro Pamplona Freitas, Aline Santos de Oliveira, Joel Campos De-Paula, Renato Crespo Pereira, Diana Negrão Cavalcanti and Valéria Laneuville Teixeira</td>
<td></td>
</tr>
<tr>
<td>Analysis and Antiproliferative Activity of Bark Extractives of <em>Betula neoalaskana</em> and <em>B. papyrifera</em>. Synthesis of the Most Active Extractive Component - Betulin 3-Caffeate</td>
<td>17</td>
</tr>
<tr>
<td>Igor V. Kolomitsyn, Jon Holy, Edward Perkins and Pavel A. Krasutsky</td>
<td></td>
</tr>
<tr>
<td>Three Pregnane Glycosides from <em>Pergularia pallida</em></td>
<td>27</td>
</tr>
<tr>
<td>Sangeeta Srivastava, Naveen K. Khare and Anakshi Khare</td>
<td></td>
</tr>
<tr>
<td>Steroidal Glycosides from the Underground Parts of <em>Agapanthus inapertus</em> and Their Cytotoxic Activity</td>
<td>35</td>
</tr>
<tr>
<td>Akihito Yokosuka and Yoshihiro Mimaki</td>
<td></td>
</tr>
<tr>
<td>New Neuritogenic Steroid Glycosides from the Vietnamese Starfish <em>Linckia laevigata</em></td>
<td>41</td>
</tr>
<tr>
<td>Alla A. Kicha, Natalia V. Ivanchina, Anatoly I. Kalinovsky, Pavel S. Dmitrenok, Natalia V. Palyanova, Tatyana M. Pankova, Marina V. Starostina, Margherita Gavagnin and Valentín A. Stonik</td>
<td></td>
</tr>
<tr>
<td>Synthesis of Polyhydroxylated Δ^15^-17,17-dialkyl-18-norsteroids by BF$_3$·Et$_2$O/Ac$_2$O-promoted Wagner-Meerwein Rearrangement of Furostanols</td>
<td>47</td>
</tr>
<tr>
<td>Martin A. Iglesias-Arteaga, José. M. Mendez-Stivalet and Nury Pérez</td>
<td></td>
</tr>
<tr>
<td>Abruptoside A, A Novel Glycolipid from the Kenyan Soft Coral <em>Sinularia abrupta</em></td>
<td>51</td>
</tr>
<tr>
<td>Guy Shmul, Yehuda Benayahu and Yoel Kashman</td>
<td></td>
</tr>
<tr>
<td>Phenolic Constituents of Leaves of <em>Diospyros montana</em></td>
<td>55</td>
</tr>
<tr>
<td>Toshiyuki Tanaka, Miyuki Furusawa, Tetsuro Ito, Ibrahim Iliya, Masayoshi Oyama, Munekazu Inumma, Nobuyuki Tanaka and Jin Murata</td>
<td></td>
</tr>
<tr>
<td>The Effect of Cinnamtannin B1 on Cell Proliferation and Glucose Uptake of 3T3-L1 Cells</td>
<td>61</td>
</tr>
<tr>
<td>Muhammad Taher, Fadzilah Adibah Abdul Majid and Mohamad Roji Sarmidi</td>
<td></td>
</tr>
<tr>
<td>Synthesis of Hypericin via Emodin Anthrone Derived from a Two-fold Diels-Alder Reaction of 1,4-Benzoxquinone</td>
<td>67</td>
</tr>
<tr>
<td>Jiro Motoyoshiya, Yusuke Masue, Yoshinori Nishi and Hiromu Aoyama</td>
<td></td>
</tr>
<tr>
<td>Naturally Occurring 1,1’-Trimethylenebisuracil from the Marine Sea Hare <em>Dolabella auricularia</em></td>
<td>71</td>
</tr>
<tr>
<td>Tadigoppula Narender, Tanvir Khaliq and M. N. Srivastava</td>
<td></td>
</tr>
</tbody>
</table>

*Continued inside back page*