Pharmacognosy Journal 5 (2013) 143–148

Contents lists available at ScienceDirect

Pharmacognosy Journal

journal homepage: www.elsevier.com/locate/phcgj

Original article

Pharmacognostic standardization of leaves of *Melaleuca leucadendron*

Gagan Shah [a,*], Amit Chawla [b], Uttam Singh Baghel [b], Sandeep Rahar [b], Prabh Simran Singh [b], Ravi Kumar Dhawan [c]

[a] Department of Pharmacognosy, Khalsa College of Pharmacy, Amritsar 143001, India
[b] Department of Pharmaceutical Chemistry, Khalsa College of Pharmacy, Amritsar 143001, India
[c] Department of Pharmacology, Khalsa College of Pharmacy, Amritsar 143001, India

ARTICLE INFO

Article history:
Received 11 May 2013
Accepted 17 July 2013
Available online 21 August 2013

Keywords:
Melaleuca leucadendron
Standardization parameters
Phytochemical screening

ABSTRACT

Introduction: Essential oil of *Melaleuca leucadendron* used as antimycotic and antimicrobial. The oil also used as antiinflammation and antifungal.
Methods: The present study will assist in standardization for quality, purity and sample identification. Various standardization parameters like morphological characters, microscopic evaluation, physico-chemical evaluations) preliminary phytochemical screening and TLC chromatographic profile of the extract were carried out and the qualitative parameters were reported.
Result: In the pharmacognostical studies, in an attempt to standardize the leaves of *M. leucadendron* have been shown which will be definitely useful to the future scientist for the identification of the plants.
Conclusion: These studies provide referential information for correct identification and standardization of this plant material.

1. Introduction

The World Health Organization estimates that 80% of the world's population relies on herbal medicine. Meanwhile, the use of herbs in the United States is expanding rapidly, to the point where herbal products are readily found in most pharmacies and supermarkets. From 1990 to 1997, as the use of complementary and alternative medicine rose from 34 to 42%, herbal use quadrupled from 3 to 12%. It is worth remembering that these rapid changes have come not through the medical profession, but by popular demand. The public has discovered that natural medicines often provide a safe, effective, and economical alternative to pharmaceuticals, and research validates this finding. The majority of those who use herbal and high-dose vitamin products fail to tell their physicians. Either they assume that these products are harmless and not worth mentioning or they fear being ridiculed by doctors sceptical about their use. These same doctors, however, must begin to familiarize themselves with the subject. Aside from the advantages of the natural products, herb–drug interactions are a growing concern: almost one in five prescription drug users also using supplements.[1] India has a rich heritage of traditional medicines and the traditional health care systems have been flourishing for many centuries. It mainly consist of three major systems namely Ayurveda, Siddha and Unani systems of Medicine.[2] In almost all the traditional systems of medicine, the quality control aspect has been considered from its inspection itself by the Rishis and later by the Vaidya and Hakims. However, in modern concept it requires necessary changes in their approach. Quality control and quality assurance is an integral part of traditional medicines, which ensures that it delivers the required quantity of quality medicament.[3] Essential oil of *Melaleuca leucadendron* possessed antimicrobial and antifungal activities.[4] chloroform and methanol extracts of the fruits of *M. leucadendron* strongly inhibited histamine release from rat mast cells induced by compound 48/80 or concanavalin A. Ursolic acid, a triterpene, was the most active compound contained in the chloroform extract and two stilbenes, piceatannol and oxyresveratrol, were isolated as active compounds from the methanol extract.[5] New lupane-type nortriterpene and 13 known compounds from the leaves of *M. leucadendron.* Based on chemical and spectral methods, the structure of the new compound was elucidated as 28-norlup-20(29)-ene-3beta,17beta-diol, while the known compounds were identified as (2E,6E)-farnesol, phytol, squalene, alloaromadendrene, ledene, palustrol, viridiflorol, ledol, betulinaldehyde, betulinic acid, 3beta-acetyl-lup-20(29)-en-28-oic acid, 3-oxolup-20(29)-en-28-oic acid, and platanic acid.[6] Four new triterpenes, eupha-7,24-diene-3beta,22beta-diol (1), 20-tarax astene-3alpha,28-diol (2), 3alpha,27-dihydroxy-28, 20beta-tarax-astanolide (3), and 3alpha-hydroxy-13(18)-oleanene-27, 28-dioic

* Corresponding author.
E-mail addresses: gaganshah83@gmail.com, gaganshah83@yahoo.com (G. Shah).

0975-3575/$ – see front matter
http://dx.doi.org/10.1016/j.phcgj.2013.07.008

acid (4) have been isolated from the heartwood of *M. leucadendron.* The structures and stereochemistry of 1–4 have been determined by spectroscopic analysis, with compounds 3 and 4 being investigated in the forms of their diacetate (3a) and dimethyl (4a) derivative, respectively.[7] The essential oil of *M. leucadendron.* (tea tree) against the Gram-negative bacterium *Escherichia coli* AG100, the Gram-positive bacterium *Staphylococcus aureus* NCTC 8325, and the yeast *Candida albicans* has been using a range of methods. The essential oil of *M. leucadendron.* (tea tree) exhibits broad-spectrum antimicrobial activity.[8] The tree oil reduces histamine-induced skin inflammation.[9] In vitro susceptibility of oral bacteria to *M. leucadendron* oil and found that a range of oral bacterial are susceptible to tea tree oil. Investigation was also made on the in vitro antifungal activity of the components of *M. leucadendron* oil and found active.[10] However no scientific standards or pharmacognostic parameters are yet available to determine the quality of this crude drug. Thus the present study was designed to evaluate the pharmacognostic parameters of *M. leucadendron* leaves.

2. Materials and methods

Fresh leaves of. *M. leucadendron* were collected from MPDA, Doddabetta and Sims Park at Coonoor in Nilgiris respectively in the month of February. The collected material were identified, confirmed and authenticated by botanist Dr. D. Suresh Baburaj, Botanical survey of India, Central Council for Research in Homeopathy, Govt. Arts College campus, Ootacamund.

2.1. Phytochemical screening

The various extracts of *M. leucadendron* were subjected to qualitative chemical examination.[11,12]

2.2. Thin layer chromatographic profile

TLC glass plates (5 × 15 cm), 0.25 mm thick were prepared using silica gel G. The plates were activated at 110 °C for 30 min. The TLC profiles of the extracts were studied using different solvent systems. TLC plates were developed in TLC chamber. Thin layer chromatograms were visualized under 254/366 nm UV light and in iodine chamber. Spraying reagent 5% methanolic-sulphuric acid is used.

2.3. Organoleptic evaluation

Organoleptic evaluation of leaves was done by observing fruits and seeds with naked eyes.

2.4. Microscopic and histological techniques of leaves

2.4.1. Study of transverse sections

The leaves of *M. leucadendron* were boiled with water until soft. Free hand sections of both fruits and seeds were cut transferred on slides cleared by warming with chloral hydrate and mounted in glycerin. The lignified and cellulosic tissues were distinguished using differential staining techniques.[13]

2.4.2. Photomicrography

Microscopic evaluation of tissues was supplemented with micrographs. Photographs of different magnifications were taken with Nikon Labpot 2 microscopic unit. For normal observations bright field was used. For the study of crystals, starch grain and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars.[14]

2.4.3. Powder microscopy

A few drops of chloral hydrate solution was added to a sample of powered plant material on a slide, covered with a glass slip and heated gently over a microbunsen. Vigorous boiling was avoided. The slide was examined under the microscope. When the clearing process is completed a drop of glycerol solution was added which will prevent crystallization of the mounting agent on cooling.

2.5. Physicochemical parameters

Physicochemical analysis i.e., alcohol (90% ethanol) and water soluble extractive values, total ash, acid-insoluble ash, and loss on drying of the powdered drug were determined.[15,16]

2.6. Phytochemical screening

The various extracts of *M. leucadendron* were subjected to qualitative chemical examination.[11,17]

2.7. Quantitative microscopy

Numbers of leaf measurements were used to study microscopic features not easily characterized by general microscopy. These included – stomatal number vein islet number – veinlet termination number – stomatal index, palisade ratio. The average number of palisade cells beneath each upper epidermal cell is termed the palisade ratio.[18]

2.8. Evaluation of volatile oil

Volatile oil was evaluated for optical rotation and refractive index as per the standard procedure.[19]

3. Results

3.1. Organoleptic features of leaves (Fig. 1)

Condition	Fresh adult leaves
Colour	Dark green
Odour	Characteristic
Shape	Lanceolate
Dimensions	Length – 10–15 cm, Width – 2–4 cm
Leaf base	Exstipulate
Margin	Entire
Apex	Acuminate
Base	Symmetrical
Surface	Glabrous
Texture	Coriaceous
Venation	Pinnate lateral veins anastomose near the margin to a continuous line
Petiole	Short and twisted

3.2. Microscopic features of the leaves

3.2.1. Anatomy of leaf

In TS view, the leaf is flat with more or less uniform lamina and fairly prominent midrib (Fig. 2). The lateral veins are thick and prominent, lent do not project beyond the surface of the lamina (Fig. 3). The marginal part is thick and semicircular (Fig. 4). The following parts of the leaf are seen.