Standardization of the Homeopathic Mother Tincture of *Calendula officinalis* Linn.

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Homeopathic medicine
***Calendula officinalis* Linn.**
Mother tincture
Standardization
HPTLC fingerprinting

Summary

The quality of two samples (Indian and German mother tincture) of *Calendula officinalis* Linn. prepared from marigold flowers have been evaluated on the basis of homeopathic literature. In addition to non-specific standards, two biomarkers, rutin and quercitin, have been used for microfingerprinting analysis of the samples of mother tincture. The results indicated the total absence of rutin in the Indian mother tincture whereas both rutin and quercitin were present in the German mother tincture. On the basis of these results a ratio calculation was performed. The study will be useful to manufacturers of homeopathic mother tinctures.

1 Introduction

In India alternative systems of medicine, including homeopathy, have been practiced for a long time. Although homeopathic formulations are considered to be safe, stable, and efficacious, these aspects must be documented after experimentation. Because data in the Homeopathic Pharmacopoeia of India [1] and the British Herbal Pharmacopoeia [2] is far from complete, more data on standardization and validation of mother tinctures must be generated.

Because of the multiconstituent nature of plant extracts, results from fingerprinting, preferably using two biomarkers, are acceptable. A model drug, marigold (*Calendula officinalis* Linn.), the tincture of which is extensively used in ointments for wound healing [3] and also has other properties, for example anti-inflammatory [2] and antiprotozoal [4] activity, were selected in this study. This drug contains an important flavonoid, rutin, and a product of its hydrolysis, quercitin, [5] along with calendulin, esters of higher fatty acids, triterpene saponins, resins, bitter glycosides, tannins, carotenes, essential oil, sterols, and mucilage [6]. Rutin and quercitin could be employed as biomarkers for quantitative estimation of mother tinctures of *Calendula officinalis* Linn. prepared by extraction of florets or flowers. Some details of the tincture have been published in a textbook [7]. Recent requirements for standardization of herbal remedies demand use of two markers instead of one. This paper reports the fingerprint analysis of mother tincture of *Calendula officinalis* Linn. using working standards of rutin and quercitin (the latter synthesized from the former in this laboratory) as two calibrator biomarker standards.

2 Experimental

Samples of mother tincture of *Calendula officinalis* Linn. were procured from two different sources – indigenous (SBL, India; Sample I) and imported from Germany (Dr Reckeweg, Bensheim; Sample II). The working standard of rutin trihydrate was procured from Fluka, Switzerland and sample of *Rutin* NF was kindly supplied by Ornate Pharma, Ahmedabad. *n*-Butanol, glacial acetic acid, chloroform, and acetone were GR grade from Merck (India); toluene was spectroscopic grade from Merck and sulfuric acid was GR grade from Qualigen. Distilled water (parenteral grade) was prepared by use of a MilliQ unit.

2.1 Characterization of the Mother Tincture

All the volumetric ware used in the study was calibrated; instruments were calibrated and/or validated. Both samples of mother tincture were processed identically throughout the study.

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