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Characterisation of polyphenolic compounds in *Clerodendrum petasites* S. Moore and their potential for topical delivery through the skin

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ABSTRACT

Ethnopharmacological relevance: Clerodendrum petasites S. Moore (CP) has been widely prescribed in Thailand and neighbouring countries for both oral and topical administration to treat asthma, fever, cough, vomiting and skin diseases, for at least 30 years. However, the nature of the active species remains poorly characterized and there have been no clinical trials concerning the topical delivery of this medicine. The study aims to characterise polyphenolic compounds in the plant, to predict the feasibility of their topical absorption and to test their ability to penetrate the skin.

Materials and methods: Identification and quantification of flavonoids and phenolic acid derivatives in an ethanolic extract of the aerial parts of the plant were carried out using high performance liquid chromatography (HPLC) with photodiode array (PDA) and mass spectrometry (MS) detection. Ambiguous isomeric compounds were distinguished by nuclear magnetic resonance (NMR) spectroscopy. The feasibility of the compounds' topical permeability was evaluated by predicting their maximum fluxes from their physicochemical properties. The skin penetration of compounds in the plant extract was measured *in vitro* over 24 h.

Results: Vanillic acid, verbascoside, 4-coumaric acid, ferulic acid, nepetin, luteolin, apigenin, naringenin, hispidulin, hesperetin and chrysin, were identified in CP. All compounds except apigenin and hispidulin are reported in this species for the first time. Hispidulin is the predominant compound (1.2% w/w in a dried ethanolic extract) followed by nepetin, verbascoside, vanillic acid, and apigenin. Across mammalian skin, hispidulin was percutaneously absorbed within 3 h and vanillic acid and nepetin permeated the skin after 6 h. These experimental observations were consistent with the predicted maximum fluxes of these compounds calculated from their physicochemical properties.

Conclusions: Many of the phenolic compounds reported in this study are well-known to possess antimicrobial, anti-inflammatory and anti-oxidant activities. The skin permeation studies reported here support traditional topical uses of the plant in skin treatments and are useful for further topical formulation optimisation.

1. Introduction

Clerodendrum petasites (English name: One Root Plant) is one of the ~700 species of this genus in the family Lamiaceae (*Clerodendrum petasites* S. Moore, 2005; The plant list, 2010). The plant is widespread in the middle, north-eastern, and southern parts of Thailand. There are numerous Thai names from each region, for instance, Ping-Khom and Ping-Luang in the north, Phaya-Rak-Deaw in the south, Nang-Shon and Phom-Phee in the northeast. However, Thao-Yaai-Mom from the midlands is the best known.

Thai traditional practitioners usually prepare aerial parts, leaves, or roots of *Clerodendrum petasites* as a tea, alcoholic extract or cigarette to treat asthma (Hazekamp et al., 2001; Panthong et al., 1986, 2003). Leaves and roots are also ground into powders for treatment of inflammation (Panthong et al., 1986) as well as to treat fever, cough, and vomiting (Panthong et al., 2003; Thai traditional medical textbook: Paet-Ta-Ya-Saat-Song-Kror (แพทยศาสตร์สงเคราะห์), 2007) (S. Tungjitaruen, pers. comm., 2011). The plant is widely prescribed for oral administration and generally formulated into multi-herb recipes. The most famous recipe is “Ha-Rak” (synonyms: Ben-Cha-Lo-Ka-Wi-Chian, Kaew-Ha-Dueng, Phed-Sa-Wang), containing equal amounts by weight of five roots from *Clerodendrum petasites*, *Ficus racemosa* Linn,

Abbreviations: CP, *Clerodendrum petasites* S. Moore; HPLC, high performance liquid chromatography; PDA, photodiode array; UV, ultraviolet; MS, mass spectrometry; NMR, nuclear magnetic resonance spectroscopy

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Capparis micracantha DC, *Harrisonia perforata* Merr, and *Tiliacora triandra* Diels (Pichaensoonthon et al., 2005). The recipe is currently registered by the Thai Food and Drug Administration (FDA) for antipyretic activity (List of herbal medicinal products, 2006; National list of essential medicines: Ha-Rak, 2012). Dosage forms of Ha-Rak are powders, tablets and capsules, but decoction is conventionally served. There are fewer records for topical remedies. Poultices are most often formulated for skin diseases, such as rash, abscess, urticaria, snakebites and insect bites (Pongboonrot, 1965; Panthong et al., 2003; Thai traditional medical textbook: Paet-Ta-Ya-Saat-Song-Kror (แพทยศาสตร์สงเคราะห์), 2007) (T. Tipcharoentham, pers. comm., 2011; S. Tungjitaruen, pers. comm., 2011). Many recipes are dispersed in alcohol, especially Thai rice whisky, before application.

Clerodendrum petasites is also widely distributed in many other countries, *e.g.*, Malaysia, India, Southern China, Sri Lanka, and Vietnam. Ethnomedical uses of the plant are found in their medical systems. For example, root and leaf extracts of *Clerodendrum petasites* have been documented for the treatment of rheumatism, asthma and other inflammatory diseases (Shrivastava and Patel, 2007). In India, fruits are reportedly used to reduce fertility in males and the plant is used to cure malaria in China (Hazekamp et al., 2001; Panthong et al., 2003; Shrivastava and Patel, 2007).

Although the chemical constituents in the genus *Clerodendrum* have been widely investigated, there have been only a few studies on *Clerodendrum petasites*. The compounds previously reported in the aerial parts and roots of *Clerodendrum petasites* include apigenin, hispidulin, 6,4′-dimethoxyscutellarin, hispidulin 7-methylglucuronide, nevadensin 7-glucoside, arbutin and bungene A (Hazekamp et al., 2001; Klaiklay, 2009; Singharachai et al., 2011; Thongchai et al., 2007). There have been no clinical trials that identify and verify the compounds that elicit useful pharmacological effects following topical delivery. Thus, in this study, flavonoids and other phenolic compounds, which are well-known as strong antioxidants with free radical scavenging and metal chelating activities (Perron and Brumaghim, 2009; Robak and Gryglewski, 1996; Wuguo et al., 1997), and are extensively used in dermatological and cosmetological applications (Arct et al., 2002; Arct and Pytkowska, 2008; Bonina et al., 1996; Cimino and Saija, 2005; Lin et al., 2008), were characterised and their topical absorption were determined using a pig skin model. Experimental values were compared with theoretical percutaneous fluxes calculated from the physicochemical properties of the compounds (Potts and Guy, 1992) to evaluate the predictive value of the theoretical equations to the complex mixtures present in a herbal preparation.

2. Materials and methods

2.1. Plant materials

Dried samples of the aerial parts of *Clerodendrum petasites* were authenticated by macroscopic identification and obtained from the Ayurved Siriraj Manufacturing Unit of Herbal Medicines and Products, Center of Applied Thai Traditional Medicine (CATTM), Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. Extracts were produced by maceration using 80% ethanol and subsequently evaporated to dryness. Five batches of ethanolic extracts were kept separately in light protective and airtight containers and stored in a desiccator at room temperature.

The ethanolic extracts were separated into water, butan-1-ol, ethyl acetate and petroleum ether soluble fractions by liquid–liquid partition. Only the butanol and ethyl acetate fractions were further separated by column chromatography using a step gradient of 100% ethyl acetate followed by 1%, 2%, 5%, 10%, 20%, and 50% methanol in ethyl acetate and 100% methanol. All the fractions were kept in light protective and airtight containers and stored at 4 °C. The fractions were subsequently examined by NMR to elucidate the structure of ambiguous isomers.

2.2. Chemicals and reagents

Caffeic acid, 4-coumaric acid, naringin, chrysin, 5,7-dimethoxycoumarin, gallic acid, rosemarinic acid, kaempferol, cinnamic acid (Sigma-Aldrich, USA), vanillic acid, ferulic acid, apigenin (Fluka Analytical, China), rutin, quercetin (Koch-Light Laboratories Ltd., UK), verbascoside, naringenin, chrysoeriol, hesperetin, luteolin, diosmetin, nepetin, scutellarein (Extrasynthese, France), hispidulin (Tocris Bioscience, UK), cirsimaritin (BioBioPha. Co; Ltd.), were of analytical grade.

Mobile phases for HPLC–MS and HPLC–PDA consisted of HPLC grade acetonitrile (Fisher Scientific, UK), HPLC grade water obtained from a deionized water treatment system (Milli-pore, MA, USA) and MS grade acetic acid (Fluka Analytical, Germany). Deuterated-methanol (methanol-D_4, CD_3OD), deuterated-chloroform (chloroform-D, $CDCl_3$) and deuterium oxide (D_2O) were used for NMR analysis and purchased from Cambridge Isotope Laboratories, Inc., UK. Other chemicals and reagents, methanol, ethanol (Sigma-Aldrich, USA), butan-1-ol (Fisher Scientific, UK), ethyl acetate, and petroleum ether, tris (hydroxymethyl) aminomethane hydrochloride (Tris–HCl, Acros Organics, USA), tris aminomethane (Trizma® base, Sigma-Aldrich, USA), were of analytical grade.

Excipients of the preliminary topical formulations comprised propylene glycol (Acros Organics, UK) and Vaseline white (Riedel-de Haën, Germany).

2.3. Skin

Fresh porcine abdominal skin was obtained from B&J Pigs Ltd., Somerset, UK. Excessive hair was carefully trimmed using scissors. After cleaning with running cold water, the skin was dermatomed (Zimmer electric dermatome, Oklahoma, USA) to a nominal thickness of 750 μm. The dermatomed skin was sealed in a plastic bag and stored at −20 °C until use.

2.4. Preparation of standard solutions

Stock solutions (0.1 mg mL^{-1}) of the phenolic standards were prepared by dissolution in methanol followed by sonication for 30 min where necessary (Fisherbrand® FB11002, Thermo Fisher Scientific Inc., UK). Each analyte stock solution was diluted with methanol to appropriate concentrations for the establishment of calibration curves and validation tests. All standard solutions were filtered through a 0.45 μm nylon membrane (Chronus® filter, LabHut Ltd., UK) before HPLC–MS or HPLC–PDA analysis. Both stock and diluted solutions were stored at 4 °C.

2.5. Preparation of plant sample solutions

The dried extract of *Clerodendrum petasites* was accurately weighed and dissolved in methanol at a concentration of 50 mg mL^{-1} and sonicated for 30 min. After centrifugation at 4000 rpm for 20 min (U-32, Boeco, Germany), the supernatant was filtered through a 0.45 μm nylon membrane and diluted with methanol to appropriate concentrations prior to HPLC–MS or HPLC–PDA analysis. The filtered plant sample solution was stored at 4 °C.