Journal of Ethnopharmacology 166 (2015) 18–22

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Hispidulin, a constituent of *Clerodendrum inerme* that remitted motor tics, alleviated methamphetamine-induced hyperlocomotion without motor impairment in mice

Wei-Jan Huang [a,b], Hsin-Jung Lee [c,1], Hon-Lie Chen [d,1], Pi-Chuan Fan [d,e], Yuan-Ling Ku [g], Lih-Chu Chiou [c,d,f,*]

[a] Graduate Institute of Pharmacognosy, Taipei Medical University, Taipei, Taiwan
[b] School of Pharmacy, National Defense Medical Center, Taipei, Taiwan
[c] Department of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan
[d] Graduate Institute of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan
[e] Department of Pediatrics, College of Medicine, National Taiwan University, Taipei, Taiwan
[f] Graduate Institute of Brain and Mind Sciences, College of Medicine, National Taiwan University, Taipei, Taiwan
[g] Medical and Pharmaceutical Industry Technology and Development Center, New Taipei City, Taiwan

ARTICLE INFO

Article history:
Received 13 August 2014
Received in revised form
17 February 2015
Accepted 1 March 2015
Available online 9 March 2015

Chemical compounds studied in this article:
Hispidulin (PubChem CID: 5281628)
Acacetin (PubChem CID: 5280442)

Keywords:
Clerodendrum inerme
Hispidulin
Flavonoids
Motor tics
Tourette syndrome
Methamphetamine

ABSTRACT

Ethnopharmacological relevance: Previously, we found a patient with an intractable motor tic disorder that could be ameliorated by the ground leaf juice of *Clerodendrum inerme* (*CI*). Furthermore, the ethanol extract of *CI* leaves effectively ameliorated methamphetamine-induced hyperlocomotion (MIH) in mice, an animal model mimicking the hyper-dopaminergic status of tic disorders/Tourette syndrome, schizophrenia, or obsessive–compulsive disorder. Here, we for the first time identified a constituent able to reduce MIH from the *CI* ethanol extract that might represent a novel lead for the treatment of such disorders.

Materials and methods: The ethanol extract of *CI* was sub-divided into *n*-hexane, dichloromethane, *n*-butanol and water fractions. Using MIH alleviation as a bioassay, active compounds were identified in these fractions using silica gel chromatography, recrystallization and proton NMR spectroscopy.

Results: The dichloromethane and *n*-hexane fractions were active in the bioassay. Further subfractionation and re-crystallization resulted in an active compound that was identified to be hispidulin by proton NMR spectroscopy. Hispidulin significantly alleviated MIH in mice at doses that did not affect their spontaneous locomotor activity or performance in the rotarod test, a measure for motor coordination.

Conclusions: Hispidulin is a flavonoid that has been isolated from several plants and reported to have anti-oxidative, anti-inflammatory and anti-cancer activities. Here, we for the very first time found that hispidulin can also alleviate MIH at doses that did not impair motor activity, suggesting a therapeutic potential of hispidulin in hyper-dopaminergic disorders.

1. Introduction

In a clinical case report, we have found that a female pediatric patient with an intractable chronic motor tic disorder responded dramatically to the grounded leaf juice of a local herb, *Clerodendrum inerme* (L.) Gaertn (*CI*) (Fan et al., 2009). This patient has also suffered from recurrent seizures and her parent originally tried to use this herb to relieve her seizures since *CI* is anecdotally believed to have anti-seizure activity. However, the *CI* leaf juice was ineffective for her seizures. Therefore, she keeps visiting our pediatric clinic for seizure control. On her latest visit to our clinic, 13 years after she first took *CI*, she reported that she had almost no more tics and the tic attack, if occurred occasionally, would subside 1 hour after taking *CI*. The latter observation supports the conclusion that *CI* was effective in remitting her tic attacks. However, a contribution of more mature brain circuits to her almost tic-free status after growing up (Singer, 2005) cannot be ruled out.

CI is a shrub distributed in estuaries and coasts of southern China, Australia, the Pacific island, Southeast Asia, and India (Wiart, 2006). It is a herbal medicine claimed for remitting skin diseases and fever (Gopal and Sengottuvelu, 2008). The *CI* extracts have also been reported to have antinociceptive (Parveena et al.,

* Corresponding author at: Department of Pharmacology, College of Medicine, National Taiwan University, No. 1, Jen-Ai Rd., Section 1, Taipei 100, Taiwan. Tel.: +886 2 2312 3456x88323; fax: +886 2 2341 4788.
E-mail address: lcchiou@ntu.edu.tw (L.-C. Chiou).
[1] Equal contribution.

http://dx.doi.org/10.1016/j.jep.2015.03.001
0378-8741/

2010), hepato-protective (Anitha and Kannan, 2006), antiviral (Gopal and Sengottuvelu, 2008) and antifungal (Prasad et al., 2007) activities. However, none of these activities is relevant to neuropsychiatric disorders. Nevertheless, Murugesan et al. (2001) reported that another herb belonging to the same genus, *Clerodendrum phlomidis* Linn, has been used by the rural people in Tamilnadu for treating mental tension and mental disturbance and they found the methanolic extract of this plant's leaves had sedative and muscle relaxant effects in rodents.

The tic disorder is a neuropsychiatric disorder manifesting involuntary, sudden, rapid, repetitive, non-rhythmic, stereotyped movements (motor tics) or phonic tics (Singer, 2005). Tourette syndrome (TS) is an idiopathic spectrum of tic disorders with multiple motor tics and at least one phonic tic lasting for at least 1 year, and often comorbid with obsessive–compulsive disorder (OCD) and attention deficit hyperactivity disorder. Hyper-dopaminergic reactivity in the cortico-thalamic-pallido-striatal circuits is believed to contribute to the pathogenesis of tic disorders/TS (Singer, 2005), mainly based on the effectiveness of antipsychotic treatments, neuroimaging studies, and limited postmortem morphological studies (Singer, 2006; Singer and Minzer, 2003).

To elucidate why *CI* could remit tic attacks in our patient, we have used an animal model based on the hyper-dopaminergic hypothesis of tic disorders/TS by giving mice methamphetamine, a dopamine releaser, to induce hyperlocomotion. We have found that the ethanol extract of *CI* effectively alleviated methamphetamine-induced hyperlocomotion (MIH) at doses that did not impair motor functions (Chen et al., 2012). In this study, we further identified the active constituents from the *CI* ethanol extract using MIH alleviation as a bioassay-guided isolation strategy.

2. Materials and methods

2.1. Plant material

The name of the plant studied in this article has been checked with The Plant list. *Clerodendrum inerme* (L.) Gaertn is an accepted name in version 1 of The Plant List (http://www.theplantlist.org/tpl/record/kew-42703?ref=tpl2), while it is now a synonym of *Volkameria inermis* L (family *Lamiaceae*) (http://www.theplantlist.org/tpl1.1/record/kew-42703).

CI leaves were collected from mangrove marshes in the riverside of southern Taiwan and stored as reported in our previous study (Chen et al., 2012). A *CI* leave sample from the same batch was deposited as a voucher specimen (TMU27423) in the herbarium of College of Pharmacy, Taipei Medical University.

2.2. Isolation and fractionation of CI active constituents

The aerial part of *CI* (3.3 kg) was extracted with 95% ethanol (10 L × 3), and the solvent was removed *in vacuo*. The ethanol extract was then triturated with *n*-hexane (1 L × 3) and CH_2Cl_2 (1 L × 3), respectively. Then, the residue was dissolved with *n*-BuOH (1 L) and partitioned with distilled H_2O (400 mL × 3). The CH_2Cl_2 extract (16.40 g) was further fractioned via silica gel chromatography (400 g) by eluting 0, 1%, 2%, 10% MeOH/CH_2Cl_2 successively to yield subfractions 1–9. Recrystallization of subfraction 7 using EtOH gave two pure compounds **1** (500 mg) and **2** (183 mg). The structures of these two compounds, as compared with previously reported compounds in terms of the NMR spectroscopic analysis, were identified as hispidulin (Hase et al., 1995) and acacetin (Seijas et al., 2005), respectively (Fig. 1). The purity of hispidulin was estimated at least > 95% as judged by HPLC. The structure of hispidulin was also confirmed by two-D NMR spectra using Nuclear Overhauser Effect Spectroscopy (Fig. S1), Heteronuclear Single Quantum Correlation (Fig. S2) and Heteronuclear Multiple Bond Correlation (Fig. S3). The ^1H and ^{13}C NMR spectra for hispidulin and acacetin obtained on Bruker Fourier 300 and Bruker AV500 spectrometers, respectively, using standard pulse programs were:

Hispidulin (6-methoxy-4′,5,7-trihydroxyflavone, **1**): ^1H NMR (300 MHz, DMSO-d_6) δ 13.08 (s, 1H, OH), 10.71 (s, 1H, OH), 10.35 (s, 1H, OH), 7.93 (d, J=8.8 Hz, 2H, H-2′, H-6′), 6.92 (d, J=8.8 Hz, 2H, H-3′, H-5′), 6.78 (s, 1H, H-3), 6.59 (s, 1H, H-8), 3.75 (s, 3H, 6-OMe); ^{13}C NMR (125MHz, DMSO-d_6) δ 182.2, 163.8, 161.2, 157.3, 152.8, 152.4, 131.4, 128.5, 121.2, 116.0, 104.1, 102.4, 94.3, 60.0.

Acacetin (5,7-dihydroxy-4′-methoxyflavone, **2**): ^1H NMR (500 MHz, DMSO-d_6) δ 12.92 (s, 1H, OH), 10.86 (s, 1H, OH), 8.02 (d, J=8.5 Hz, 2H, H-2′, H-6′), 7.10 (d, J=8.5 Hz, 2H, H-3′, H-5′), 6.86 (s, 1H, H-3), 6.50 (d, J=2.0 Hz, 1H, H-8), 6.20 (d, J=2.0 Hz, 1H, H-6), 3.85 (s, 3H, 4′-OMe); ^{13}C NMR (125 MHz, DMSO-d_6) δ 181.9, 164.4, 163.5, 162.5, 161.6, 157.5, 128.5, 123.0, 114.7, 103.9, 103.7, 94.2, 55.7.

2.3. Behavioral studies

All animal experiments were carried out in accordance with the guidelines established by the Institutional Animal Care and Utilization Committee of National Taiwan University, College of Medicine. The animal model used is similar to that reported in our previous study (Chen et al., 2012). Male mice (ICR, 6–9 weeks) were housed (3–5 mice per cage) in the holding room on a 12-h/12-h reversed light schedule with free access to rodent chow and water. On the day conducting behavioral tests, animals were transferred with their home cages from the holding room to the behavioral room and acclimated for 1 h before conducting experiments. Animals were randomly divided into several groups of 3–8 mice each, as indicated in Fig. 3, each bar representing one group. The testing chamber in each behavioral apparatus was cleaned with 70% alcohol after each

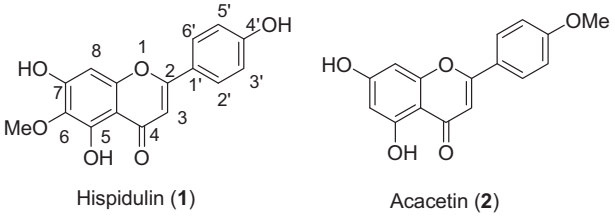

Fig. 1. Chemical structures of hispidulin and acacetin.

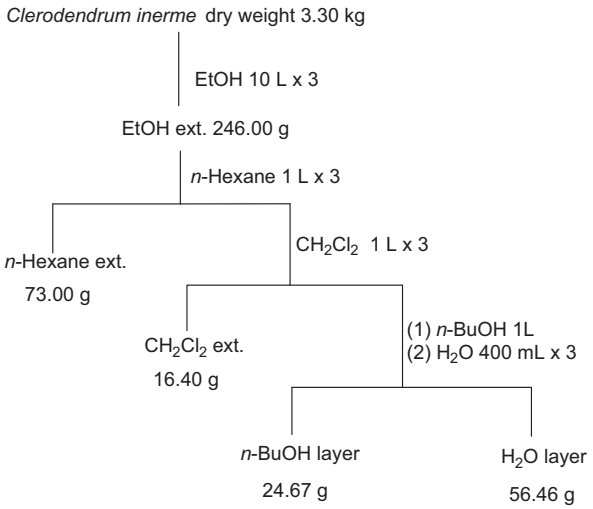

Fig. 2. Diagrammatic representation for the fractionation of the *CI* ethanol extract by *n*-hexane, dichloromethane, *n*-butanol and water.