CHEMICAL STRUCTURE AND ANTIVIRAL ACTIVITY OF AERIAL PART FROM *LAGGERA PTERODONTA*

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ABSTRACT: *Laggera pterodonta* has been used in a variety of practical applications in natural medicine. Preliminary studies showed that the hexane extract of *L. pterodonta* exhibited antiviral activity against herpes simplex type I and II. Subsequent bioassay-guided fractionation led to the isolation five compounds with three triterpenes and two steroids from aerial part of *L. pterodonta*. Among the two compounds, taraxeryl acetate exhibited antiviral activity against herpes simplex virus type II and taraxerone exhibited weak antiviral activity against herpes simplex virus (type I and II) while two steroids exhibited no effect on herpes simplex virus. To our knowledge, taraxeryl acetate is the first natural product from which an effect on the HSV response has been reported.

Keywords: Asteraceae, *Laggera pterodonta,* triterpenes, taraxeryl acetate, antiviral activity, cytotoxicity, bioassay-guided

INTRODUCTION: Many species in the family of Asteraceae have been reported to exhibit antiviral activity against herpes simplex type I[1-6]. The ethanolic extract of *Laggera pterodonta* (DC.) Benth. (Asteraceae) have also been shown antiviral activity against respiratory syncytial virus (RSV) in previous screening[7].

In Chinese traditional medicine, the aerial parts of *L. pterodonta* have been used as an anti-inflammatory, antibacterial and antileukemia activities[8]. The ethanolic extract of *L. pterodonta* (local named "Naat Doi" in Thai) has been assessed for their antiviral and cytotoxic properties. The hexane extract of *L. pterodonta* showed topical antiviral potential and was selected for further investigation as part of our continuing efforts to discover antiviral agents from natural sources. Chemical constituents from leaves of *L. pterodonta* has been reported to contain the essential oil[9, 10], sesquiterpenoid glycosides[11] and eudesmane derivatives[12, 13].

The phytochemical screening and biological activity testing of *L. pterodonta* are the objective of this study in order to isolate pure compounds from the aerial parts of *L. pterodonta* (i.e. taraxeryl acetate, taraxerone, taraxerol, β-sitosterol and stigmasterol). To our knowledge, taraxeryl acetate showed the significant antiviral activity against herpes simplex virus (type II) and taraxerone showed weak antiviral activity against herpes simplex virus (type I and II), along with taraxerol, β-sitosterol and stigmasterol showed the inactive components.

MATERIALS AND METHODS:

Plant material

The aerial parts of *Laggera pterodonta* (DC.) Benth. (Asteraceae) was collected in April, 1998 at Kanchanaburi Province, Thailand and was identified by comparison with herbarium specimens in the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand. A voucher specimen of the plant is deposited in the herbarium of the Faculty of Pharmacognosy and Pharmaceutical Botany, Chulalongkorn University.

Isolation and characterization of chemical constituents

Air-dried and ground aerial part of *L. pterodonta* (3.5 kg) was extracted three times with 95% ethanol at room temperature to yield the

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extract (520 g), which was dissolved in aqueous methanol and the residue was partitioned with *n*-hexane and $CHCl_3$, to yield *n*-hexane (120 g) and $CHCl_3$ (150 g), finally to yield aqueous MeOH (230 g). The hexane extract of *L. pterodonta* was subject to bioassay-guided isolation of active compounds by silica gel column chromatography (CC), (400 g; 4.5 x 60 cm; Merck) using $CHCl_3$-hexane 73 : 27 to furnish 3 fractions (F1–F3) after monitoring their TLC. Fraction F1 (2.2 g) was subjected to sephadex LH- 20 with $CHCl_3$-MeOH 1 : 1 to give compound **1** (38.0 mg). Fraction F2 (3.5 g) was subjected to CC eluted with $CHCl_3$-hexane 15 : 85 to give compound **2** (41.0 mg) after eluting CC with MeOH and follow by sephadex LH 20 with $CHCl_3$-MeOH 1 : 1 to give compound **3** (42.3 mg). Fraction F3 (2.8 g) was subjected to CC eluted with $CHCl_3$-hexane 60: 40 to give compounds **4** (45.0 mg) and **5** (48.0 mg).

Structural elucidation

The structural identification of pure compounds **1**-**5** were all performed with EI-MS, IR, ^{1}H NMR,^{13}C NMR and DEPT-135 spectra.

The chemical structures for **1**-**5** were determined and further comparing the data with the reported literature and in agreement with taraxeryl acetate (**1**)[14, 15], taraxerone (**2**)[15-17], taraxerol (**3**)[15, 17], and two known steroids compounds; β-sitosterol (**4**)[18] and stigmasterol (**5**)[19]. As far as we know the three triterpene compounds were obtained from *L. pterodonta* for the first time. Their antiviral activity against HSV-1 and HSV-2 were assayed.

Antiviral and Cytotoxicity Assays

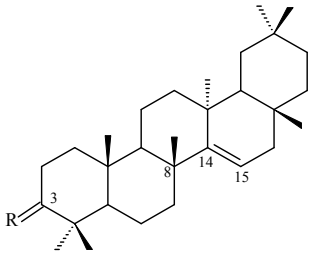
The antiviral and cytotoxic activities of the described compounds and control antiviral compounds were determined using the cytopathic effect (CPE) assay. The procedures used for the antiviral and cytotoxicity assays were as reported previously[20, 21]. The antiviral activity of each substance was expressed in μg/ml as 50% effective concentration (EC_{50}) and cytotoxicity was expressed as 50% inhibitory concentration (IC_{50}).

Anticancer Assay.

Assays were performed as previously described [21].

RESULTS AND DISCUSSION:

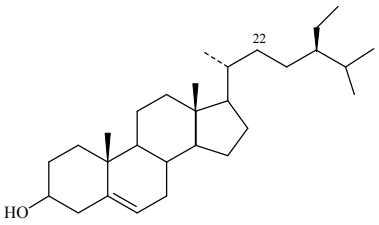
The initial crude hexane extract induced reduction in the herpes simplex virus cell and further fractionation revealed that the activity was distributed over chloroform fractions. The notably topical antiviral activity of the hexane justified their further subjection to a bioassay-guided fractionation to yield the pure compounds.



taraxeryl acetate (**1**) ; R = *α*-H, *β*-OAc

taraxerone (**2**) ; R = O

taraxerol (**3**) ; R = *α*-H, *β*-OH



β-sitosterol (**4**)

Δ ^{22} stigmasterol (**5**)

The antiviral activity of these compounds is warranted. As summarized in Table 1, taraxeryl acetate and taraxerone were the triterpenes isolated from the active fractions of *L. pterodonta*, the first compound, showed topical antiviral activity against herpes simplex virus type II (ED_{50}

Table 1. *In vitro* antiherpes activity and cytotoxicity of *Laggera pterodonta* components

Compounds	Cytotoxicity μg/ml	HSV I[a]	HSV II[a]
hexane extract	>50	+++	+++
taraxeryl acetate (**1**)	>50	-	+++
taraxerone (**2**)	>50	+	+
taraxerol (**3**)	>50	-	-
β-sitosterol (**4**)	>50	-	-
stigmasterol (**5**)	>50	-	-

Note; The cell were tested at maximum non-cytotoxic concentration with various concentrations of extracts and pure compounds (0, 0.05, 0.5, 1, 20 μg/ml)

[a] = Testing by Sulforhodamine B (pre-teatment 30 min). +++, active > 50%, ++, active > 35%, (+), active > 20%, (-), inactive

= 50 μg/ml) whilst taraxerone showed weak antiviral activity against herpes simplex virus type I and II.

In taraxeryl acetate, acetate group was introduced at the C-3 hydroxy group of taraxerol, taraxerone was found to have weak in herpes simplex virus type I and II. This seems to suggest that an intact ring A with C-3 acetate group is required for antivirus activities of these compounds. The anticancer effects were tested against two human cancer cell lines KB and BC. Five natural compounds, three triterpenes and two known steroids were inactive against two of the cancer cell lines utilized (>50 μg/ml).

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