

CHAPTER I

INTRODUCTION

Herpes simplex virus type 2 is a member of the family *Herpesviridae*, which comprises herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus 6 (HHV-6), human herpesvirus 7 (HHV-7) and human herpesvirus 8 (HHV-8) (Roizman, 1996).

Herpes simplex viruses are the first of human herpesviruses (Roizman and Sears, 1996). Infections of these viruses occur worldwide and have been reported in both developed and developing countries. Consequently, the infection has been recognized as a public health concern (Corey et al., 1983).

In addition, herpes simplex viruses (HSV) are intensely human pathogens and responsible for a broad range of human diseases. The clinical specimens of HSV-induced disease have been recognized as eczema herpeticum, vulvovaginitis, keratoconjunctivitis, encephalitis and meningitis (Mattison, Eisenberg and Reichman, 1991). The existence of two distinct antigenic types of HSV was revealed in the early 1960s, and, under the formal designation of the International Conference for Taxonomy of Viruses (ICTV), they are now designated as human herpesviruses 1 (HHV-1) and human herpesviruses 2 (HHV-2) (Roizman et al., 1981). Type 1 and 2 herpes simplex viruses differ significantly in their pathogenic potential. Infection with HSV-1 generally is limited to the oropharynx and is transmitted by direct contact of a susceptible individual with infected secretions. Thus, initial replication of virus will occur in the oropharyngeal mucosa. The trigeminal ganglion becomes colonized and harbors latent virus. Acquisition of HSV-2 infection is usually the consequence of transmission by genital contact. Virus replicates in the genital, perigenital, or anal skin sites with seeding of the sacral ganglia (Whitley, 1996). Changes in sexual mores, however, have somewhat

altered these common patterns: occasionally, type 2 viruses are isolated from oral lesions and type 1 viruses from genital lesions (Ginsberg, 1980).

Among various populations, between 60% and more than 95% are infected with HSV-1, and between 6% and 50% with HSV-2 (Cunningham and Mikloska, 2001). Recurrences of both oral labial and genital herpes simplex virus infections in human occur frequently. For more than 60% of patients with initial HSV-2 infection, the infection recurs within 6 months, and patients with recurrent genital disease have a median of five recurrences per year. The disease is often painful, sometimes debilitating, and causes considerable social and psychological stress. Moreover, a number of clinical and epidemiological studies have shown a significant correlation between genital HSV-2 infections and a higher incidence of cervical carcinoma (Whitley, 1996). In Thailand, HSV infections have been frequently found in various populations and it is an important public health problem. Investigators from virus research institute, Department of Medical Sciences, Ministry of Public Health had surveyed HSV-1 and HSV-2 antibodies of the people in Bangkok and Chainart provinces. They concluded that (1) 10-17% of young people ever had symptomatic herpes and 5% of them were genital herpes; (2) 0.21-0.35% (4-7 cases per 2,000 people) had HSV infection in vagina and cervix, and this had increased in the past years; (3) HSV infection incidence had increased in the women with genital infections; (4) In Bangkok, almost upward 30-year old people had HSV-1 infections and had a little lower HSV-2 infection incidence. In Chainart where the population density is lower than Bangkok, there were also lower incidences of HSV infection (ประเสริฐ ทองเจริญ 2528).

A study by Chalobon Yoosook, Wasun Chuntratita, and Pongpen Rimdusit (1989) reported that herpes simplex viruses were isolated from 40.8 to 56.0 percent of the patients with genital herpes. The frequencies of recovery from HSV diseases seemed to be higher in female than in male, particularly during the first episode of infection. Asymptomatic shedding of the viruses from female genitalias was approximately 0.7 percent. Herpes simplex virus type 2 represented 98.4 percent of all isolates and the remaining isolates were type 1.

Antiviral agents for the treatment of HSV-infections such as idoxuridine, trifluridine, vidarabine are toxic and poorly effective, whereas acyclovir (ACV) is efficient but expensive. However, Leung and Sacks (2000) reported that acyclovir in topical, oral or intravenous forms was highly effective only at first episode of herpes simplex infection and ineffective with latent infection. Resistances of HSV to ACV were reported in many parts of the world (Pottage and Kessler, 1995; Shin et al., 2001). Trisodium phosphonoformate (PFA, foscarnet), an antiviral agent which inhibits HSV-DNA polymerase, was used to treat ACV-resistant virus, but it showed strong side effect. So, foscarnet is recommended for only severe infection treatment (Hasegawa and Kaeagushi, 1994). In addition, the development of clinical resistance to foscarnet and the isolation of foscarnet-resistant virus after several courses of the drug had been reported (Birch et al., 1990). Therefore, a new approach to explore of alternative drugs, especially constituents from medicinal plants, is very interesting worldwide. Thus, several plant extracts were screened for antiherpes simplex as shown in Table 1.

The purpose of this study was to screen for antiviral activity of some Thai medicinal plant extracts against HSV-2 strain Baylor 186 compared with that of ACV. In this study, seventeen medicinal plants were investigated, including (1) *Andrographis paniculata* (Burm) Wall. Ex Nees, [ฟ้าทะลายโจร] (2) *Cissus quadrangularis* Linn., [เพชรสังฆาต] (3) *Citrus reticulata* Blanco, [ส้มเขียวหวาน] (4) *Clinacanthus siamensis* Brem., [ลิ้นงูเห่า] (5) *Cocos nucifera* Linn., [มะพร้าว] (6) *Costus speciosus* (Koen.) J. E. Smith, [เอื้องดิน] (7) *Momordica charantia* Linn. var minima William & NG, [มะระขี้นก] (8) *Momordica charantia* Linn. var maxima William & NG, [มะระ] (9) *Nephelium lappaceum* Linn., [เงาะ] (10) *Bridelia ovata* Decne., [มะกา] (11) *Orthosiphon aristatus* (Blume) Miq., [หญ้าหนวดแมว] (12) *Phyllanthus amarus* Schum., [ลูกใต้ใบ] (13) *Schefflera leucantha* Viguier, [หนุমানประดานกาย] (14) *Thunbergia laurifolia* Linn. [รางจืด] and (15) *Vitis vinifera* Linn. [องุ่น], (16) *Barleria lupulina* Lindl. [เสลดพังพอน] and (17) *Clinacanthus nutans* [พญาขอ] The last two medicinal plants were previously reported for anti-HSV activity. Therefore, they were investigated for the comparison of the activity. *C. siamensis*, *A. paniculata* and *T. laurifolia* are members of the same plant family of *B. lupulina* and *C. nutans* (Acanthaceae) and are used for some skin diseases therefore,

these three plants may have anti-HSV-2 activity . The pericarps of *N. lappaceum* and *C. reticulata* were less useful parts. However, they were previously investigated for some antiviral activities. Also, other selected plants had been no reported for anti-HSV-2 activity but some plants in their family were reported for antiviral activities. In this study, fresh plants were extracted into five fractions including ethanol fraction, hexane fraction, aqueous-ethanol fraction, ethyl acetate fraction and aqueous-ethyl acetate fraction. Each fraction was investigated for anti-HSV-2 activity *in vitro* using plaque reduction assay.

The results from this study could provide a preliminary information on the *in vitro* anti-HSV-2 activities of some Thai medicinal plant extracts. Furthermore, effective medicinal plant(s) would be interesting candidate(s) for the anti-HSV-2 drug development in the future.



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Table 1 List of plants with antiherpes simplex activities.

Plants	Family	Type of extracts or fluid from plant	Reference
<i>Achyrocline alata</i>	Compositae	ethanol	Garcia et al., 1990
<i>Achyrocline flaccida</i>	Compositae	ethanol, aqueous	Garcia et al., 1990; 1995; 1999
<i>Achyrocline vautheriana</i>	Compositae	ethanol	Garcia et al., 1990
<i>Acorus gramineus</i>	Araceae	ethanol	Abou-Karam and Shier, 1990
<i>Adansonia digitata</i>	Bombacaceae	methanol	Anani et al., 2000.
<i>Allium sativum</i>	Alliaceae	fresh extract	Weber et al., 1992
<i>Aloe barbardensis</i>	Liliaceae	80%ethanol	Sydiskis et al., 1991
<i>Alternanthera brasiliana</i>	Amaranthaceae	aqueous, 95%ethanol	Lagrota et al., 1994.
<i>Anemone coronaria</i>	Ranunculaceae	ethanol	Abou-Karam and Shier, 1990
<i>Annona cherimola</i>	Annonaceae	methanol	Betancur-Galvis et al., 1999.
<i>Annona muricata</i>	Annonaceae	ethanol, methanol	Padma et al., 1998; Betancur-Galvis et al., 1999
<i>Annona squamosa</i>	Annonaceae	aqueous, alcohol	Chutinan Kuntasuk, 1992.
<i>Anthyllis vulneraria</i>	Leguminosae	ethyl acetate, butanol	Suganda et al., 1983
<i>Aristolelia chilensis</i>	Elaeocarpaceae	hydroalcohol	Pacheco et al., 1993.
<i>Artemisia anomala</i>	Compositae	aqueous	Zheng, 1989.
<i>Artemisia princeps</i>	Compositae	aqueous	Kurokawa et al., 1996.
<i>Atalantia monophylla</i>	Rutaceae	chloroform	Chansakaow, Ruangrunsi and Ishikawa, 1996.
<i>Baccharis genistelloides</i>	Compositae	aqueous	Abad et al., 1999a.
<i>Baccharis trinervis</i>	Compositae	aqueous	Abad et al., 1999b.
<i>Barleria lupulina</i>	Acanthaceae	aqueous, alcohol	Chutinan Kuntasuk, 1992.
<i>Bauhinia vahlii</i>	Fabaceae	methanol	Taylor et al., 1996.
<i>Beta vulgaris</i>	Chenopodiaceae	aqueous	Betancur-Galvis et al., 1999.
<i>Bryonia dioica</i>	Cucurbitaceae	butanol	Suganda et al., 1983.
<i>Callisia gracilis</i>	Commelinaceae	ethanol	Betancur-Galvis et al., 1999.
<i>Camellia sinensis</i>	Theaceae	hot aqueous	Suganda et al., 1983.
<i>Cardamine angulata</i>	Cruciferae	methanol	McCutcheon et al., 1995.
<i>Carissa carandas</i>	Apocynaceae	methanol	Taylor et al., 1996.
<i>Cassia alata</i>	Fabaceae	95%ethanol	Chutinan Kantasuk, 1992.
<i>Cassia angustifolia</i>	Fabaceae	80%ethanol	Sydiskis et al., 1991.

Table 1 (continued)

Plants	Family	Type of extracts or fluid from plant	Reference
<i>Cassia stipulacea</i>	Fabaceae	hydroalcohol	Pacheco et al., 1993.
<i>Centella asiatica</i>	Umbelliferae	aqueous, alcohol	Chutinan Kuntasuk, 1992., Yingmanee Boonyakiat 1994., Yoosook et al., 2000; Zheng, 1989
<i>Cerbera odollam</i>	Apocynaceae	aqueous-hexane	Vimolmas Lipipun, et al., 1999
<i>Citrullus vulgaris</i>	Cucurbitaceae	aqueous	บุญเกิด คงยิ่งยศ, 2533.
<i>Clausena excavata</i>	Futaceae	chloroform	Vimolmas Lipipun et al., 1999
<i>Clérodendron myricoides</i>	Verbenaceae	80%ethanol	Vlietinck et al., 1995.
<i>Clinacanthus nutans</i>	Acanthaceae	aqueous	Chutinan Kuntasuk, 1992., ชื่นฤดี ไทยวสุ และคณะ, 2535.
<i>Clivia cyrtanthiflora</i>	Amaryllidaceae	80%ethanol	Beghe et al., 1978.
<i>Clivia miniata</i>	Amaryllidaceae	80%ethanol	Beghe et al., 1978.
<i>Coleus amboinicus</i>	Labiatae	chloroform	Vimolmas Lipipun et al., 1999
<i>Combretum micranthum</i>	Combretaceae	methanol	Ferrea et al., 1993.
<i>Comptonia peregrina</i>	Myrtaceae	NaCl solution	Abou-Karam and Shier, 1990.
<i>Conocephalum conicum</i>	Conocephalaceae	methanol	McCutcheon et al., 1995.
<i>Conyza aegyptiaca</i>	Asteraceae	methanol	Anani et al., 2000.
<i>Cordia salicifolia</i>	Boraginaceae	70%ethanol	Hayashi, Hayashi, Morita, et al., 1990.
<i>Coreopsis verticillata</i>	Asteraceae	NaCl solution	Abou-Karam and Shier, 1990.
<i>Crassocephalum multicorybosum</i>	Asclepiadaceae	80%ethanol	Vlietinck et al., 1995.
<i>Crepis tectorium</i>	Asteraceae	NaCl solution	Abou-Karam and Shier, 1990.
<i>Cryptolepis sanguinolenta</i>	Periplocaceae	80% ethanol	Cimanga et al., 1996.
<i>Cudrania javanensis</i>	Moraceae	aqueous	Chutinan Kuntasuk, 1992., Yingmanee Boonyakiat, 1994.
<i>Curcuma domestica</i> (C. longa)	Zingiberaceae	alcohol, hexane	Chutinan Kuntasuk, 1992; อัมพวัน อภิสริยะกุล, เยาวลักษณ์ พันธุ์พิสุทธิชัย และ ปราณีย์ ลิ้นนะภัย, 2538.
<i>Cynometra cloiselii</i>	Fabaceae	ethanol	Hudson, Lee, and Rasoanaivo, 2000.
<i>Cynometra madagascariensis</i>	Fabaceae	ethanol	Hudson, Lee, and Rasoanaivo, 2000.
<i>Derris thorelii</i>	Papillionaceae	methanol	จุฬารัตน์ จันทร์ทวนะ และ สุพรรณภา ทิวากรพรรณราย, 2537.
<i>Dittrichia viscosa</i> [Inula viscosa]	Compositae	aqueous	Abad et al., 2000.
<i>Drimys winteri</i>	Winteraceae	hydroalcohol	Pacheco et al., 1993.
<i>Dryopteris inaequalis</i>	Asclepiadaceae	80%ethanol	Vlietinck et al., 1995.

Table 1 (continued)

Plants	Family	Type of extracts or fluid from plant	Reference
<i>Elytropus chilensis</i>	Apocynaceae	hydroalcohol	Pacheco et al., 1993.
<i>Epimedium sagittatum</i>	Berberidaceae	aqueous, alcohol	Zheng, 1989.
<i>Erythrina abyssinica</i>	Fabaceae	80%ethanol	Vlietinck et al., 1995.
<i>Escallonia illinita</i>	Grossulariaceae	hydroalcohol	Pacheco et al., 1993.
<i>Eugenia caryophyllata</i>	Myrtaceae	aqueous	บุญเกิด คงยิ่งยศ, 2533.
<i>Eupatorium articulatum</i>	Asteraceae	aqueous	Abad et al., 1999b.
<i>Eupatorium buniifolium</i> (<i>Acanthostyles buniifolius</i>)	Compositae	ethanol	Garcia et al., 1990
<i>Euphorbia hirta</i>	Euphorbiaceae	80%ethanol	Vlietinck et al., 1995.
<i>Eurycoma longifolia</i>	Simaroubaceae	methanol	Nawawi, Nakamura, et al., 1999.
<i>Evonymopsis longipes</i>	Celastraceae	ethanol	Hudson, Lee, and Rasoanaivo, 2000.
<i>Filicium decipiens</i>	Sapindaceae	methanol	Nawawi, Nakamura, et al., 1999.
<i>Filipendula ulmaria</i>	Rosaceae	ethanol, NaCl solution	Vlietinck et al., 1995; Abou-Karam and Shier, 1990
<i>Flaveria bidentis</i>	Compositae	ethanol	Garcia et al., 1990
<i>Fragaria virginiana</i>	Rosaceae	ethanol, NaCl solution	Abou-Karam and Shier, 1990.
<i>Fritillaria imperialis</i>	Liliaceae	ethanol	Abou-Karam and Shier, 1990.
<i>Gamochaeta simplicicaulis</i>	Asteraceae	aqueous	Cavallaro et al., 1995.
<i>Garcinia mangostana</i>	Guttiferae	methanol	Nawawi, Nakamura, et al., 1999.
<i>Geranium sanguineum</i>	Geraniaceae	aqueous	Serkedjjeva and Ivancheva, 1999.
<i>Geum japonicum</i>	Rosaceae	hot aqueous	Abou-Karam and Shier, 1990.
<i>Geum triflorum</i>	Rosaceae	NaCl solution, ethanol	Abou-Karam and Shier, 1990.
<i>Glycine javanica</i>	Fabaceae	80%ethanol	Vlietinck et al., 1995.
<i>Gynura pseudochina</i> <i>var.hispida</i>	Compositae	aqueous, alcohol	Chutinan Kantasuk, 1992.
<i>Hamamelis virginiana</i>	Hamamelidaceae	hydroalcohol	Erdelmeier et al., 1996.
<i>Heisteria acuminata</i>	Olaceae	ethanol	Abad et al., 1999b.
<i>Helichrysum aureonitens</i>	Asteraceae	aqueous, exudate	Meyer et al., 1996; Afolayan and Meyer, 1995
<i>Heuchera sanguinea</i>	Saxifragaceae	NaCl solution	Abou-Karam and Shier, 1990.
<i>Hibiscus mutabilis</i>	Malvaceae	aqueous	Zheng, 1989.
<i>Hippeastrum rhodophiala</i>	Amaryllidaceae	ethanol	Abou-Karam and Shier, 1990.
<i>Holoptelia integrifolia</i> (<i>Holoptelea integrifolia</i>)	Ulmaceae	methanol, methanol-aqueous	Rajbhandari et al., 2001.

Table 1 (continued)

Plants	Family	Type of extracts or fluid from plant	Reference
<i>Hosta plantaginea</i>	Liliaceae	aqueous	Zheng, 1989.
<i>Houttuynia cordata</i>	Saururaceae	steam distillate	Hayashi, Kamiya and Hayashi, 1995.
<i>Hypericum cordifolium</i>	Clusiaceae	methanol	Taylor et al., 1996.
<i>Hypericum japonicum</i>	Clusiaceae	aqueous, alcohol	Zheng, 1989.
<i>Inula japonica</i>	Asteraceae	aqueous	Zheng, 1989.
<i>Juniperus sabina</i>	Cupressaceae	n-hexane	San Feliciano et al. 1993.
<i>Lagerania leucantha</i>	Cucurbitaceae	aqueous, alcohol	Chutinan Kuntasuk, 1992.
<i>Lagerstroemia flosreginae</i>	Lythraceae	aqueous	บุญเกิด คงยิ่งยศ, 2533.
<i>Lithraea molleoides</i>	Anacardiaceae	aqueous	Kott et al., 1999.
<i>Luma apiculata</i>	Myrtaceae	hydroalcohol	Pacheco et al., 1993.
<i>Lychnis coronata</i>	Caryophyllaceae	fresh leaf juice	Yue-Yu et al., 1998.
<i>Lysichiton americanum</i>	Araceae	methanol	McCutcheon et al., 1995.
<i>Machilus thunbergii</i>	Lauraceae	aqueous	Kurokawa et al., 1996.
<i>Maclura cochinchinensis</i>	Moraceae	ethyl acetate, methanol, aqueous	Sudarat Dechsree, 1998; Yoosook et al., 2000; Bunyapraphatsara et al., 2000
<i>Maesa lanceolata</i>	Maesaceae (Myrsinaceae)	methanol	Sindambiwe et al., 1998; 1999
<i>Mallotus philippensis</i>	Euphorbiaceae	methanol	Taylor et al., 1996.
<i>Mandevilla pentlandiana</i>	Apocynaceae	ethanol	Cabrera et al., 1993.
<i>Mangifera indica</i>	Anacardiaceae	aqueous	Chutinan Kuntasuk, 1992., Yingmanee Boonyakiat, 1994; Yoosook et al., 2000
<i>Markhamia lutea</i>	Bignoniaceae	80%ethanol	Vlietinck et al., 1995.
<i>Matricaria chamomilla</i>	Compositae	ethyl acetate, butanol	Suganda et al., 1983.
<i>Matricaria inodora</i>	Compositae	ethyl acetate, butanol	Suganda et al., 1983.
<i>Melia azedarach</i>	Meliaceae	aqueous	Claus, Coto and Torres, 1998.
<i>Melicope sessiliflora</i>	Rutaceae	hexane	Chan et al., 1989.
<i>Millettia extensa</i>	Fabaceae	methanol	Taylor et al., 1996.
<i>Mosla punctata (M.punctulata)</i>	Lamiaceae	aqueous, alcohol	Zheng, 1989.
<i>Myrsine australis</i>	Myrsinaceae	methanol	Bloor, 1994.
<i>Myrsine salicina</i>	Myrsinaceae	methanol	Bloor, 1994.

Table 1 (continued)

Plants	Family	Type of extracts or fluid from plant	Reference
<i>Narcissus poeticus</i>	Amaryllidaceae	ethanol	Abou-Karam and Shier, 1990.
<i>Nepeta coerulea</i>	Lamiaceae	aqueous	Abad et al., 2000.
<i>Nepeta nepetella</i>	Lamiaceae	aqueous	Abad et al., 2000.
<i>Nepeta tuberosa</i>	Lamiaceae	aqueous	Abad et al., 2000.
<i>Nephelium lappaceum</i>	Sapindaceae	methanol, aqueous	Nawawi, Nakamura, et al., 1999.
<i>Nerium indicum</i> [<i>N. oleander</i>]	Apocynaceae	methanol, methanol-aqueous	Rajbhandari et al., 2001.
<i>Oenothera missouriensis</i>	Onagraceae	NaCl solution	Abou-Karam and Shier, 1990.
<i>Ononis vaginalis</i>	Fabaceae	ether	Abdel-Kader, 2001.
<i>Pachysandra terminalis</i>	Buxaceae	ethanol	Abou-Karam and Shier, 1990.
<i>Palisota hirsuta</i>	Commelinaceae	methanol	Anani et al., 2000.
<i>Persea americana</i>	Lauraceae	leaf infusion, aqueous	Almeida et al., 1998; Miranda et al., 1997
<i>Petunia axillaris</i> (<i>P. nyctaginiflora</i>)	Solanaceae	aqueous	Padma et al., 1998.
<i>Phyla nodiflora</i>	Verbenaceae	hexane	Vimolmas Lipipun et al., 1999
<i>Phyllanthus orbicularis</i>	Euphorbiaceae	aqueous	del Barrio, 2000.
<i>Piliostigma thonningii</i>	Fabaceae	methanol	Cristoni et al., 1996.
<i>Pinus parviflora</i>	Pinaceae	NaOH solution	Fukuchi et al., 1989.
<i>Plectranthus hereroensis</i>	Lamiaceae	acetone	Batista et al., 1995.
<i>Polugonum cuspidatum</i>	Polygonaceae	ethanol	Abou-Karam and Shier, 1990.
<i>Polygonum glycyrrhiza</i>	Polypodiaceae	methanol	McCutcheon et al., 1995.
<i>Polygonum punctatum</i>	Polygonaceae	aqueous	Kott et al., 1999.
<i>Pongamia pinnata</i>	Papilionaceae	aqueous	Elanchezhiyan et al., 1993
<i>Prunella vulgaris</i>	Lamiaceae	hot aqueous	Hong-Xi et al., 1999.
<i>Psychotria serpens</i>	Rubiaceae	ethanol	Kuo et al., 2001.
<i>Punica granatum</i>	Punicaceae	methanol, aqueous	Nawawi, Nakamura, et al., 1999.
<i>Ravensara retusa</i>	Lauraceae	ethanol	Hudson, Lee, and Rasoanaivo, 2000.
<i>Rhamnus frangula</i>	Rhamnaceae	80%ethanol	Sydiskis et al., 1991.
<i>Rhamnus purshianus</i>	Rhamnaceae	80%ethanol	Sydiskis et al., 1991.
<i>Rhododendron simsii</i>	Ericaceae	aqueous	Zheng, 1989.

Table 1 (continued)

Plants	Family	Type of extracts or fluid from plant	Reference
<i>Rhus chinensis</i>	Anacardiaceae	aqueous	Zheng, 1989.
<i>Rhus javanica</i> (<i>Brucea javanica</i>)	Anacardiaceae	hot aqueous	Abou-Karam and Shier, 1990.
<i>Rhus vulgaris</i>	Anacardiaceae	80%ethanol	Vlietinck et al., 1995.
<i>Rumex hastatulus</i>	Polygonaceae	methanol	Taylor et al., 1996.
<i>Sanguisorba minor</i>	Rosaceae	aqueous	Abad et al., 2000.
<i>Satureja boliviana</i>	Lamiaceae	aqueous	Abad et al., 1999a
<i>Sebastiania brasiliensis</i>	Euphorbiaceae	aqueous	Kott et al., 1999.
<i>Sebastiania klotzschiana</i>	Euphorbiaceae	aqueous	Kott et al., 1999.
<i>Spatholobus suberectus</i>	Papilionaceae	aqueous	Kurokawa et al., 1996.
<i>Spondias mombin</i>	Anacardiaceae	80%ethanol	Corthout et al., 1991.
<i>Stephania cepharantha</i>	Menispermaceae	methanol	Nawawi, Ma-ChaoMei et al., 1999.
<i>Streblus asper</i>	Moraceae	methanol	Taylor et al., 1996.
<i>Syzygium aromaticum</i>	Myrtaceae	hot aqueous, ether, ethyl acetate	Abou-Karam and Shier, 1990; Takechi and Tanaka, 1981
<i>Terminalia alata</i>	Combretaceae	methanol	Taylor et al., 1996.
<i>Terminalia chebula</i>	Combretaceae	hot aqueous	Abou-Karam and Shier, 1990.
<i>Terminalia monoceros</i>	Combretaceae	ethanol	Hudson, Lee, and Rasoanaivo, 2000.
<i>Thevetia peruviana</i>	Apocynaceae	chloroform	Vimolmas Lipipun, et al., 1999
<i>Thujae orientalis</i>	Cupressaceae	Aqueous, methanol, butanol	บุญเกิด คงยิ่งยศ, 2533; Ho-Kyoung et al., 1998
<i>Thunbergia laurifolia</i>	Thunbergiaceae	aqueous	บุญเกิด คงยิ่งยศ, 2533.
<i>Toona sureni</i>	Meliaceae	methanol	Nawawi, Nakamura et al., 1999.
<i>Tridax procumbens</i>	Asteraceae	methanol	Taylor et al., 1996.
<i>Verbascum thapus</i>	Scrophulariaceae	methanol	McCutcheon et al., 1995.
<i>Waldstenia fragarioides</i>	Rosaceae	ethanol, NaCl solution	Abou-Karam and Shier, 1990.
<i>Woodwardia orientalis</i>	Blechnaceae	hot aqueous, methanol	Xu et al., 1993.
<i>Zanthoxylum bungeanum</i>	Rutaceae	aqueous	Kurokawa et al., 1996.
<i>Zizyphus spina-christi</i> (<i>Ziziphus spina-christi</i>)	Rhamnaceae	ethyl alcohol, aqueous	vanden Berghe, 2001.