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Review

Chemical Constituents and Pharmacological Activities of *Hedyotis diffusa*

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Abstract – The chemical constituents from *Hedyotis diffusa* Willd and their pharmacological activities were summarized. It has been known data that this herb contains anthraquinones, terpenoids, steroids, flavonoids, organic acid, and polysaccharides. The studies of pharmacology have shown that *Hedyotis diffusa* Willd possess various levels of activities such as anticancer, anti-inflammatory, immunostimulatory, antioxidative, neuroprotective, and hepatoprotective activities.
Keywords – *Hedyotis diffusa*, chemical constituents, pharmacological activities

Introduction

Hedyotis diffusa Willd. (Rubiaceae), other latin scientific names: *Hedyotis herbacea* Lour., *Oldenlandia diffusa* (Willd.) Roxb., an annual herb distributed in subtropical area in Asia, is known in oriental folk medicines used for the treatment of various diseases including pneumonia in children, pelvitis, hepatitis, tonsillitis, sore throat, appendicitis, urethral infection, contusions, furunculosis, especially for various kinds of tumors, such as tumors of digestive tract, carcinoma of liver, pancreas and urinary bladder, lymphoma, hysteromyoma, in China, Korea, Japan and Malaysia. According to the traditional Chinese medicine theory, *H. diffusa* is sweet and bland in taste, cool in nature, and attributive to lung, liver, urinary bladder and large intestine channels, possess efficacy of clearing away heat and toxic material, promoting blood circulation and removing blood stasis. Taking into account its extensive clinical applications in cancer and other diseases (Wu, 2000; Zhong *et al.*, 2001), the present paper summarizes the chemical constituents from *H. diffusa* and their pharmacological activities.

Chemical constituents

Anthraquinones, terpenoids, flavonoids, steroids, coumarins, organic acid, and polysaccharides have been isolated from *H. diffusa* and showed to have various levels of activities such as anticancer, anti-inflammatory, antioxidant, immunostimulatory, neuroprotective and hepatoprotective effects. The structures of these compounds and their bioactivity information are listed in Table 1 and Fig. 1.

Anthraquinones – Anthraquinone deriviates, a class of important natural pigments distributed widely in plants, are effective compositions of many herbs. Phytochemical studies revealed 8 anthraquinone compounds existed in the herb of *H. diffusa.* Firstly, the Taiwan scholar Tai isolated three anthraquinones from *H. diffusa* and identified them as 2-methyl-3-hydroxyanthraquinone (**1**), 2-methyl-3-methoxy-anthraquinone (**2**), and 2-methyl-3-hydroxy-4-methoxyanthraquinone (**3**) (Tai *et al.*, 1979). Subsequently, another anthraquinone compound named as 2,3-dimethoxy-6-methyanthraquinone (**4**) was isolated from *H. diffusa* (Ho *et al.*, 1986). More recently, two 1,4-anthraquinone compounds, named as 2-hydroxymethyl-10-hydroxy-1,4-anthraquinone (**5**) and 2,3-dimethoxy-9-hydroxy-1,4-anthraquinone (**8**), were isolated from *Hedyotis herbacea* along with two 9,10-anthraquinone compounds named as 1,4-dihydroxy-2-hydroxymethyl-anthraquinone (**6**) and 1,4-dihydroxy-2,3-dimethoxy- anthraquinone (**7**) (Dharma *et al.*, 1999).

Terpenoids – Terpenoids are the biggest class of compounds among various classes natural substances. So far, 14 terpenoids were found in *H. diffusa.* These compounds were attributed to two basic classes of iridoids and triterpenoids. 12 iridoids, named as asperuloside (**9**), asperulosidic acid (**17**), deacetylasperulosidic acid (**19**), scandoside (**20**), *E*-6-*O*-*p*-methoxycinnamoyl scandoside

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Table 1. The chemical information and activities of compounds from *H. diffusa*

No.	Compounds	Compound type	Activities	References
1	2-methyl-3-hydroxyanthraquinone	anthraquinones		Tai *et al.*, 1979
2	2-methyl-3-methoxyanthraquinone	anthraquinones		Tai *et al.*, 1979
3	2-methyl-3-hydroxy-4-methoxyanthraquinone	anthraquinones		Tai *et al.*, 1979
4	2,3-dimethoxy-6-methyanthraquinone	anthraquinones		Ho *et al.*, 1986
5	2-hydroxymethyl-10-hydroxy-1,4-anthraquinone	anthraquinones		Dharma *et al.*, 1999
6	1,4-dihydroxy-2-hydroxymethylanthraquinone	anthraquinones		Dharma *et al.*, 1999
7	1,4-dihydroxy-2,3-dimethoxyanthraquinone	anthraquinones		Dharma *et al.*, 1999
8	2,3-dimethoxy-9-hydroxy-1,4-anthraquinone	anthraquinones		Dharma *et al.*, 1999
9	asperuloside	terpenoids		Nishihawa *et al.*, 1981; Huang *et al.*, 1981; Takagi *et al.*, 1982
			antitumor	Kim *et al.*, 1999
			antioxidative	Lu *et al.*, 2000
				Dharma *et al.*, 2003
10	*E*-6-*O*-*p*-methoxycinnamoyl scandoside methyl ester	terpenoids		Nishihawa *et al.*, 1981; Wu *et al.*, 1991
			neuroprotective	Kim *et al.*, 2001
11	*Z*-6-*O*-*p*-methoxycinnamoyl scandoside methyl ester	terpenoids		Wu *et al.*, 1991
			neuroprotective	Kim *et al.*, 2001
12	*E*-6-*O*-*p*-feruloyl scandoside methyl ester	terpenoids		Nishihawa *et al.*, 1981 Huang *et al.*, 1981; Wu *et al.*, 1991
13	*Z*-6-*O*-*p*- feruloyl scandoside methyl ester	terpenoids		Wu *et al.*, 1991
14	*E*-6-*O*-*p*-coumaroyl scandoside methyl ester	terpenoids		Nishihawa *et al.*, 1981; Huang *et al.*, 1981; Wu *et al.*, 1991
			neuroprotective	Kim *et al.*, 2001
15	*Z*-6-*O*-*p*- coumaroyl scandoside methyl ester	terpenoids		Wu *et al.*, 1991
			neuroprotective	Kim *et al.*, 2001
16	scandoside methyl ester	terpenoids		Takagi *et al.*, 1982
17	asperulosidic acid	terpenoids		Takagi *et al.*, 1982
18	geniposidic acid	terpenoids		Takagi *et al.*, 1982
19	deacetylasperulosidic acid	terpenoids		Takagi *et al.*, 1982
20	scandoside	terpenoids		Takagi *et al.*, 1982
21	ursolic acid	terpenoids		Fu *et al.*, 1963; Cai *et al.*, 1964; Yang *et al.*, 1971; Lv *et al.*, 1996 Kim *et al.*, 1995 Ahmad *et al.*, 1996
			antitumor	Kim *et al.*, 1999
22	oleanolic acid	terpenoids		Cai *et al.*, 1964; Lv *et al.*, 1996
23	β-sitosterol	steroids		Fu *et al.*, 1963; Cai *et al.*, 1964; Yang *et al.*, 1971; Kim *et al.*, 1995
24	γ-sitosterol	steroids		Cai *et al.*, 1964
25	β-sitosterol-β-D-glucoside	steroids		Cai *et al.*, 1966; Kim *et al.*, 1995
26	kaempferol-3-*O*-[2-*O*-(*E*-6-*O*-feruloyl)-β-D-glucopyranosyl]-β-D-galactopyranoside	flavonoids	antioxidative	Lu *et al.*, 2000
27	quercetin	flavonoids		Lu *et al.*, 2000
28	quercetin-3-*O*-glucopyranoside (isoquercitrin)	flavonoids		Lu *et al.*, 2000
			antioxidative	Dharma *et al.*, 2003
29	quercetin-3-*O*-sambubioside	flavonoids		Lu *et al.*, 2000
30	quercetin-3-*O*-sophoroside	flavonoids		Lu *et al.*, 2000
31	kaempferol-3-*O*-[2-*O*-(6-*O*-*E*-feruloyl)-β-D-glucopyranosyl]-β-D-galactopyranoside	flavonoids	neuroprotective	Kim *et al.*, 2001
32	quercetin-3-*O*-[2-*O*-(6-*O*-*E*-feruloyl)-β-D-glucopyranosyl]-β-D-galactopyranoside	flavonoids	neuroprotective	Kim *et al.*, 2001

Table 1. Continued

No.	Compounds	Compound type	Activities	References
33	quercetin-3-*O*-[2-*O*-(6-*O*-*E*-feruloyl)-β-D-glucopyranosyl]-β-D-glucopyranoside	flavonoids	neuroprotective	Kim *et al.*, 2001
34	kaempferol-3-*O*-(2-*O*-β-D-glucopyranosyl)-β-D-galactopyranoside	flavonoids	neuroprotective	Kim *et al.*, 2001
35	quercetin-3-*O*-(2-*O*-β-D-glucopyranosyl)-β-D-galactopyranoside	flavonoids	neuroprotective	Kim *et al.*, 2001
36	quercetin-3-*O*--rutinoside	flavonoids	antioxidative	Dharma *et al.*, 2003
37	kaempferol-3-*O*-arabinopyranoside	flavonoids		Ahmad *et al.*, 1996
38	kaempferol-3-*O*-rutinoside	flavonoids		Ahmad *et al.*, 1996
39	4, 4-dihydroxy-α-truxillic acid	organic acid		Lv and He, 1996
40	hentriacontane	alkane		Cai *et al.*, 1964
41	*p*-coumaric acid	coumarins		Cai *et al.*, 1966

methyl ester (**10**), *Z*-6-*O*-*p*-methoxycinnamoyl scandoside methyl ester (**11**), *E*-6-*O*-*p*-feruloyl scandoside methyl ester (**12**), *Z*-6-*O*-*p*-feruloyl scandoside methyl ester (**13**), *E*-6-*O*-*p*-coumaroyl scandoside methyl ester (**14**), *Z*-6-*O*-*p*-coumaroyl scandoside methyl ester (**15**), scandoside methyl ester (**16**), and geniposidic acid (**18**), were isolated successively from *H. diffusa* by Japanese, Chinese, Korean and Malaysia researchers (Nishihawa *et al.*, 1981; Huang *et al.*, 1981; Takagi *et al.*, 1982; Wu *et al.*, 1991; Lu *et al.*, 2000, Kim *et al.*, 2001; Dharma *et al.*, 2003). Two triterpenoids were isolated and identified as ursolic acid (**21**) and oleanolic acid (**22**) from *H. diffusa* (Fu *et al.*, 1963; Cai *et al.*, 1964; Yang *et al.*, 1971; Lv *et al.*, 1996; Kim *et al.*, 1995; Ahmad *et al.*, 1996).

Steroids – As one class of ubiquitous compounds in the vegetable kingdom, steroids compounds were most early isolated and identified as β-sitosterol (**23**), γ-sitosterol (**24**), and β-sitosterol-β-D-glucoside (**25**) from *H. diffusa* by Chinese researchers (Fu *et al.*, 1963). After that, these steroid compounds have been isolated several times (Cai *et al.*, 1964; 1966; Yang *et al.*, 1971; Kim *et al.*, 1995).

Flavonoids – Two flavonol glycosides were first isolated from *H. diffusa* in 1996, and were identified as kaempferol-3-*O*-arabinopyranoside (**37**) and kaempferol-3-*O*-rutinoside (**38**) (Ahmad *et al.*, 1996). In succession, a new acylated flavonol di-glycoside was isolated and characterized as kaempferol-3-*O*-[2"-*O*-(*E*-6"'-*O*-feruloyl)-β-D-glucopyranosyl]-β-D-galactopyranoside (**26**) together with four known flavonol glycosides, quercetin (**27**), quercetin-3-*O*-glucopyranoside (**28**), quercetin-3-*O*-sambubioside (**29**), and quercetin-3-*O*-sophoroside (**30**) by spectral and chemical methods from the methanol extract of *H. diffusa* (Lu *et al.*, 2000). In a bioassay-guided search for neuroprotective compounds from medicinal plants, two new acylated flavonol glycosides were isolated and eluciated as kaempferol-3-*O*-[2-*O*-(6-*O*-*E*-feruloyl)-β-D-glucopyranosyl]-β-D-galactopyranoside (**31**), and quercetin-3-*O*-[2-*O*-(6-*O*-*E*-feruloyl)-β-D-glucopyranosyl]-β-D-galactopyranoside (**32**) from the methanol extract of whole plants of *H. diffusa*, together with three known flavonoid glycosides named as quercetin-3-*O*-[2-*O*-(6-*O*-*E*-feruloyl)-β-D-glucopyranosyl]-β-D-glucopyranoside (**33**), kaempferol-3-*O*-(2-*O*-β-D-glucopyranosyl)-β-D-galactopyranoside (**34**), and quercetin-3-*O*-(2-*O*-β-D-glucopyranosyl)-β-D-galactopyranoside (**35**) (Kim *et al.*, 2001). In another bioassay-guided search for antioxidative compounds from *H. diffusa* (Dharma *et al.*, 2003), two flavonol glycosides were isolated and eluciated as quercetin-3-*O*-β-rutinoside (**36**), and quercetin-3-*O*-glucopyranoside (**28**) from the methanol extract of whole plants.

Others – In addition, one cyclobutane derivative, 4,4-dihydroxy-α-truxillic acid (**39**) (Lv *et al.*, 1996); one long chain alkane, hentriacontane (**40**) (Cai *et al.*, 1964); one coumarin precusor, *p*-coumaric acid (**41**) (Cai *et al.*, 1966); one preliminary defined structure, a polysaccharide fraction HD-W-3-B with immune activity (Wu *et al.*, 1992); and one oxytocic peptide consisting of 11 amino acid residues were also obtained from *H. diffusa.*

Pharmacological activities

Anticancer – *H. diffusa* has been used as an anticancer agent for several decades in oriental medicine. Recently, *H. diffusa* was claimed to have an antitumor effect on human malignancies such as hepatoma, cervical, gastric and intestinal carcinoma. Previous investigations (Kim, 1997; Kim *et al.*, 1998; 1999) suggested that *H. diffusa* possessed the anticancer activity capable of suppressing the growth of some cancer cell lines. Further studies (Woo *et al.*, 1998) were performed to investigate the inhibitory effect of a methanol extract of *Oldenlandia diffusa* (OD) on the proliferation and differentiation of human cancer cell line U937. The results showed that the conditioned

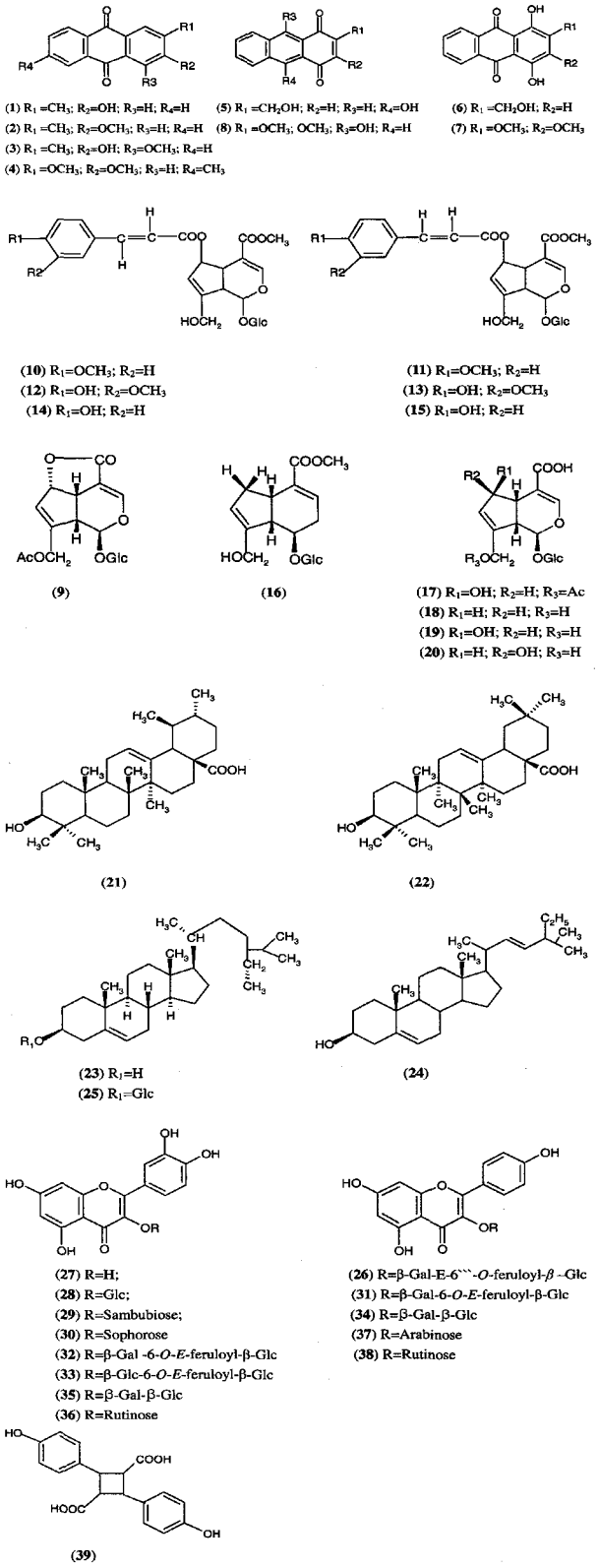

Fig. 1. Structures of the compounds from isolated *H. diffusa*.

medium from OD-stimulated blood mononuclear cells (OD-MNC-CM) treatment induced about 50% of the cells differentiating into mature monocytes/ macrophages of CD 11b, CD 14, and CD 68. The levels of interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-1 were very low in normal MNC-CM, and they were greatly increased in MNC-CM prepared with OD stimulation. These results suggested that OD could inhibit the growth of leukemic U937 cells and induce them to differentiate into mature monocytes/macrophages through stimulating the production of differentiation-inducing cytokines by mononuclear leukocytes.

Korean researchers (Pak *et al.*, 2000) investigated the effect of the methanol extract of *H. diffusa* on transcriptional activation factors. The result suggested that the methanol extract of *H. diffusa* exerted anticancer effects to induce the death of human leukemic HL-60 cells via activation of transcriptional factors such as NF-*k*B and AP-1, increase in expression of Fas mediated signaling proteins, and induction of the tumor suppressor gene, p53. Further study was carried out to examine the expression of cell cycle related genes in HL-60 cells undergoing apoptosis by the methanol extract of *H. diffusa* (Han *et al.*, 2000). The results provided the evidences that expression of cell cycle related genes in HL-60 cells ungoing apoptosis by the methanol extract of *H. diffusa* mainly resulted from a decreased level of p21/ Cipl and an increased level of p27/Kipl of the cell cycle related genes. At the same time, the effects of aqueous and methanol extracts of *H. diffusa* on the induction of apoptotic cell death were investigated (No *et al.*, 2000) in a human lymphoid leukemia cell line (HL-60). The results showed that the death of HL-60 cells was markedly induced by the addition of the methanol extract of *H. diffusa* in a dose and time-dependent manner. The activity of caspase 3-like proteases remained in a basal level in HL-60 cells treated with an aqueous extract of *H. diffusa*. However, it was markedly increased in HL-60 cells treated with a methanol extract of *H. diffusa*. In addition, the phosphotransferase ativity of JNK1 was increased in HL-60 cells treated with the methanol extract of *H. diffusa*. Furthermore, the activation of transcription activator, NF-*k*B was markedly induced by methanol extract of *H. diffusa*. Anti-apoptotic Bcl-2 was cleaved into a 23 Kda fragment by treatment of the methanol extract of *H. diffusa*. However, expression of proapoptotic Bax protein was increased by treatment of the methanol extract of *H. diffusa* in a time-dependent manner. These results suggested that methanol extract of *H. diffusa* induced the apoptotic death of human leukemic HL-60 cells via activations of Caspase-3 proteases, JNK1, transcriptional activator NF-*k*B. The methanol extract of

H. diffusa reduced malignant potential of HL-60 cells via down regulation of colony forming efficiency through cleavage of Bcl-2 as well as induction of Bax. A latest research (Gupta *et al.*, 2004) on anticancer activities of water extract of *O. diffusa* showed that *O. diffusa* extract effectively inhibited the growth of all the eight cancer cell lines and induced significant increase of apoptosis, meanwhile the extract exhibited minimum toxic effect on normal pancreatic cells. Furthermore, there was a significant inhibition of lung metastases in the animal model with no noticeable adverse effects. These results indicated that *O. diffusa* extract could be a potential anticancer agent.

To date, most of studies on anticancer activity of *H. diffusa* still lingered at crude extract level, a few researches were carried out at single compound level. Several investigating indicated that the anticancer activity of *H. diffusa* was associated with its ursolic acid (**21**) and ursolic acid fractions of the extract (Kim, 1997; Kim *et al.*, 1998; 1999). The cytotoxicity-guided fractionation from the MeOH extract of *O. diffusa* led to the isolation of **21** as an active principle. **21** demonstrated a significant inhibition of the proliferation of cultured tumor cells, i.e. A594, SK-OV-3, SK-MEL-2, XF498, HCT-15, SNU-1, L1210, and B16-F_0. The results from further investigation suggested that the cytotoxicity or the apoptotic effect of **21** on tumor cells might be related to the activation of the endonucleolytic enzyme and subsequent activation of poly (ADP-ribose) polymerase in tumor cells and these could eventually lead to cell lysis (Kim *et al.*, 1998). More recently, for the evaluation of antitumor and antimetastatic effects of either ursolic acid alone or in combination with asperuloside (**9**), several experimental parameters such as cytotoxicity against various cancer cell lines, clonogenic assay, antiadhesion assay, CAM assay, pulmonary colonization assay, and MMP-2 expression were investigated (Kim *et al.*, 1999), the results showed that ursolic acid possessed a potent cytotoxic activity in a dose dependent manner against SF295, SK-OV-3, HCT15, and UN-2 cancer cell lines, while combined treatment of **21** and **9** did not. However, combination of **21** and **9** was more effective than **21** alone in the inhibitory effect on cell adhesion of A549 cells to complex extracellular matrix *in vitro* and pulomonary colonization assay by B16BL/6 *in vivo*. In the clonogenic assay, combination of **21** and **9** inhibited more effectively colony formation by A549 synergistically to 97% than **21** to 78%. A combination of **21** and **9** suppressed MMP-2 gene expression more effectively than **21** only in a dose dependent manner. These results suggested that combined therapy of ursolic acid and asperuloside showed much stronger antitumor and antimestastatic effects than ursolic acid alone. The implanted subcutaneous tumor and hepatoma cell line hep-2B were used to study (Hsu, 1998) the tumor-inhibitory effect of ursolic acid (**21**), oleanolic acid (**22**) from *H. diffusa*, through the gradient administrator doses in vivo and in vitro, respectively. The results showed that **21** and **22** are more prominent in inhibiting the growth of cultured hep-2B cells and the enlargement of subcutaneous tumor.

In addition, some derivates of ursolic acid were artificially synthesized for the development of new better anticancer agents. Among them, ursonic acid, 3-oxo-12-ursen-28-oic acid, a derivative of ursolic acid showed good cytotoxicity against various tumor cell lines, IC_{50} value for B16-FO, SK-OV3, HCT15, XF498, SK-MEL, and A549 were 3.9, 11.3, 2.1, 10.1, 11.8, and 3.4 μg/ml, respectively. Ursonic acid showed 50% inhibiting rate for DNA cleavage by topoisomerase I at 50 μg. These results suggested that ursonic acid also has the possibility to be applied as an anticancer agent (Kim, 1997).

Immune activity – The effect of *H. diffusa* on immunological function of mice was investigated (Qin *et al.*, 1990), and the results indicated that *H. diffusa* was able to enhance the proliferation responses of spleen cells to mitogen ConA and LPS, as well as to induce the mitogenesis of the spleen cells. Furthermore, at the doses of 100 mg/kg/day, *H. diffusa* was able to improve the functions of specific antibody forming splenic cells against SRBC and augment the delayed type hypersensitivity (DTH) induced by allogenic splenocytes as well as the killing activity of cytotoxic T-lymphocyte (CTL). Administration of *H. diffusa* was found to enhance the lymphocytic recovery in mice whose hematopoietic tissue was heavily depleted by sublethal irradiation, *H. diffusa* also ameliorated the leukopenia and splenic cellular immunity induced by sublethal irradiation, and slightly increased the immunocompetence of splenic cells after being stimulated by mitogens (Liao *et al.*, 1979; Yang *et al.*, 1997a; 1997b; Shan *et al.*, 1999; 2001). The effects of *O. diffusa* on oxidative burst as an indicator of phagocytic function were determined (Wong *et al.*, 1996) in a murine macrophage cell line J774 using an automated microfluorometric assay. A dose-dependent augmentation of oxidative burst was observed with *O. diffusa.* This indicated that *O. diffusa* was capable of enhancing macrophage function *in vitro* and inhibiting tumor growth *in vivo.* The effects of 8 traditional Chinese medicines on lymphocytes from mice spleen were investigated *in vitro* (Yoshida *et al.*, 1997), the results showed that *H. diffusa* stimulated lymphocytes, and stimulated macrophages to

produce IL-6 and TNF, these indicated that *H. diffusa* possessed immunostimulatory effect. An immunological analysis was performed at molecular level by FACS (Kim *et al.*, 1999), the results showed that a combination of **21** and **9** activated CD4 (helper cell) and CD8 (cytotoxic T cell) as well as macrophage, while **21** activated macrophage only. More recently, the effect of *O. diffusa* on nitric oxide (NO) production by using mouse peritoneal macrophages was investigated (Chung *et al.*, 2002). Treatment of macrophages with rINF-γ plus *O. diffusa* (1 mg/ml) caused a significant increase in tumor necrosis factor-α (TNF-α) production (4.49±1.43 ng/ml) by enzyme-linked immunosorbent assay. The results demonstrated that *O. diffusa* increased the production of NO and TNF-α by rINF-γ-primed macrophages and suggested that NF-*k*B played a critical role in mediating these effects of *O. diffusa.*

Anti-inflammatory – Chronic diseases such as rheumatism, arthritis, and allergy are well known to be associated with inflammation. In traditional Chinese medicine, despite the lack of scientific evidence, many crude drugs have been used or claimed to have anti-inflammatory activities. *H. diffusa* is believed to possess this activity. However, few studies have been conducted to validate these claims. Recently, inflammatory-related diseases are increasingly common in many parts of the world. Therefore, the anti-inflammatory properties of *H. diffusa* were investigated (Lin *et al.*, 2002), the results showed that an extract of *H. diffusa* possessed inhibitory effect against carrageenan-induced rat paw edema at concentrations of 100 and 300 mg/kg, and 10 mg/kg indomethacin showed significant anti-inflammatory activity ($p < 0.05$). Therefore, confirmed that *H. diffusa* possessed anti-inflammatory activity.

Antioxidative activity – A study on the bioactive principles of *H. diffusa* led to the isolation of four flavonol glycosides and six iridoid glycosides, these compounds were tested for antioxidant effects on xanthine oxidase inhibition, xanthine-xanthine oxidase cytochrome C and TBA-MDA systems (Lu *et al.*, 2000). The results showed that asperuloside (**9**) and derivative of kaempferol-3-*O*-[2"-*O*-(*E*-6'"-*O*-feruloyl)-β-D-glucopyranosyl]-β-D-galactopyranoside (**26**) possessed minor anti-lipid peroxidation effects, while other compounds did not. These results demonstrated that the antioxidant effects of *H. diffusa* might due to flavonol and iridoid glycosides. More recently, a measurement of antioxidant activity using ferric thiocyanate (FTC) and DPPC radical scavenging techniques were conducted on the isolated compounds (Dharma *et al.*, 2003). The results of antioxidative measurement using the FTC method showed that quercetin 3-*O*-β-rutinoside (**36**) was a stronger antioxidant than quercetin 3-*O*-β-glucoside (**28**) and α-tocopherol while asperuloside (**9**) was found to be inactive. The activity measured using the DPPH technique showed that **36** and **28** were slightly less active than vitamin C and α-tocopherol as radical scanvengers. These results supported the medicinal use of *H. diffusa* as a tonic for health maintenance. Recently, the antioxidative activities of a traditional Chinese medicine preparation named as "Peh-Hua-Juwa-Chi-Cao", which was consisted of *H. diffusa*, *H. corymbosa* and *Mollugo pentaphylla*, were reported by Taiwanese researchers (Lin *et al.*, 2004), the investigation results showed that *H. diffusa* possessed the strongest inhibition on the $FeCl_2$-ascorbic acid induced lipid peroxidation in rat liver homogenate, meanwhile showed strong superoxide anion scavenging activity from electron spin resonance (ESR) assay.

Neuroprotective activity – An investigation were performed to find neuroprotective compounds from natural sources by employing glutamate-insulted primary cultures of rat cortical neurons as a screening system (Kim *et al.*, 2001). *H. diffusa* extract showed significant neuroprotective activity from deleterious effects of glutamate in an *in vitro* assay system. Bioactivity-guided isolation was further performed to yield four known acylated iridoid glycosides, *Z*-6-*O*-*p*-methoxycinnamoyl scandoside methyl ester (**11**), *E*-6-*O*-*p*-feruloyl scandoside methyl ester (**12**), *E*-6-*O*-*p*-coumaroyl scandoside methyl ester (**14**) *Z*-6-*O*-*p*- coumaroyl scandoside methyl ester (**15**) as well as five flavonoid glycosides kaempferol-3-*O*-[2-*O*-(6-*O*-*E*-feruloyl)-β-D-glucopyranosyl]-β-D-galactopyranoside (**31**), quercetin-3-*O*-[2-*O*-(6-*O*-*E*-feruloyl)-β-D-glucopyranosyl]-β-D-galactopyranoside (**32**), quercetin-3-*O*-[2-*O*-(6-*O*-*E*-feruloyl)-β-D-glucopyranosyl]-β-D-glucopyranoside (**33**), kaempferol-3-*O*-(2-*O*-β-D-glucopyranosyl)-β-D-galactopyranoside (**34**), quercetin-3-*O*-(2-*O*-β-D-glucopyranosyl)-β-D-galactopyranoside (**35**). The results suggested that all nine compounds exhibited significant neuroprotective activity in primary cultures of rat cortical cells damaged by L-glutamate.

Hepatoprotective activity – Taiwanese researchers (Lin *et al.*, 2002) investigated hepatoprotective properties of *H. diffusa* on a CCl_4- and D-Galactosamine (D-GalN)-induced liver damage model, the hepatotoxic effect of *H. diffusa* was evaluated by assessing serum glutamate oxalate transaminase (sGOT) and serum glutamate pyruvate transaminase (sGPT) activities in experimental animals. The results indicated that *H. diffusa* significantly reduced the acute elevation of sGOT and sGPT

concentration, and alleviated the degree of liver damage 24 hours after the intraperitoneal administration of the hepatotoxins.

Anti-mutagenesis – Anti-mutagenesis of Chinese medicinal herbs was investigated by the Wong research group (Wong *et al.*, 1992a; 1992b), and the results showed that *O. diffusa* inhibited mutagenesis, DNA binding and metabolism of aflatoxin B1 (AFB1) and benzo(a)pyrene (Bap), and further demonstrated that *O. diffusa* might possess antimutagenic activity towards AFB1 and BaP through an inhibition of CYP3-mediated metabolism of AFB1 and BaP (Wong *et al.*, 1993a; 1993b). Anti-mutagenic effects of some Chinese herbal medicines and green tea antagonizing cigarette tar were studied with an unscheduled DNA synthesis (UDS) test in human peripheral lymphocytes (Han *et al.*, 1997). Results showed that 125 g/L *H. diffusa* could inhibit the damage to DNA in lymphocytes caused by the total particle material (TPM) extract from cigarette tar.

Perspective

In summary, numerous chemical constituents were isolated and identified from *H. diffusa*, which provided a sound base for further pharmacological researches. However, some commercial available compounds still can not be obtained, thus further activity-guided isolation should provide enough amounts of compounds for the research and development of a potential drug candidate from *H. diffusa*. Among the various pharmacological activities of *H. diffusa*, antitumor activity was paid much attention to and investigated extensively at crude extract level; while other activities such as immune, antioxidative, neuroprotective, hepatoprotective activities have not been deeply investigated. Although various pharmacological activities have been investigated, to date most of studies still linger at crude extract level, only a few researches have been carried out at single compound level.

H. diffusa possesses a long folk medicinal history and extensive clinical application, as well as characteristics of prominent curative effect, relatively non-toxic and low side-effects and abundant resources, it is worthy of Farther research and development of a drug candidate. Summarizing the above review, author considers that further researches are needed to do as below several fields: 1) reinforce pharmacological studies, elucidate the mechanisms of action, provide scientific data for clinical application with reason; 2) further isolate effective components, definitely reveal structure-activity relationships combining with pharmacology, and further develop high efficacy and low toxicity novel drug candidate by structural amelioration; 3) according to oriental medicinal custom and objective efficacy, carry out preparation type amelioration and develop complex formula patent medicine; 4) develop food and cosmetics with a preventive and health care function from *H. diffusa*.

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