Herba Andrographis
Chuanxinlian

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition, 2000/2005

Official drug[1]: Common Andrographis Herb is the dried aerial part of *Andrographis paniculata* (Burm. f.) Nees (Fam. Acanthaceae). The drug is collected in early autumn when foliage branch growing luxuriantly, sliced and dried in the sun.

Descripition of the drug[1]: Stems square and frequently branched, 50 – 70 cm long, nodes slightly swollen; texture fragile, easily broken. Leaves simple, opposite, short petioled or nearly sessile; lamina crumpled and easily broken, when whole, lanceolate or ovate-lanceolate, 3 – 12 cm long, 2 – 5 cm wide, with acuminate apex and cuneate-decurrent base, margin entire of undulate; the upper surface green, the lower surface greyish-green, glabrous on both surfaces. Odour, slight; taste, extremely bitter.

Pretreatment of the raw drug[1]: Foreign matters and legumes are eliminated, washed clean, cut into sections and dried.

Medicinial use[1,2]: Inflammations, hepatitis, febrile diseases, common cold, laryngitis, cough, diarrhoe, mastitis, externally carbuncles, sores and nodules

Effects and indications according to Traditional Chinese Medicine[1]

Taste:	extremely bitter
Temperature:	cold
Channels entered:	acts on the lung, stomach, large intestine and small intestine channels
Effects:	clears pathogenic heat, relieves depressed liver, removes dampness, alleviates pain and promotes diuresis
Symptoms and indications:	jaundice with hypochondriac distress, epigastric distensions and pain, acute and chronic hepatitis, mastitis

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Main constituents[2]**:**

- **diterpene lactones**:
  andrographolide, neoandrographolide, deoxy-didehydroandrographolide, deoxy-oxoandrographolide, deoxyandrographolide, dideoxyandrographolide (andrograpanin), andrographiside, deoxyandrographoside (andropanoside), deoxy-methoxyandrographolide
- **flavone derivatives:**
  oroxylin, wogonin, andrographidine A, B, C, D, E, F
- sesquiterpen lactones:
  paniculide A, B, C
- acidic polysaccharides PA, PB

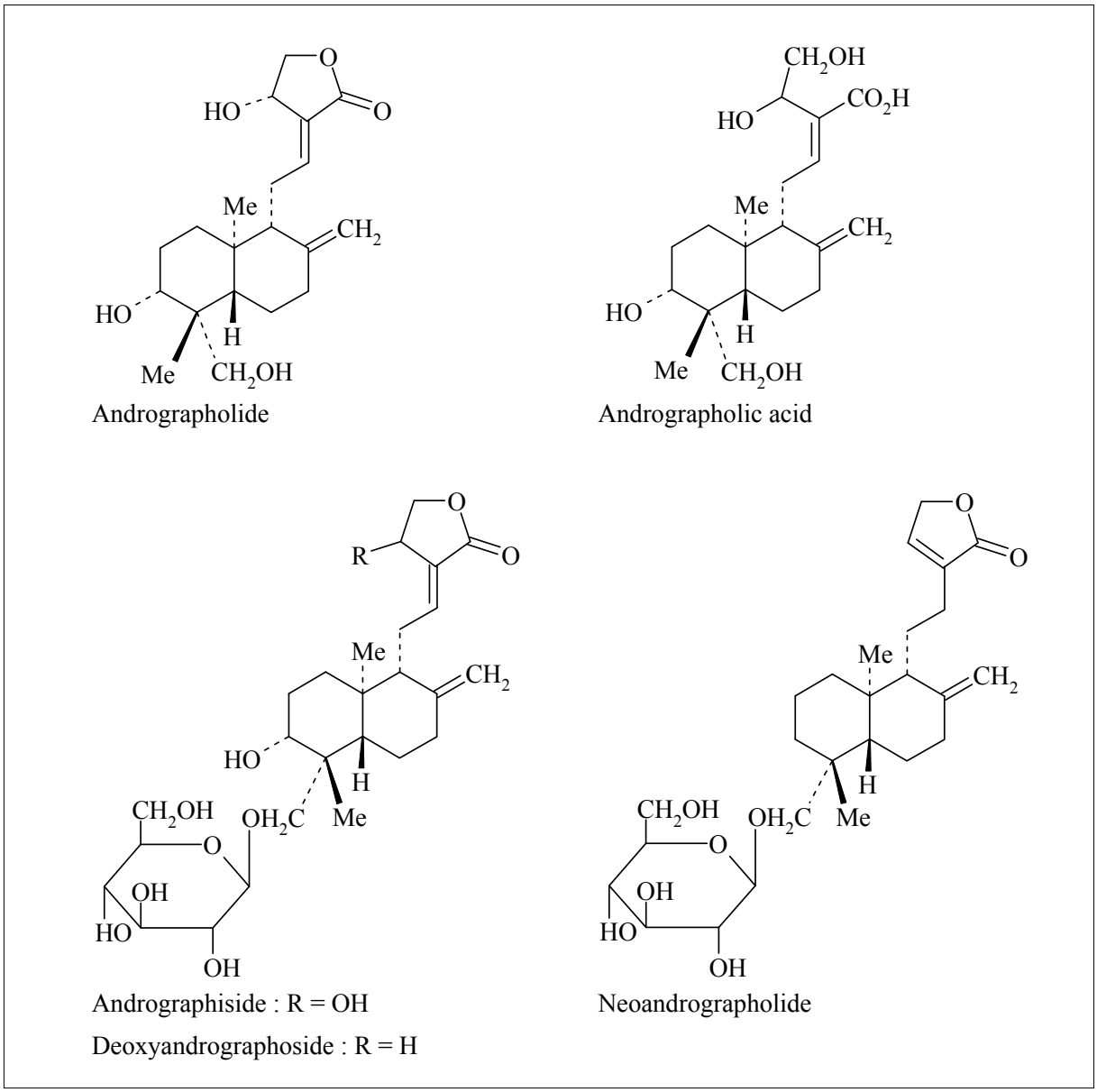
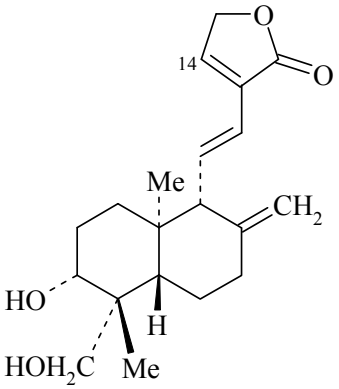
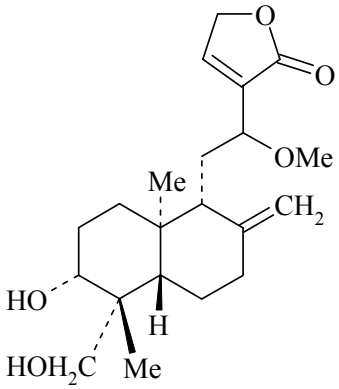


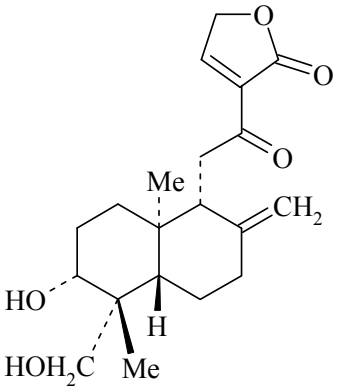
Fig. 1: Formulae of the main constituents



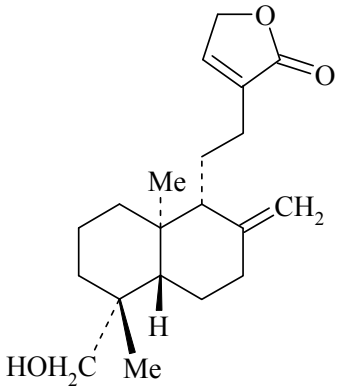
14-deoxy-11,12-didehydroandrographolide



Deoxy-methoxy-andrographolide



Deoxy-oxo-andrographolide



Dideoxy-andrographolide

Pharmacology: (in vitro/in vivo)

extracts, andrographolide and derivatives:

– antiinflammatory [3]
– antihepatoxic and liverprotective [4,5]
– NO inhibitory in macrophages (neoandrographolide) [6]
– superoxide scavenging effect [7,8]
– antihyperglycemic [9,10]
– antithrombotic [11,12]
– hypotensive [13]
– immunstimulatory [14]

Clinical trial: Common cold [15]

TLC fingerprint analysis

1) Extraction: 0.5 g of the powdered drug is macerated for 30 minutes with 30 ml 96 % ethanol. Afterwards the macerate is ultrasonicated for 30 minutes, filtered and the residue washed thrice with 10 ml of ethanol 96 %. The washings are combined to the filtrate and the total solution evaporated to dryness. The residue is dissolved in a small amount of ethanol 96 %, transferred to a 5 ml volumetric flask and filled up to the 5 ml mark with ethanol 96 %.

2) Reference compound: andrographolide (T 1): 1 mg is dissolved in 1 ml 96 % ethanol

3) Separation parameters:

Plates: Silica gel F_{254} Merck

Applied amounts: Andrographis herba-ethanol-extract: each 25 µl
reference compound: 20 µl

Solvent system: chloroform : ethyl acetate : methanol
4 3 0.4

Detection: Vanillin-sulphuric acid reagent:
Solution I: 1 % ethanolic vanillin solution
Solution II: 50 % ethanolic sulphuric acid

The plate is intensively sprayed with 10 ml solution I followed immediately by 10 ml solution II. Afterwards the plate is heated for 5 – 10 minutes at 105 °C. The evaluation is carried out in VIS.

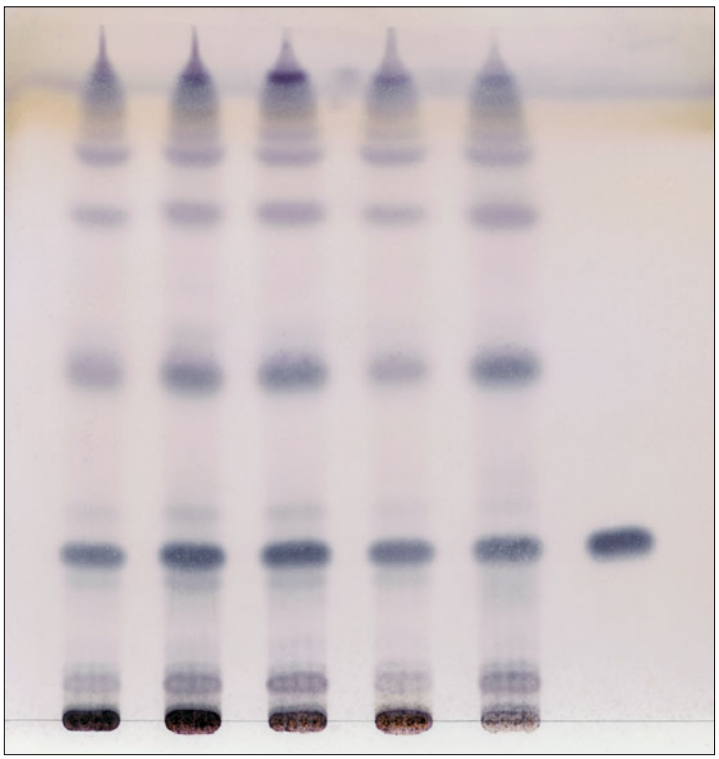


Fig. 2: Thin layer chromatogram of ethanolic extracts of Andrographis herba after spraying with vanillin-sulphuric acid reagent in VIS

Drug samples		Origin
1	Andrographis herba	province Fujian, China
2	Andrographis herba	sample of commercial drug, China
3	Andrographis herba	sample of commercial drug, China
4	Andrographis herba	sample of commercial drug, China
5	Andrographis herba	sample of commercial drug, China

Reference compound		R*f*
T 1	andrographolide	0.25

4) Description of the TLC-chromatogram:

The chromatograms of all investigated samples of *Andrographis* extracts show a very homogeneous pattern of six violett grey zones at R*f* = 0.06, 0.25, 0.50, 0.73, 0.81 and R*f* = 0.92. The most prominent are andrographolide (R*f* = 0.25) and 14-deoxy-11,12-didehydroandrographolide (R*f* = 0.50). The zone with the R*f* = 0.06 is one of the diterpenglucosides (andrographiside, neoandrographolide or andrographolic acid).

HPLC-fingerprint analysis:

1) Sample preparation: The same extract, used for the TLC, is filtered over Millipore® filtration unit type 0.45 µm and injected into the HPLC.

2) Injection volume: 20.0 µl extract

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 125-4 with LiChrospher® 100 RP 18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 100 RP 18, Merck
Solvent:	A: dist. water (Acros Organics) B: methanol (Acros Organics)
Gradient:	40 – 60 % B in 5 minutes 60 % B in 10 minutes 60 – 100 % B in 5 minutes total runtime: 20 minutes
Flow rate:	0.7 ml/min
Detection:	229 nm

Retention times of the main peaks:

peak	Rt (min.)	compound
1/2	1.2 – 1.6	andrographiside, neoandrographolide or andrographolic acid
3	6.9	14-deoxy-andrographolide
4	9.9	andrographolide
5	15.1	14-deoxy-11,12-didehydroandrographolide

4) Description of the HPLC chromatogram:

The chromatograms are characterized by the dominant andrographolide peak **4** at Rt = 9.9. Peak **1** and **2** at Rt = 1.2 and 1.5 can be assigned to andrographiside, neoandrographolide or andrographolic acid, whereas peak **5** (Rt = 15.1) must be identical with 14-deoxy-11,12-didehydroandrographolide. Peak **3** (Rt = 6.9), which shows an UV-spectrum superimpossible to andrographoside, could be 14-deoxy-andrographolide.

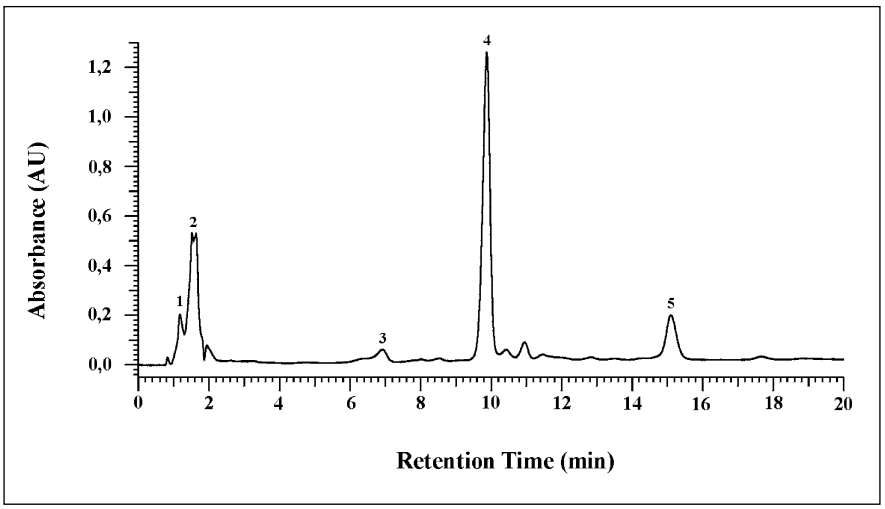


Fig. 3: HPLC-fingerprint chromatogram of Andrographis herba

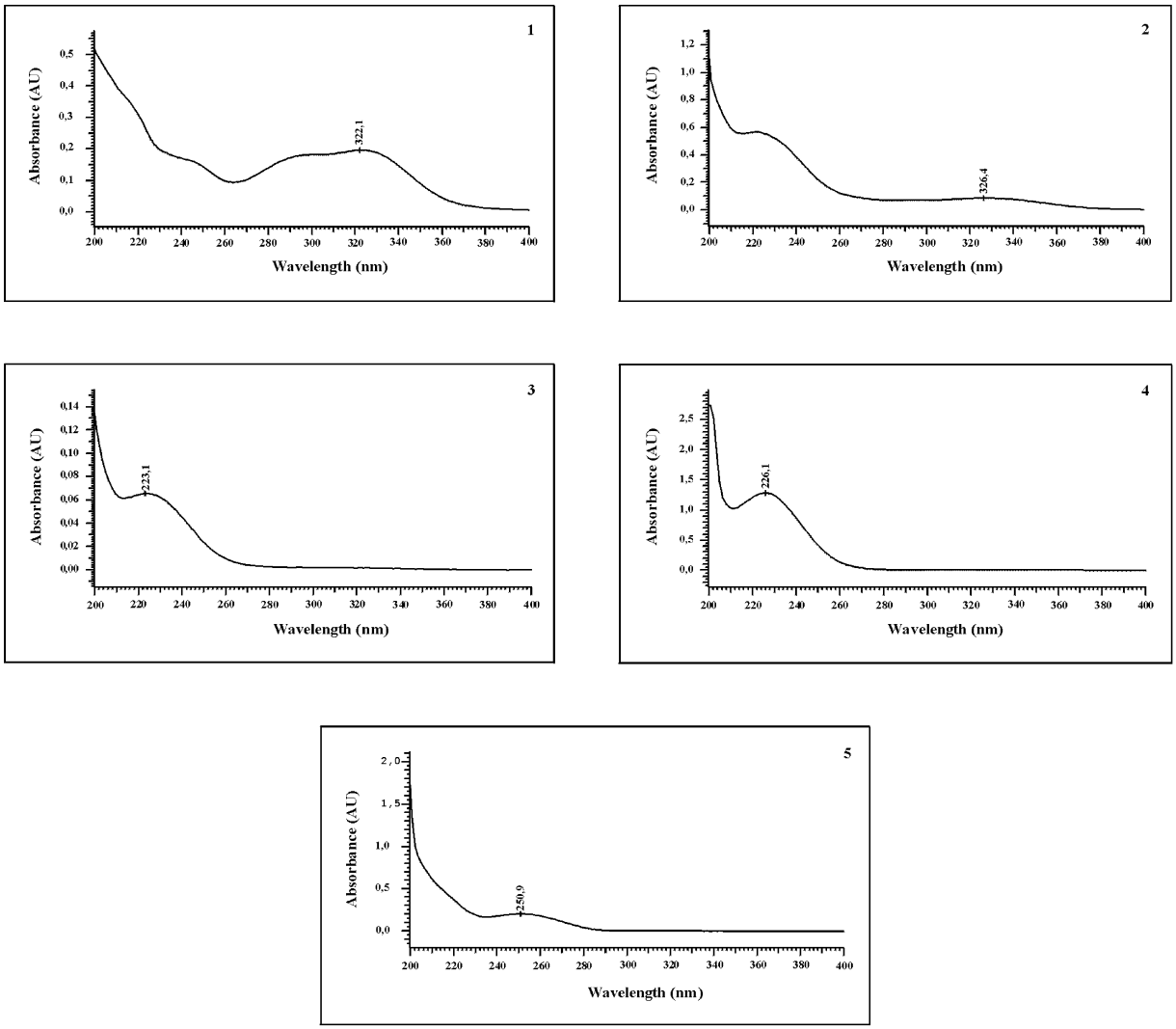


Fig. 4: UV-spectra of the main compounds (peak) of Andrographis herba

Note: According to the Pharmacopoeia of the People's Republic of China, English Edition, 2000 and 2005 Andrographis herba should contain not less than 0.80 % of total amount of andrographolide and dehydroandrogrpholide, calculated on the dried raw drug.

References

(1) Pharmacopoeia of the People's Republic of China 2000 and 2005 (English Edition): People's Medical Publishing House, Beijing, China

(2) Chinese drugs of plant origin: Tang. W., Eisenbrand, G.; Springer-Verlag, Berlin, Heidelberg, New York (1992)

(3) Deng WL, Nie RJ, Lin JY, Comparison of pharmacological effects of four andrographolides, Chin Pharm Bull. 17: 195 – 198 (1982)

(4) Trivedi NP, Rawal UM, Indian J Exp Biol. 39 (1): 41 – 6 (2001)

(5) Visen PK, Shukla B, Patnaik GK, Dhawan BN, J Ethnopharmacol. 40 (2): 131 – 6 (1993)

(6) Batkhuu J, Hattori K, Takano F, Fushiya S, Oshiman K, Fujimiya Y, Suppression of NO production in activated macrophages in vitro and ex vivo by neoandrographolide isol. from *Andrographis paniculata*. Biol Pharm Bull. 25 (9): 1169 – 74 (2002)

(7) Kandem RE, Sang S, Ho CT, Mechanism of the superoxide scavenging activity of neoandrographolide – a natural product from *Andrographis paniculata* Nees. J. Agric Food Chem. 50 (16): 4662 – 5 (2002)

(8) Shen YC, Chen CF, Chiou WF, Andrographolide prevents oxygen radical production by human neutrophils: possible mechanism(s) involved in its antiinflammatory effect. Br J Pharmacol. 135 (2): 399 – 406 (2002)

(9) Zhang XF, Tan BK, Anti-diabetic property of ethanolic extract of Andrographis paniculata in streptozotocin diabetic rats, Clin Exp Pharmacol Physiol 27 (5 – 6): 358 – 363

(10) Bu-Chin Yu, Chen-Road Hung, Wang-Chuan Chen, Juei-Tang, Cheng, Planta Med. 69: 1075 – 79 (2003)

(11) Amroyan E, Gabrielian E, Panossian A, Wikman G, Wagner H, Phytomedicine 6 (1): 27 – 31 (1999)

(12) Zhao HY, Fang WY, Chin Med J. 104 (9): 770 – 5 (1991)

(13) Zhang CY, Tan BK, Clin Exp Pharmacol Physiol. 23 (8): 675 – 8 (1996)

(14) Puri A, Saxena R, Saxena RP, Saxena KC, Srivastava V, Tandon JS, J Nat Prod. 56 (2): 995 – 9 (1993)

(15) Cáceres DD, Hancke JL, Burgos RA, Wikman GK, Prevention of common colds with Andrographis paniculata dried extract. A pilot double blind trial. Phytomedicine 4 (2): 101 – 104 (1997)

Additional references (Chromatography/clinical trial/Pharmacology)

Saxena RC, Singh R, Kumar P, Yadav SC, Negi MP, Saxena VS, Joshua AJ, Vijayabalaji V, Goudar KS, Venkateshwarlu K, Amit A, A randomized double blind placebo controlled clinical evaluation of extract of *Andrographis paniculata* (KalmCold) in patients with uncomplicated upper respiratory tract infection, Phytomedicine 17(3-4):178-85 (2010)

Dong HJ, Zhang ZJ, Yu J, Liu Y, Xu FG, Chemical fingerprinting of *Andrographis paniculata* (Burm. f.) Nees by HPLC and hierarchical clustering analysis, J Chromatogr Sci. 47(10):931-5 (2009)

Burgos RA, Hancke JL, Bertoglio JC, Aguirre V, Arriagada S, Calvo M, Cáceres DD, Efficacy of an *Andrographis paniculata* composition for the relief of rheumatoid arthritis symptoms: a prospective randomized placebo-controlled trial, Clin Rheumatol. 28(8):931-46 (2009)

Kligler B, Ulbricht C, Basch E, Kirkwood CD, Abrams TR, Miranda M, Singh Khalsa KP, Giles M, Boon H, Woods J, *Andrographis paniculata* for the treatment of upper respiratory infection: a systematic review by the natural standard research collaboration, Explore (NY) 2(1):25-9 (2006)

Pholphana N, Rangkadilok N, Thongnest S, Ruchirawat S, Ruchirawat M, Satayavivad J, Determination and variation of three active diterpenoids in *Andrographis paniculata* (Burm.f.) Nees, Phytochem Anal. 15(6):365-71 (2004)

Coon JT, Ernst E, *Andrographis paniculata* in the treatment of upper respiratory tract infections: a systematic review of safety and efficacy, Planta Med. 70(4):293-8 (2004)

Poolsup N, Suthisisang C, Prathanturarug S, Asawamekin A, Chanchareon U, *Andrographis paniculata* in the symptomatic treatment of uncomplicated upper respiratory tract infection: systematic review of randomized controlled trials, J Clin Pharm Ther. 29(1):37-45 (2004)

Hovhannisyan AS, Abrahamyan H, Gabrielyan ES, Panossian AG, The effect of Kan Jang extract on the pharmacokinetics and pharmacodynamics of warfarin in rats, Phytomed 13(5): 318-23 (2006)

Panossian A, Davtyan T, Gukassyan N, Gukasova G, Mamikonyan G, Gabrielian E, Wikman G, Effect of Andrographolide and Kan Jang – fixed combination of extract SHA-10 and extract SHE-3 – on proliferation of human lymphocytes, production of cytokines and immune activation markers in the whole blood cells culture, Phytomed 9(7): 598-605 (2002)