CHAPTER II

HISTORICAL

1. The Occurrence of Chemical Compounds in *Andrographis paniculata* Nees

Andrographis paniculata is widely investigated, especially in the field of chemical studies. Many type of chemical compounds, isolated from this plant, have been reported such as lactone, flavone and miscellaneous compounds. The occurrence of chemical compounds in *Andrographis paniculata* are shown in Table 1.

Table 1 The Occurrence of Chemical Compounds in *Andrographis paniculata*

Type of Compound	Compound	Part of the Plant	References
Lactone	Andrographolide	leaves, root whole plant stem	14, 17,28 35
	Neoandrographolide	leaves whole plant	16, 17, 35
	Deoxyandrographolide--19β-D-glucoside	leaves	16
	14-Deoxy-11,12-didehydroandrographolide	whole plant	17

Type of Compound	Compound	Part of the Plant	References
Lactone	14-Deoxyandrographolide	leaves	16,17
		whole plant	
	Andrographiside	stem	33
	Andrographoside	stem	34
	14-Deoxyandrographoside	stem	34
	14-Deoxy-12-methoxy--andrographolide	Leaves	7
	Andrograpanin	leaves	7
	Homoandrographolide	leaves	26
	Panicolide	leaves	26, 28
	14-Deoxy-11-oxoandrographolide	whole plant	17
	Paniculide A	leaves,tissue culture	18, 27
	Paniculide B	leaves,tissue culture	18, 27
	Paniculide C	leaves,tissue culture	18, 27
	3,14-Dideoxyandrographolide	stem	33
Flavone	Andrographin	root	29
	Panicolin	root	29
	Mono-O-methywightin	root	29
	Apigenin-4',7-dimethyl ether	root	29

Type of Compound	Compound	Part of the Plant	Reference
	Apigenin-4-,7-di-O-methyl ether	root	30
	5-Hydroxy-2',3',7,8-tetramethoxy flavone	root	30, 31
	5-Hydroxy-7,8-dimethoxy flavone	root	31, 32
	(*dl*)-5-Hydroxy-7,8-dimethoxy flavone	root	31
Miscellaneous	Andrographan	leaves	26
	Andrographon	leaves	26
	Panicula-wachs	leaves	26
	Andrographosterin	leaves	26
	2-*cis*-6-*trans* Farnesol	whole plant	36
	2-*trans*-6-*trans* Farnesol	whole plant	36
	Caffeic acid (3,4-dihydroxycinnamic acid)	leaves	19
	Chlorogenic acid	leaves	19
	3,5-Dicaffeoyl-*d*-quinic acid	leaves	19
	KH_2PO_4	whole plant	31
	KCL	whole plant	26
	NaCl	whole plant	26

2. Chemistry

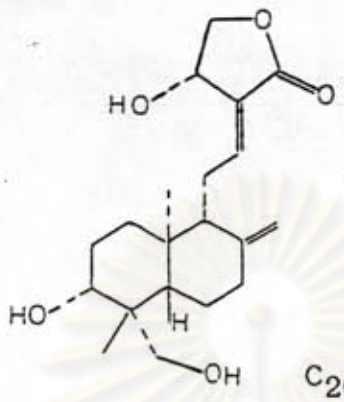
2.1 Lactone compounds

2.1.1 Andrographolide

An attempt to isolate the active principle was made as early as in 1896, Boorsma was the first who isolated the main crystalline bitter principle of *Andrographis paniculata*. This compound is a colorless neutral, crystalline substance which was called andrographide. Latter in 1911 the bitter principle was isolated in a pure crystalline by Gorter, who found it to be a diterpene lactone. Gorter changed the compound's name from andrographide to andrographolide which is still in use at present (37,38).

Andrographolide which is an unsaturated trihydroxy lactone having the molecular formula $C_{20}H_{30}O_5$, gives a positive legal reagent, Kedde's reagent and 50% KOH-methanol. Andrographolide appeares as colorless plates crystal with bitter taste. It is freely soluble in methanol, ethanol, pyridine, acetic acid, sparingly soluble in benzene, chloroform and acetone, insoluble in ether and water. Its physical properties were reported as follows : mp 230-231 ^{o}C (from methanol), $[\alpha]_D^{25}$ -96.2 (C=1.00,C_5H_5N), ultraviolet spectrum in ethanol : λ max 223 nm (ϵ 12300), IR spectrum in KBr (cm^{-1}) : 3448 and 3390, 3279 (hydroxy group), 1828, 1647, 909 (exocyclic methylene), 1727 (α,β-unsaturated-γ-lactone), 1672

(conjugated C=C) cm^{-1}.

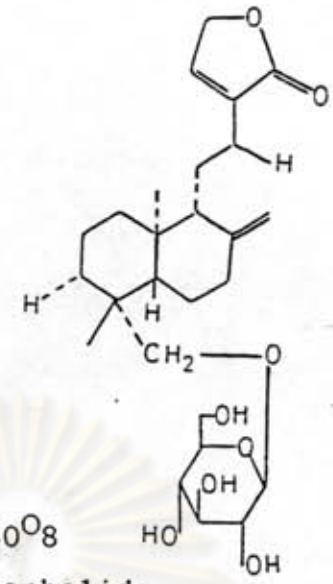


$C_{20}H_{30}O_5$

Andrographolide

2.1.2 Neoandrographolide

The isolation of neoandrographolide from *Andrographis paniculata* was reported in 1952 by leipool (35,39). Neoandrographolide, a diterpene glucoside, gives a positive legal reagent, Kedde's reagent and 50% KOH-methanol. Neoandrographolide which has molecular formula $C_{26}H_{40}O_8$, appears as colorless needles crystal with bitter taste. It is soluble in methanol, ethanol, acetone,pyridine, sparingly soluble in chloroform and water, insoluble in ether and petroleum ether. Its physical properties were reported as follows : mp 167-168°C (from methanol), $[\alpha]_D$ -48 (pyridine), ultraviolet spectrum in ethanol: λ max 205 nm (ϵ10400), IR spectrum in KBr cm^{-1} : 3400 (hydroxy group), 1640, 909 (exocyclic methylene), 1748 (α,β-unsaturated-γ- lactone) cm^{-1}.

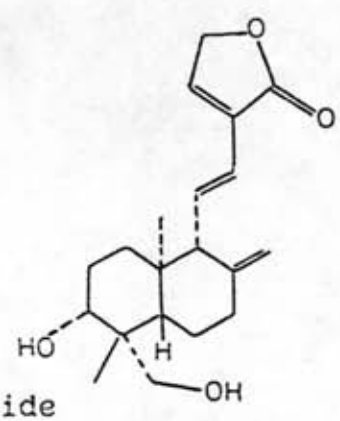


$C_{26}H_{40}O_8$

Neoandrographolide

2.1.3 Dehydroandrographolide

Dehydroandrographolide, a diterpene lactone with molecular formula $C_{20}H_{28}O_4$, gives a positive legal reagent, Kedde's reagent and 50% KOH-methanol. Dehydroandrographolide appeares as colorless fine needles crystal with bitter taste. It is soluble in methanol, ethanol, sparingly soluble in chloroform and water, insoluble in ether. Its physical property were reported as follows : mp 203-204 ^{o}C (from methanol) (17), ultraviolet spectrum in ethanol λ max 248 nm (ϵ11000), IR spectrum in KBr cm^{-1} : 3,300 (hydroxy group), 1758 (α,β-unsaturated-γ - lactone), 1640 and 900 (exocyclic methylene) cm^{-1}.



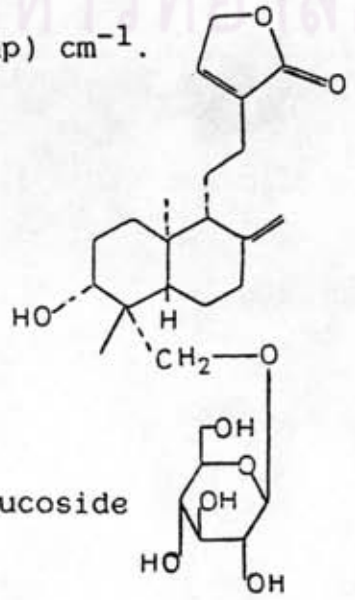
$C_{20}H_{28}O_4$

Dehydroandrographolide

2.1.4 Deoxyandrographolide-19β-D-glucoside

This new diterpene glucoside has been isolated from the leaves of *Andrographis paniculata* and it structure elucidated as deoxyandrographolide-19B-D-glucoside on the basis of chemical and spectral evidence (15). In 1981 Hu,C.Q and B.N.Zhae (1981) called this compound 14-deoxyandrographiside (33,34). In 1982 Meng, Zhengmu called the compound ninandrographolide (40). In 1984 Fujita *et al.*, isolated this compound and called it andropanoside (7).

Deoxyandrographolide-19β-D-glucoside which is white crystals, with molecular formula $C_{26}H_{40}O_9$, gives a positive legal reagent, Kedde's reagent and 50% KOH-methanol. It is soluble in methanol, ethanol, sparingly soluble in chloroform and water, insoluble in ether. Its physical properties were reported as follows mp 187-188 °C (from methanol), $[\alpha]_d^{20}$ -30 (C=1.00, methanol), ultraviolet spectrum in ethanol: λ max 205 nm (ϵ13803), IR spectrum in KBr cm^{-1} : 3500, 3400 and 3362 (hydroxy group), 1755 (α,β-unsaturated-γ-lactone), 1640, 898 (exocyclic methylene group) cm^{-1}.

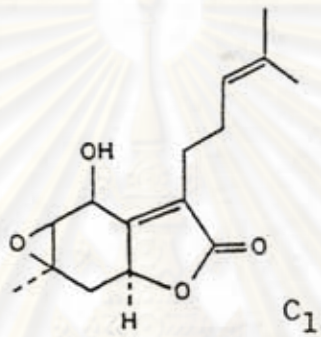


$C_{26}H_{40}O_9$

Deoxyandrographolide-19β-D-glucoside

2.1.5 Paniculide A

Paniculide A which is the constituent of *Andrographis paniculata* when grown in tissue culture was reported by Allison *et al.*,(27). The compound has molecular formula $C_{15}H_{20}O_4$, crystals from ethanol, mp 120-121 ^{o}C, ultraviolet spectrum : absorption maximum at 216 nm in ethanol ($\in$15500).

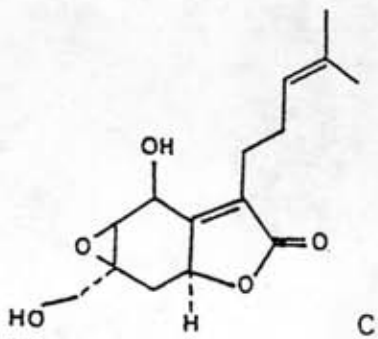


$C_{15}H_{20}O_4$

Paniculide A

2.1.6 Paniculide B

Paniculide B which is the constituent of *Andrographis paniculata* when grown in tissue culture was reported by Allison *et al.* (27). The compound has molecular formula $C_{15}H_{20}O_5$, needle crystal crystallization from ethanol, mp 145-146 ^{o}C, ultraviolet spectrum : absorption maximum at 217 nm in ethanol ($\in$15500).

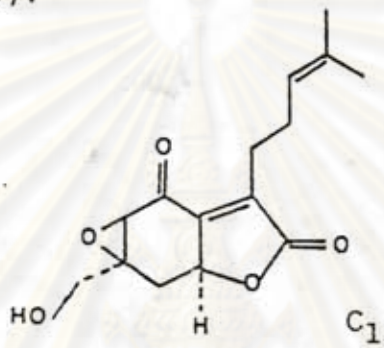


$C_{15}H_{20}O_5$

Paniculide B

2.1.7 Paniculide C

Paniculide C which is the constituent of *Andrographis paniculata* when grown in tissue culture was reported by Allison *et al.* (27). The compound with molecular formula $C_{15}H_{18}O_5$, was oily matterial and gave ultraviolet spectrum : absorption maximum at 250 nm in ethanol ($\in$ 4500).

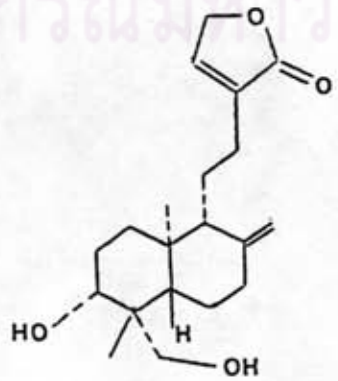


$C_{15}H_{18}O_5$

Paniculide C

2.1.8 14-Deoxyandrographolide

In 1973 Balmain *et al.*(15),isolated 14-deoxyandrographolide from the leaves of *Andrographis paniculata*. It is colorless nedles crystal, mp 172-173 ^{o}C (from methanol), with molecular formula $C_{20}H_{30}O_4$.

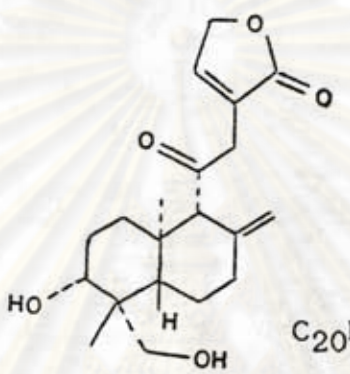


$C_{20}H_{30}O_4$

14-Deoxyandrographolide

2.1.9 14-Deoxy-11-oxoandrographolide

In 1973 the isolation of 14-deoxy-11-oxoandrographolide from the whole part of *Andrographis paniculata* was reported by Balmain (15). The compound has molecular formula $C_{20}H_{28}O_5$, mp 98-100 °C (from chloroform-ether), max 227 nm (∈9000).

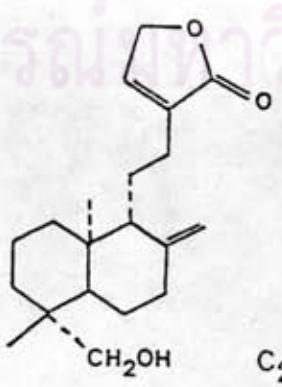


$C_{20}H_{28}O_5$

14-Deoxy-11-oxoandrographolide

2.1.10 3,14-Dideoxyandrographolide

Hu,C.Q and B.N. Zhao (33) isolated 3,14-dideoxyandrographolide from the aerial part of *Andrographis paniculata*. The compound has molecular formula $C_{20}H_{30}O_3$, with mp 92-94 °C.

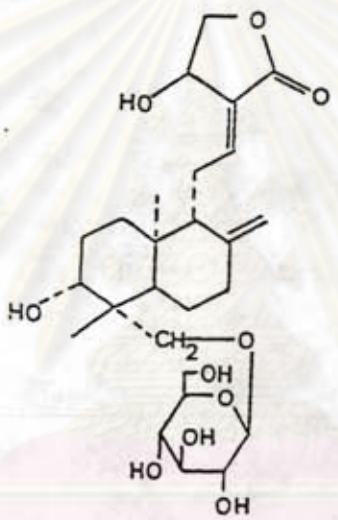


$C_{20}H_{30}O_3$

3,14-Dideoxyandrographolide

2.1.11 Andrographiside

Hu.C.Q and B.N. Zhao (34) isolated andrographiside from the aerial part of *Andrographis paniculata*. The compound has molecular formula $C_{26}H_{40}O_{10}$, mp 203-204 ^{o}C. it was also called andrographolide-19β-glucoside.

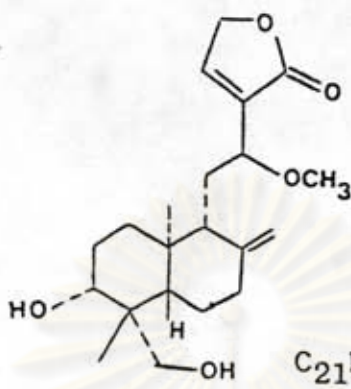


$C_{26}H_{40}O_{10}$

Andrographiside

2.1.12 14-Deoxy-12-methoxy-andrographolide

In 1984, Fujita *et al.*(7) isolated 14-Deoxy-12-methoxy-andrographolide from the leaves of *Andrographis paniculata*. The compound with molecular formola $C_{21}H_{32}O_5$, was colorless needle crystals, mp 121-124 ^{o}C (from methanol). It is belived to be an artefact due to the course of methanolic extraction.

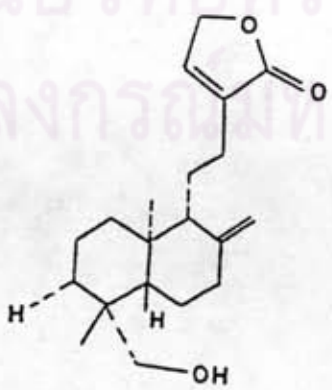


$C_{21}H_{32}O_5$

14-Deoxy-12-methoxy-andrographolide

2.1.13 Andrograpanin

Fujita *et al.* (7), isolated andrograpanin from the leaves of *Andrographis paniculata*. This compound with molecular formula $C_{20}H_{30}O_3$, was colorless needles crystal, mp 104-106 ^{o}C (from Me_2CO-hexane), ultraviolet spectrum in methanol : absorption 220 nm ($\in$ 4567).



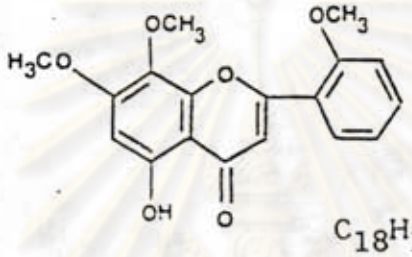
$C_{20}H_{30}O_3$

Andrograpanin

2.2 Flavone compound

2.2.1 Andrographin

In 1964, Qudrat-i-khuda (28) isolated andrographin from the root of *Andrographis paniculata*. The compound has molecular formula $C_{18}H_{16}O_6$, mp 190-191^{o}C.

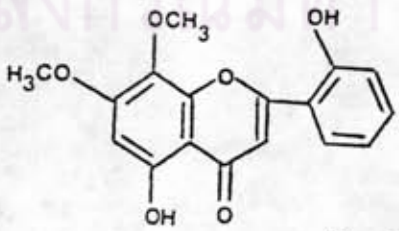


$C_{18}H_{16}O_6$

Andrographin

2.2.2 Panicolin

The isolation panicolin from the root of *Andrographis paniculata* was reported by Qudrat-i-khuda (26,28) in 1964. The compound with moleccular formula $C_{17}H_{14}O_6$, mp 263-264 ^{o}C, was pale yellow fluffy needles crystal.

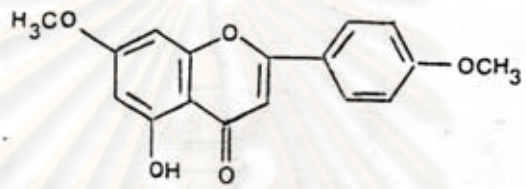


$C_{17}H_{14}O_6$

Panicolin

2.2.3 Apigenin-4',7-dimethyl ether

In 1969 Govindachari *et al.*(26,28), isolated apigenin 4',7-dimethyl ether from the root of *Andrographis paniculata*. The compound with molecular formula $C_{17}H_{16}O_4$ mp 170-171 ^{o}C, was pale yellow haring needle crystal.

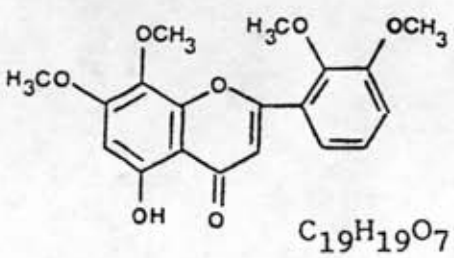


$C_{17}H_{16}O_4$

Apigenin-4',7-dimethyl ether

2.2.4 Mono-O-methylwightin

In 1969 Govindachari *et al.*(26,28), isolated mono-O-methylwightin from the root of *Andrographis paniculata*. The compound has molecular formula $C_{19}H_{19}O_7$ mp 156 ^{o}C.

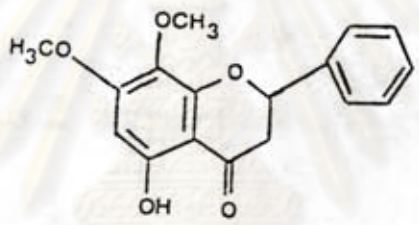


$C_{19}H_{19}O_7$

Mono-O-methylwightin

2.2.5 5-Hydroxy-7,8-dimethoxyflavone

In 1979, Mahubul (32) isolated 5-hydroxy-7,8-dimethoxyflavone from the root of *Andrographis paniculata*. The compound with molecular formula $C_{17}H_{14}O_5$, was yellow needle crystals crystallized from mixture of chloroform and hexane, mp 179-180 ^{o}C, ultraviolet spectrum in methanol showed strong absorption at 276, ($\in$ 31300), 340 ($\in$ 6750).

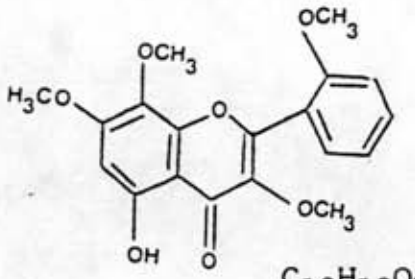


$C_{17}H_{14}O_5$

5-Hydroxy-7,8-dimethoxyflavone

2.2.6 5-Hydroxy-3,7,8,2'-tetramethoxyflavone

Chromatographic separation of the petrol extract of *Andrographis paniculata* roots resulted in the isolation and characterization of 5-hydroxy-3,7,8,2'-tetramethoxyflavone was reported by Gupta *et al.*(31) in 1983. The compound has molecular formular $C_{19}H_{18}O_7$, mp 209-211 ^{o}C, ultraviolet spectrum in methanol showed strong absorptions at 272, 362 and an inflexion at 302 nm.



$C_{19}H_{18}O_7$

5-Hydroxy-3,7,8,2'-tetramethoxyflavone

3. Pharmacological Activity

3.1 Crude Extract of *Andrographis paniculata*

3.1.1 Antimicrobial Activity

George *et al.* (41), reported the antibacterial activity against *Staphylococcus aureus* and *Escherechia coli* in vitro, by the alcoholic extract of the leaves of *Andrographis paniculata*.

Nakanishi *et al.* (42), reported that the alcoholic extract of whole part of *Andrographis paniculata* exhibited antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Proteus vulgaris* in vitro. In addition this extraction exhibited antityphoid activity active against *Salmonella typhosa* in vitro (43).

Ray P.G. (44) reported the alcoholic extraction of root part of *Andrographis paniculata* exhibiting antimicrobial activity against *Staphylococcus aureus* in vitro.

The aqueous extraction and alcoholic extract of

whole part of the plant exhibited antimicrobial activity with high potency against *Staphylococcus aureus* and *Proteus vulgaris*, but has low potency against *Escherechia coli* and *Shigella dysenteriae* (26).

3.1.2 Anticoagulant Activity

Pilai *et al.* (45), reported the aqueous extract of the powder of stems and roots of *Andrographis paniculata* possesses a very low anticoagulant activity when testing against plasma, but not against the whole blood.

3.1.3 Antifertility Activity

Antifertility activity of a medicinal plant from the genus *Andrographis* family Acanthaceae was reported by Shamsuzzoha *et al.*(46). In this report, six weeks old male and female Wister strain mice were put on diets supplemented to the extent of 0.75% of the stem powder of the plant for one, two, three and four weeks and the effect on fertility and gestational period was observed. Significant reduction in fertility was observed after three and four weeks of feeding in the group containing the treated males and the untreated females. In the same group the gestation period was also prolonged after four weeks of feeding. There was virtually no change in fertility and gestational period in the treated females and the untreated males during any stage of the experiment.

Zhang xing *et al.*(47), reported the effect of *Andrographis paniculata* on the cell vitality and hormone secretion of the cultured human trophoblast tissue at ages between 6 to 8 weeks of pregnancy. The experimental results obtained showed that the chloroform extract of the plant is effective in inhibiting human chorionic gonadotrophin (hcg) and progesterone production by the trophoblast tissue. Morphologically, the treated cells become pyknotic, rolled up and finally dying off.

3.1.4 Anthelmintic Activity

The alcoholic extracts of whole part of *Andrographis paniculata* exhibited anthelmintic action against human *Ascaris lubricoides* in vitro. The treated *Ascaris lubricoides* were paralysed after 18 hours and dead after 24 hours of treatment (48).

Filaricidal properties of *Andrographis paniculata* was reported by Dutta and Sukul (49). In this report, water decoction of the leaves of the plant gave a 100% killed in vitro to the microfilaria of *Dipetalonema reconditum* within 40 minutes. Three subcutaneous injections of the extract onto infected dogs at 0.06 ml per Kg body weight reduced more than 85 % of the number of microfilariae in blood. The larvae were not totally eliminated with more infection but the reduced microfilarial level persisted. No toxic effect of the extract was observed in rabbits. The treated dogs become

lethargic initially for a week probably due to the mass killing of microfilariae.

Bandyopadhyay *et al.*(50), reported nematocidal effect of the aqueous extract of the leaves of *Andrographis paniculata* on soil nematodes.

3.1.5 Hypotensive Activity

Column chromatographic fraction of the ethyl ether extract from the leaves of *Andrographis paniculata* resulted in the isolation of a slightly coloured crystalline substance which possess hypotensive activity in mice (51).

Nazimudeen *et at.*(52), observed a 50 mm of Hg fall of blood pressure in dog's experiment after treatment of the extract of the plant. In addition, the aqueous extract and alcoholic extract of whole plant showed hypotensive activity in cat (26).

3.1.6 Antiinflammatory Activity

Tajuddin *et al.* (53), reported that aqueous extracts of whole part of *Andrographis paniculata* exhibited antiinflammatory activity in the carragenin treated rat and the mechanism of action was belived to differ from those of the NSAIDS.

3.1.7 Antispasmodic Activity

The aqueous extract of the leaves of

Andrographis paniculata decrease propulsive movement of small intensive which preparation of the guinea pig ileum (54).

3.1.8 Miscellaneous

Chaudhuri (55), reported the aqueous extract of *Andrographis paniculata* increased both biliary flow and liver weight. The extract also reduced the duration of action of hexabarbital in Wister strain male rats. This report indicated that the plant can induce hepatic drug metabolising enzyme. In 1984 Choudhury (56) reported that carbontetrachloride induced hepatic microsomal lipid peroxidation was decreased when the rats were pretreated with the extraction of the leaves of *Andrographis paniculata* for 4 hours. And the extraction of the leaves of the plant has more protective action on carbontetrachloride-induced hepatic toxicity than its bitter principle, andrographolide in vitro.

Nazimudeen *et al.*(52), reported the effect of alcoholic extract of whole part of *Andrographis paniculata* on cobra venom. It prolonged, the life time of venom injected mice. The extract showed cholinergic activity but not nicotinic activity.

Choudhury *et al.* (57), reported that oral administration of a single dose of *Andrographis paniculata* leaves extract (0.5 g/Kg and 1.0g/Kg) to adult male albino rats (100-120 g.), accelerated intestinal digestion and

absorption of carbohydrate by activating the intestinal disaccharidase enzymes.

A systematic pharmacological investigation of the effects of *Andrographis paniculata* on the blood-sugar level of rats was carried out (58). Different doses of leaves and stem extract were administered to the normal and diabetic rats. No significant change in the blood-sugar level of the rats was observed in all cases.

3.2 Andrographolide

3.2.1 Antispasmodic Activity

Petcharat *et al.* (21), reported that andrographolide, the diterpenoid lactone isolated from the leaves of this herbal medicine, depressed the response of the isolated stomach and intestinal smooth muscle preparation of the rabbit and the guinea pig to acetylcholine, histamine and carbachol.

3.2.2 Antifertility Activity

Zhang xing *et al.*(47), reported the effect of andrographolide sodium succinate on the cell vitality and hormone secretion of the cultured human trophoblast tissue at age between 6 to 8 weeks of pregnancy. The experimental results obtained in this study show that the andrographolide sodium succinate is effective in inhibiting hcg and progesterone production by

the trophoblast tissue. Morphologically, the treated cells become pyknotic, rolled up and finally dying off. It is, also suggested that the herb appears promissing as one of the abortifacients.

3.2.3 Anthelmintic Activity

The nematocidal effect of andrographolide on soil nematodes was reported by Bandyopadhyay *et al.* in 1986 (50).

3.2.4 Miscellaneous

Oral administration of a single dose of andrographolide (5 mg/Kg and 10 mg/Kg) to adult male albino rats (100-120 g), accelerate intestinal digestation and absorption of carbohydrate by activity these intestinal disaccharidases (57).

Moniruddin Ahmed (58), reported a systematic pharmacological investigation on the effects of andrographolide on the blood-sugar level of rats. Different doses of pure andrographolide were administered to the normal and diabetic rats. No significant change in the blood-sugar level of the rats was apparent in any case.

3.3 Dehydroandrographolide

Antispasmodic Activity

Dehydroandrographolide is the diterpenoid

lactone isolated from the leves of this herbal medicine, depressed the response of the isolated stomach and intestinal smooth muscle preparation of the rabbit and the guinea pig to acetylcholine, histamine and carbachol (22).

3.4 Apigenin 7,4'-di-o-methyl ether

Antigastric ulcer

Apigenin 7,4'-di-o-methyl ether, a flavone isolated from *Andrographis paniculata* was tested for its antigastric ulcer property in various animal models. The flavone produced a significant dose dependent to antiulcer activity in Shay rats, histamine induced ulcer in guinea pigs and in aspirin induced ulcers in rats. It is suggested that the antisecretory activity and a protective effect on the gastric mucosa may be resposible for the antiulcer action of the flavone (59).

4. Toxicity

4.1 Crude Extract of *Andrographis paniculata*

The toxicity test of the aqueous extract of whole part of *Andrographis paniculata* in rat resulted this plant had an LD_{50} > 5000 mg/Kg (26).

Nakanishi *et al.*(42), studied the toxicity of the alcoholic extract of whole part of *Andrographis paniculata* in mice and found that the extract had the LD_{50} more than 1000 mg/Kg.

In the long term toxicity test condition, rats were received a single dose of *Andrographis paniculata* suspension in 1% tragacanth (100-500 mg/Kg/day) for 5 consecutive days in 3 month. The result of this examination do not found any abnormal entrails and abnormal behavior of rats (21).

In addition, Quintin L. *et al.*(60), reported various effect of *Andrographis paniculata* extracts, when given intraperitoneally to mice. Such effects are shown in table 2.

Table 2 Effect of Various Extracts from *Andrographis paniculata*

Various extracts	Dose mg/Kg	Effect
1.saponin extract	300	Body weakness, increase in respiratory rate, decrease in motor activity and altered posture lasting for 3 hours.
	1000	After 8 minutes of injection, there was convulsion, strub tail and tremors followed by death.
2.aqueous extract	300	Body weakness, increase in respiratory rat, decrease in awareness, motor activity, muscle tone and reflexes with altered posture lasting for

Various extracts	Dose mg/Kg	Effect
		24 hours.
	1000	Same manifestation as above leading to death after 24 hours.
3.ether extract	300	Decrease in awareness, motor activity, muscle tone with altered posture lasting for 1 hour.
	1000	Same manifestation as above leading to death after 45 minutes.
4.powdered ether extract	300	Temporary weakness, decrease in motor activity and muscle tone with altered posture lasting for 2 hours.
	1000	Body weakness leading to death after 24 hours.
5.alcoholic extract	300	Body weakness, decrease in motor activity, muscle tone and abnormal posture lasting for 24 hours.
	1000	5 hours and 5 minutes after injection, mouse had progessive tremores, straub tail and convulsion followed by death.

Various extracts	Dose mg/Kg	Effect
6.partially purified ether extract	1000	progressive weakness leading to death 3 hours and 30 min. after injection.

4.2 Andrographolide

Oral administration of andrographolide at 18 g/Kg in rat, did not cause death during the examination, in addition, physical abnormality was not observed in heart, kidney, liver spleen of subjects except the rat motility which was noted to be decreesed (20).

Petcharat *et al.* (21), reported that no toxic effect was observed in mice after oral administration of andrographolide at 3g/Kg.

5.Analysis of Diterpene Lactone in *Andrographis paniculata*

Many methods have been used to assay diterpene lactone compounds in *Andrographis paniculata*. Some of them are decribed as follows.

5.1 Colorimetric Method

Colorimetric method is one of the specific functional group analysis of drugs. The Pharmacopoeia of the People's Republic of China 1977 (23), had reported the determination of andrographolide in pharmaceutical

preparation by using 60% ethanolic potassium hydroxide solution as solvent. An aliquot of the sample preparation was treated with 2% solution of 3,5-dinitrobenzoic acid in ethanol and 2% methanolic potassium hydroxide solution respectivly, This mixture was standed for 25 minutes for color developing. The absorbance was measured at 540 nm against the reagent blank and the concentration of the sample preparation was determined.

5.2 UV-Spectrophotometric Method

Andrographolide contains chromophores which can absorb light in the UV region, hence the assay by using uv-spectrophotometic method can be applied.

Dar R.N. *et al.*(61) had reported the determination of andrographolide in *Andrographis paniculata* by uv-spectrophotometric method. The crude powder of *Andrographis paniculata* was firstly extracted with benzene to remove the green coloring matter. The powders were then mixed with kieselguhr and chlroform was used to extract the mixture. Chloroform extract was evaporated and then was redissolved in methanol. UV-Absorbtion at 226 nm of the methanolic solution was then measured.

5.3 Titration Method

The Division of Medical Research, Department of Medicinal Sciences, Ministry of Public Health, Thailand (24), had reported the determination of total lactones

from *Andrographis paniculata* by the acid-base titration. Five gram of the sample placed in a Soxhlet's extraction apparatus, then 100 ml of ethanol was used to extract the compound. After cooling, the alcoholic extract was added with 5 ml of 10% basic lead acetate solution. The precipitate formed was filtered and washed with proper volume of ethanol until no green color appeared on the washing filtrate. The washing and the filtrated were combined, and then shaken each time when 5 ml of 25% sodium sulfate solution was added. After further set aside for 2 hours and added with 1 gram of activated charcoal, it was refluxed in water-bath for 10 minutes, filtered through the Buchner funnel containing 1 g of activated charcoal, well-spreaded into layer, and washed with proper volume of hot ethanol. Again the filtrate and washing were combined, evaporated to approximately 50 ml, added with 80 ml of distilled water, allowed to cool, then added with a few drops of phenolpthalin TS as an indicator, and neutralized with 0.1 N. sodium hydroxide solution. After an accurate addition of 25 ml of 0.1 N. sodium hydroxide solution, it was hydrolysed by refluxing on water-bath for 30 minutes, allowed to cool and titrated with 0.1 N hydrochloric acid. The total lactones content was calculated. Each ml 0.1 N. sodium hydroxide is equivalent to 0.03504 g of andrographolide ($C_{20}H_{30}O_5$).

5.4 Thin-Layer Chromatographic Method (TLC)

The advantage of TLC is to separate each diterpene lactone from other interferenees. C. Dechang *et al.*(25), had reported the assay for andrographolide, neoandrographolide and deoxyandrographolide contents in *Andrographis paniculata* by thin layer spectral densitometry. Using silica gel (GF 254) as stationary phase and chloroform : ethanol (14:1) as mobile phase. The contents of andrographolide, neoandrographolide, deoxyandrographolde found were 1.90% 0.552% and 0.943% respectively.