

**STUDIES ON BITTER PRINCIPLES FROM
Andrographis paniculata Nees.**

Thesis

By

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**FORESTRY
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College of Forestry

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2000



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Dedicated
to
Beloved
Parents



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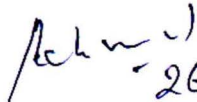
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CERTIFICATE-I

This is to certify that the thesis entitled "**Studies on bitter principles from *Andrographis paniculata* Nees**", submitted in partial fulfilment of the requirements for the award of degree of **MASTER OF SCIENCE in FORESTRY (FOREST PRODUCTS)** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan (H.P.) is a bonafide research work carried out by **MS. Chandresh Bhandari (F-98-6-M)** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

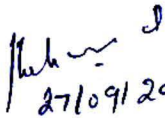
The assistance and help received during the course of investigations have been fully acknowledged.

Place : Nauri, Solan
Dated : 26th July, 2000


26.07.2000.
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Chairman
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CERTIFICATE-II

This is to certify that the thesis entitled "**Studies on bitter principles from *Andrographis paniculata* Nees)**", submitted by **Ms. Chandresh Bhandari (F-98-6-M)** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan (H.P.), in partial fulfilment of the requirements for the award of degree of **MASTER OF SCIENCE in FORESTRY (FOREST PRODUCTS)** has been approved by the Student's Advisory Committee after an oral examination of the same in collaboration with the external examiner.


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(Chandresh Bhandari)

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ABBREVIATIONS USED

<i>cm</i>	Centimeter
<i>CRD</i>	Complete Randomized Block Design
<i>g</i>	Gram
<i>hr</i>	Hour
<i>hrs</i>	Hours
<i>kg</i>	Kilogram
<i>m</i>	Meter
<i>mg</i>	Milligram
<i>ml</i>	Milliliter
<i>nm</i>	Nano-meter
<i>NMR</i>	Nuclear Magnetic Resonance
<i>ng</i>	Nanogram
<i>RBD</i>	Randomized Block Design
<i>TLC</i>	Thin Layer Chromatography

INTRODUCTION

Plants not only provide us the prime necessities of food and shelter, but certain plant species also protect us from various types of diseases. Virtually all cultures have throughout the history used a variety of plants or products derived from plants for the prevention and treatment of diseases. Evidence of the beneficial therapeutic effects of these medicinal herbs is seen in their continuous use. The knowledge of plants with healing properties started growing with passage of time and passed from one generation to another. Ultimately healing through plants became a strong system and people developed faith in plant based medications. Today we have few well developed plant based systems of medicine like Ayurveda, Unani, Homoeopathy, Sidha, etc. Thorough chemical investigation of the plants, used in these age old systems of medicine, have also given us many modern medicines which are now a days used in the allopathic system of medicine e.g. Podophyllotoxin and its semi-synthetic derivatives from *Podophyllum hexandrum*, berberine from *Berberis* spp., Colchicine from *Gloriosa superba* and *Colchicum autumnale*, ephedrine from *Ephedra gerardiana*, Taxol from *Taxus baccata*, atropine and related alkaloids from *Atropa*, vinblastine and vincristine from *Catharanthus roseus* are the few ones which are used for curing different types of diseases.

From 1935-40 onwards use of synthetic drugs became more popular than the plant based systems of medicines. However, due to realization in the recent past that synthetic drugs produce some harmful effects on the user's body system, the plant based drugs/medicines are again becoming popular the world over.

Medicinal plants are of great importance for the pharmaceutical industry. With the progress of technology an array of compounds have been isolated from

plants which form basis of various refined products. Many times pharmaceutical preparations of botanical origin constitute complex mixtures in which the active ingredients may not be known or may constitute only a small percentage of the total product. Some products are in fact believed to achieve their beneficial effect through the combined action of several ingredient chemicals, each of which account for a very small portion of the total product. The vast variation in agroclimatic conditions of India and Himachal Pradesh in particular provide favourable conditions for cultivation of large number of medicinal plants which are required by Pharmaceutical industry. Hence work on their cultivation, developing economical methods for extraction of active constituent, preparation of active constituents enriched extracts and standardization of the harvesting schedule is required so that plants can be harvested at the proper time of plant growth without causing serious loss to the active constituents.

Herbs containing bitter principles hold a place of pride in the indigenous system of medicines since ages and also form a valuable household remedy for treatment of variety of diseases. As potent pharmaceutical drugs they are much prized in India as well as in other countries in the form of folk medicines as bitter tonics, blood purifiers, carminative, digestive, expectorant, febrifuge, laxative and anthelmintic. Bitter principle containing herbs are also used for curing skin disease (leucoderma), bronchitis and urinary diseases.

Andrographis paniculata (commonly known as Kalmegh) is one of the important medicinal plant belonging to Acanthaceae family. It is an erect herb reaching a height of about three feet and is found throughout India and Ceylon. The plant as a whole is used in medicines for general bronchitis, influenza, debility, dysentery and certain forms of dyspepsia. A fresh juice of the leaves after mixing with cardamom, clove, cinnamon, etc. is dried and made into pills for use as household remedy for the minor digestive ailments of children (Anonymous, 1948). *paniculata* is also used against snake bite by local traditional medicine practitioners of Tamil Nadu (Gupta and Srivastava, 1994). The plant enjoys

considerable reputation as a febrifuge, alternative and bitter tonic and is often used as a substitute of Chirata (*Swertia chirayata*) belonging to Gentianaceae family.

A. paniculata is reported to contain andrographide (a bitter principle) as the main active constituent (Anonymous, 1948). In addition to this the plant also contains a number of flavonoids.

The growth is the result of wonderful coordination of biophysical, biochemical and organismal processes at different levels. Development implies a whole sequence of qualitative structural changes that a plant undergoes from the zygote stage to its death. Plant growth occurs by cell enlargement. Thus, growth is invariably accompanied by differentiation. All individual cells in a plant assume different shapes and perform different functions depending upon the site, where they are located in the mature plant. The end product of different reactions/functions is different and their concentration in different parts is also variable. These end products are generally secondary metabolites. In order to make efficient use of these constituents it becomes necessary to identify the best harvesting stage of the plant organ or the plant as a whole. Keeping in view, the importance of this plant, the present study was carried out with the following objectives:

- i) To standardize the extraction method for extraction of bitter principle.
- ii) To study the variation of bitter principles in the different plant parts with growth and development.



Plate 1. *Andrographis paniculata* Nees.



Plate 2. Parts of *Andrographis paniculata* Nees.
1. Fruits 2. Leaves
3. Stems 4. Root

2.1 BITTER PRINCIPLES FROM ANDROGRAPHIS AND OTHER PLANT SPECIES

Among the various taste stimuli, substances with bitter taste seem to be the most abundant. Though the bitter taste is least preferred but substances with bitter taste are widely used in curing various types of diseases. Recent findings have revealed that bitter substances produce their response of bitter taste by binding to the lipid cell membranes (Balasubramanian, 1993). The diverse nature of chemical structures of bitter component poses difficulty to point out any common structure or pattern that could be associated with bitterness causing principles.

There are many bitter compounds present in various plant sources e.g. Nimbin, Nimbidin, Nimbinin and Azadirachtin from *Azadirachta indica* belonging to family Meliaceae (Siddiqui, 1942); Sweroside, Swertiamarine, Gentropicroside from *Centaurium spicatum* (Vander Sluis and Labadie, 1981), Amarogentin from *Swertia chirayita* (Korte, 1955) belonging to family Gentianaceae; Picroside (I and II) and Kutkoside from *Picrorhiza kurroa* belonging to family Scrophulareaceae (Kaul and Kaul, 1996). *Andrographis paniculata* possesses bitter tonic and stomachic properties like Chirata and Gentian. It has been employed with benefit in case of general debility, after fever and in the advanced stage of dysentery. Bitter principle andrographolide is extracted from the whole herb of *A. paniculata* belonging to family Acanthaceae (Aminuddin *et al.*, 1997).

2.2 ANALYTICAL METHODS FOR ANDROGRAPHOLIDE AND ISOLATION OF DIFERENT COMPOUNDS

Various methods have been suggested for the quantitative estimation of andrographolide in *A. paniculata* (Kalmegh), but each method has its own merits and demerits. The gravimetric method reported in Indian Pharmacopoeia (1955) involves soxhlet extraction of the powdered drug with chloroform followed by evaporation of chloroform, washing the green crude mass with benzene and extracting the resultant mass with ethylacetate till residue is free from bitterness.

Residue obtained after evaporating ethylacetate is weighed as andrographolide. However, this method has been found to give higher results due to some yellow colouring substance other than andrographolide which is also soluble in ethylacetate (Gaind *et al.*, 1963). The colourimetric method proposed by Maiti *et al.* (1959) suffers from the disadvantage that the red colour formed with the addition of alcoholic potassium hydroxide to the solution of andrographolide is unstable and fades away quickly.

Srivastava *et al.* (1959) modified the laborious Indian Pharmacopoeia Method (1955). In the modified method, coarsely powdered drug (100 g) was extracted with alcohol (95%) for six hours in a soxhlet apparatus. The concentrated extract was mixed with water and the resultant solution extracted with benzene to remove non-bitter substances and colours. The aqueous layer, after dilution with distilled water, was extracted by ethyl acetate. Combined ethyl acetate extracts were then dried by passing through anhydrous sodium sulphate. The filtrate was evaporated and residue weighed as andrographolide. The analysis of four samples of Kalmegh by this method gave 2.46, 2.36, 2.85 and 2.10 per cent of andrographolide.

Chemical method involving lactone titration (Subba Rao, 1962) has been found to be unsuitable for detecting quantities less than 100 mg of andrographolide and, also, because of associated impurities. Talukdar *et al.* (1968) improved lactone titrimetric method for the assay of andrographolide by involving background correction due to the presence of impurities in the crude andrographolide.

Gaind *et al.* (1963) developed spectrophotometric method for estimation of andrographolide in Kalmegh which is suitable for detecting microquantities of andrographolide. The method involves removing green colour in Kalmegh with benzene and then mixing the green colour free herb with Kieselguhr. The Andrographolide from resultant combined mass is then extracted with chloroform through soxhlet extraction. Chloroform is then completely removed and resultant mass is dissolved in methanol and absorbance recorded at 226 m μ . The

andrographolide content for three samples were found to be 1.45, 1.47 and 1.45 per cent, but according to official method the values were 2.66, 2.71 and 2.66 per cent, respectively.

Xianglin *et al.* (1981) used Ion pair HPLC method for the separation and determination of various water soluble andrographolide derivatives (andrographolide sodium bisulphite additive, andrographolide sodium sulphonate, andrographolide triacetyl sodium sulphonate and their derivatives) from *A. paniculata*.

Chen *et al.* (1986) used TLC densitometric method for the estimation of andrographolide, deoxyandrographolide and neoandrographolide from *A. paniculata* with recoveries of 96.86-100, 95.68-99.44 and 94.97-97.00 per cent, respectively.

Sharma *et al.* (1992) have used HPLC for standardization of the drug Kalmegh through estimation of andrographolide.

Burgos *et al.* (1999) analyzed andrographolide in the serum of rats by HPLC using a RP-C18 lichrospher reversed phase column and mobile phase consisting of 26 per cent acetonitrile and 0.5 per cent phosphoric acid at a flow rate of 1.1 ml/min. at 228 m μ . The percentage recovery of andrographolide ranged between 70-80 per cent and the absolute detection limit was 6.4 ng.

Chauhan *et al.* (1999) estimated andrographolide in *A. paniculata* through HPTLC. The sensitivity was found to be 0.10 μ g and linearity was observed in the range of 0.1 to 1.0 μ g. The andrographolide content of 1.77 per cent was found in the test sample. The method being precise and sensitive has been suggested to be used for detection, monitoring and quantification of andrographolide in *A. paniculata*.

Talukdar and Banerjee (1968) carried out kinetic studies on the stability of andrographolide at different temperatures and different pH values. The rate of hydrolysis was found extremely slow below pH-7.0, but it considerably became

faster on alkaline side depending entirely on the pH of the medium. On the basis of calculated values of thermodynamic parameters ΔG^* , ΔH^* and ΔS^* it was concluded that around pH 10.0 andrographolide undergoes some additional structural change (possibly double bond migration) apart from simple fission of the lactone ring. This was because of extremely small negative value for ΔS^* which is more sensitive than ΔG^* for structural change that may occur at the transition state. It was suggested that there is hardly any danger of losing potentially active ingredient in a simple aqueous alcoholic preparation. The results showed that though rate constant increased with increase in temperature at every pH but the effect was not very pronounced.

Fujita *et al.* (1984) determined the crystal structure of andrographolide and also isolated three new diterpenoids of ent-labdane type from *A. paniculata* viz. andrographanin, andropanoside and 14-deoxy-12-methoxy-andrographolide together with three known compounds 14-deoxyandrographolide, neoandrographolide and andrographolide.

Kuroyanagi *et al.* (1987) isolated andrographidine A (flavanone glucoside) along with five new flavone glycosides (andrographidines B, C, D, E and F) from the roots of *A. paniculata*.

Jantan and Waterman (1994) isolated a novel diterpene from the aerial parts of *A. paniculata* which was identified as ent-14- β -hydroxy 8(17), 12-Labdadien-16, 15-oxide-3 β , 19-oxide on the basis of 2D NMR technique.

Weiming and Xiaotian (1982) isolated deoxy-andrographolide, 19 β -D glucoside (a diterpene glucoside) from the leaves of *A. paniculata*.

Hu and Zhou (1982) isolated two new glucosides viz., 14-deoxy-andrographolide-19- β glucoside and andrographolide-19- β glucoside from *A. paniculata*.

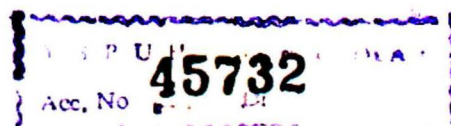
Ojha (1983) isolated carvacrol and eugenol from the benzene extracts of the plant. The crude alcoholic extract of the plant was also shown to possess promising antifungal properties.

Matsuda *et al.* (1994) isolated six new diterpenoids of ent-labdane type (14-epi-andrographolide, isoandrographolide, 14-deoxy-12-methoxy-andrographolide, 12-epi-14 - deoxy - 12 - methoxy-andrographolide, 14-deoxy-12-hydroxy-andrographolide and 14-deoxy-11-hydroxy-andrographolide) from the ethyl acetate soluble fraction of the methanol extract of *A. paniculata* along with two new diterpene glucosides (14-deoxy-11, 12-dihydro-andrographiside and 6'-acetyl- neo-andrographolide) and four new diterpene dimers (bis-andrographolides A, B, C and D).

Abeysekera *et al.* (1990) isolated a new glucoside procumbide from *A. paniculata* along with thirteen earlier known constituents of terpenoid type.

In addition to isolation of terpenoids from *A. paniculata*, some flavonoids with different oxygenation patterns have also been reported, e.g. 5 hydroxy-7,8,2',3'-tetramethoxy flavone (Govindachari *et al.*, 1969); 5-hydroxy-7,8,2'-trimethoxy flavone (Biswas and Chowdhury, 1972; Jalal *et al.*, 1979); 5-hydroxy-7,8-dimethoxyflavanone and 5 hydroxy-3,7,8,2' tetramethoxy flavone along with the known flavanoid 5-hydroxy-7,8-dimethoxy flavone (Gupta *et al.*, 1983).

Flavonoids have also been isolated from other species of *Andrographis*, e.g. echioidinin (5,2'-dihydroxy-7-methoxy flavone) and its glycoside echioidin) from *A. echioides* (Govindachari *et al.*, 1965a); wightin (5,3' dihydroxy-7,8,2'-trimethoxy



flavone) from *A. wightiana* (Govindachari *et al.*, 1965b) and serpyllin (5-hydroxy-7,8,2',3',4'-pentamethoxy flavone) from *A. serpyllifolia* (Govindachari *et al.*, 1968).

2.3 MEDICINAL USES

A. paniculata (Kalmegh), a bitter herb found in plains throughout India, is used as a household remedy for the minor digestive ailments of children (Anonymous, 1948). The plant is also used by traditional medical practitioners as stomachic, anthelmintic, anti-inflammatory, antipyretic, antiperiodic and in intermittent and remittent fevers (Chopra, 1958; Kirtikar and Basu, 1975; Tomer *et al.*, 1982; Balu and Alagesaboopathi, 1993 and Balu *et al.*, 1993). It is also used for treatment of jaundice (Hemadri and Rao, 1984), as antidote for snake bite (Gupta and Srivastava, 1994) and as immunostimulant (Puri *et al.*, 1993). It is a potential herbal drug for fever and liver diseases in Ayurveda and Homoeopathy system of medicine (Aminuddin *et al.*, 1997).

Apigenin, 7,4'-di-O-methyl ether (a flavone) isolated from *A. paniculata* has been found to produce a significant dose dependent antiulcer activity in Shay rats, histamine induced ulcer in guinea pigs and aspirin ulcer in rats (Viswanathan *et al.*, 1981).

Tajuddin and Tariq (1983) observed 65.3 per cent inhibition in carrageenin induced oedema in rat paw as compared to normal rats when an aqueous extract of *A. paniculata* was orally administered (20 mg/100 g). The results showed that this drug possessed a protective anti-inflammatory activity as compared with standard drug.

Shahid (1985) also reported anti-inflammatory effect of the aqueous extract of *A. paniculata* in albino rats. Oral administration of the extract of drug (20 mg/100 g body weight) inhibited 65.3 per cent of induced oedema in albino rats after 3 hours as compared with the control rats. The percentage inhibition of inflammation in the

group treated with phenyl butazone drug in the dose of 100 mg/100 g body weight was 76.5 per cent.

Madav *et al.* (1996) observed significant inhibition of carrageenin, kaolin and nystatin-induced paw oedema when pure andrographolide was orally administered at doses of 30, 100 and 300 mg/kg. Andrographolide also significantly inhibited the weight of granuloma induced by cotton pellet and decreased oedema in adjuvant-induced arthritis. Andrographolide at a dose of 300 mg/kg also inhibited dye leakage in acetic acid induced vascular permeability. It was devoid of ulcerogenic effect on the stomach of rat in acute and chronic tests.

Gupta *et al.* (1990) observed significant antidiarrhoeal activity in alcoholic extract of *A. paniculata* against *Escherichia coli* enterotoxin in animal models. Andrographolide and neoandrographolide were found to be similar in activity to loperamide against *E. coli* LT and LT/ST entero-toxins. Further andrographolide was found to be superior in its effects against ST enterotoxin which is the most common cause of epidemic in neonatal diarrhoea.

The screening of alcohol, hexane, chloroform, butanol and aqueous extract of different parts of 31 indigenous medicinal plants of India for their antisecretory activity against *Escherichia coli* enterotoxin have shown highly significant activity in the extracts of *A. paniculata*, *Cassia fistula*, *Coleus forskohlii*, *Tridax procumbens* and *Papaver somniferum* (Gupta *et al.*, 1993).

Anil Kumar *et al.* (1997) have found Yakrift, a polyherbal product of Dabur Ayurved Ltd., containing *A. paniculata*, *Eclipta alba*, *Picrorhiza kurroa*, *Phyllanthus niruri*, *Tephrosia purpurea*, *Tinospora cordifolia* and *Boerhaavia diffusa* to be useful in improving general condition and appetite of equines working in mines.

Considering the reputed use of *A. paniculata* in Malaysia for treatment of diabetes and hypertension, Zhang and Tan (1997) studied the cardiovascular

activities of crude water extract (WE) of the plant and its three semi-purified fractions viz., ethyl acetate (FA), n-butanol (FB) and aqueous (FC) fractions as well as of pure andrographolide in anaesthetized Sprague-Dawley (SD) rats. Ethyl acetate fraction and andrographolide elicited no drop in mean arterial blood pressure (MAP) while crude water extract, n-butanol and aqueous fractions produced a significant decrease in MAP in a dose dependent manner without significant decrease in heart rate. The ED 50 values for WE, FB and FC were 11.4, 5.0 and 8.6 mg/kg, respectively. FB seems to work via α -adrenoceptors, autonomic ganglion and histaminergic receptors.

Akbarsha *et al.* (1990) have shown the antifertility effect of *A. paniculata*. The results have suggested antispermatogenic and/or antiandrogenic effect of the plant.

Murugaion *et al.*, (1995) have shown the possibility of developing andrographolide as an antifertility compound. Andrographolide after being suspended in gum Acacia was orally administered to male rats at 10 mg/day/dose for 45 days. Reproductive system of treated rats on being subjected to histological analysis showed regression of seminiferous tubules. Also seminiferous epithelium underwent extensive lesions and vacuolation and sperms were limited. Cauda epididymal sperms decreased in count and had several abnormalities.

Rajasekaran and Kumaraswami (1985) reported antifeedant property against 3rd instar larvae of polyphagous pest *Spodoptera litura* in the whole plant extract of *A. paniculata*.

The plant extracts of *Andrographis paniculata*, *Candula officinalis*, *Enhydra fluctuans* and *Solanum khasianum* have been found to reduce root galling of tomato plants and population of *Meloidogyne incognita* (Goswami and Vijayalakshmi, 1986).

Huo and Jinzhi (1989) investigated *in vitro* and *in vivo* antiplatelet aggregation effect of *A. paniculata* on blood samples of eight volunteers. The crude extract of *A. paniculata* was found to significantly inhibit one phase and two phase platelet aggregation. The potency of *A. paniculata* appeared to be stronger than ligustrazine and persantin injection *in vitro*. The rapid effect of this drug on platelet aggregation *in vivo* suggested quick absorption of andrographolide. The study suggested *A. paniculata* to be a promising anti-thrombogenic agent which might be beneficial in preventing and treating arterial thrombotic disease.

Wang and Zhao (1994) found *A. paniculata* to significantly alleviate atherosclerotic iliac artery stenosis induced by both deendothelialization and high cholesterol diet and restenosis following angioplasty in rabbits. Fish oil with more than 70 per cent polyunsaturated fatty acids showed the same but milder effect than *A. paniculata*.

Shukla *et al.* (1992) observed significant dose dependent (1.5-12 mg/kg) choleric effect (4.8-73%) for andrographolide which was evidenced by increase in bile flow, bile salt and bile acids in conscious rats and anesthetized guinea pigs. Andrographolide was found to be more potent than silymarin, a clinically used hepato protective agent. Tripathi and Tripathi (1991) observed strong choleric action of andrographolide. The compound when intraperitoneally administered to albino rats was found to induce an increase in bile flow together with a change in the physical properties of the bile secretion.

Kanniappan *et al.* (1991) found that oral administration of the juice of *A. paniculata*, obtained by homogenizing the green plant without root, normalized induced pyrexia condition in experimental rats. The results have shown that this plant, which is commonly employed in veterinary practice for promoting digestive functions, can also be used as an antipyretic drug in febrile conditions in animals.

Vedavathy and Rao (1991) observed significant antipyretic activity in the ethanolic extracts of *A. paniculata*, *Tinospora cordifolia* and *Solanum surattense* when these extracts were orally administered to rats with yeast induced pyrexia. The results appeared to be comparable to those of the standard agent aspirin (200 mg/kg).

Misra *et al.* (1992) studied the antimalarial activity of *A. paniculata* (Kalmegh) in a four day suppressive test against *Plasmodium berghei* NK65 in *Mastomys natalensis*. A crude ethanol extract and fractions were observed to reduce the level of parasitaemia in a dose dependent manner. Four diterpenes, isolated from the n-butanol fraction also suppressed the parasitaemia level, but not in a dose dependent manner. Chemoprophylactic activity of neoandrographolide was tested using different protocols. Fifteen days of therapy with neoandrographolide before infection suppressed the parasitaemia.

Rahman *et al.* (1999) found considerable antimalarial activity of *Piper sarmentosum*, *A. paniculata* and *Tinospora crispa* both *in vitro* and *in vivo* conditions. Chloroform extract *in vitro* showed better effect than methanol extract. *In vivo* activity of *A. paniculata* also demonstrated higher antimalarial effect than the other two plant species.

Dutta and Sukul (1982) found that water decoction of *A. paniculata* leaves *in vitro* killed the microfilaria of *Dipetalonema reconditum* in 40 minutes. Three subcutaneous injections of the extract injected into infested dogs at 0.06 ml/kg body weight reduced the number of microfilaria in blood by more than 85 per cent. No toxic effect of the extract was observed in rabbits. The treated dogs became lethargic initially for a week probably due to mass killing of microfilaria.

Puri *et al.* (1993) observed significant stimulation of antibody and delayed type hypersensitivity response to sheep red blood cells in mice by andrographolide and ethanol extract of *A. paniculata*. The stimulation of both antigen specific and

non-specific immune response was, however, of lower order with andrographolide than with the ethanol extract, suggesting thereby that substances other than andrographolide present in the extract may also be contributing towards immunostimulation.

Extracts of few Indonesian plants (methanolic extract of bark of *Swietenia mahagoni*; water extracts of stem and branches of *Larenthus parasiticus*; fruit of *Helicteres isora*; fruit of *Sindora sumatrana*; leaves of *A. paniculata* and rhizome of *Curcuma aeruginosa*) have shown significant inhibition of human immunodeficiency virus type I (HIV-I) by assays using HIV-I infected MT-4 cells (Otake *et al.*, 1995).

In vitro screening of Indian medicinal plants against human immunodeficiency virus (HIV) on MT cells have shown *Ocimum sanctum* (leaf), *Strychnos nux-vomica* (seed) and *Andrographis alata* (shoot) to be effective against growth of the virus (Ramanathan *et al.* 1999).

A. paniculata is one of the few frequently used Indian medicinal plants in antihepatotoxic herbal formulations (Sankaran, 1984; Sharma *et al.*, 1991; Saraf *et al.*, 1991; Saxena, 1992). Handa and Sharma (1990) suggested andrographolide to be the major active antihepatotoxic principle present in *A. paniculata*. Andrographolide has been found to be slightly more active than the known hepatoprotective drug silymarin (Visen *et al.*, 1991; Saraswat *et al.* (1995). Shahid (1987) has found aqueous extract of *A. paniculata* to be significantly protective in liver damage in experimental male albino rats. A herbal drug containing six plants viz., *Tephrosia purpurea*, *Eclipta alba*, *Phyllanthus niruri*, *Andrographis paniculata*, *Terminalia chebula* and *Ocimum sanctum* has been found to be effective in inhibiting triglyceride accumulation in hepatic cells and in protecting liver against increased hydroxyproline content (Chauhan *et al.*, 1992).

Rana and Avadhoot (1991) found alcoholic extract of the leaves of *A. paniculata* to be effective in preventing carbon tetrachloride induced liver damage. Tefroli, an indigenous plant based preparation consisting of *Tephrosia purpurea*, *Eclipta alba*, *Andrographis paniculata*, *Terminalia chebula* and *Ocimum sanctum* has been suggested, through clinical trials, as safe and non toxic herbal preparation for routine treatment of patients suffering from viral hepatitis (Nigam *et al.*, 1982). Compound preparation of *Andrographis paniculata* (whole plant) and *Phyllanthus emblica* (fruits without seeds) has also been found to be effective in curing viral hepatitis (Ramfi *et al.*, 1992). Visen *et al.* (1992) reported picroliv (from *Picrorhiza kurroa*) and andrographolide (from *A. paniculata*) as effective hepatoprotective constituents. Crude extract of *A. paniculata* has been found to possess inactivation property against Hepatitis-B surface antigen (Jayaram *et al.*, 1989).

2.4 PROPAGATION

Wahi (1981) observed maximum accumulation of energy in *Andrographis paniculata* at 15 per cent relative light intensity through high rates of energy conserving efficiency and net energy conversion rate, followed by 30, 100 and 45 per cent light in the decreasing order. The results have suggested that *A. paniculata* should be grown under deep shade to conserve maximum energy and, thus, providing maximum dry matter.

Kapur (1996) observed significant effect of light on andrographolide content and green biomass showing a positive correlation. Fully exposed plants were found with maximum andrographolide.

Kapur (1998) also reported negative correlation of light intensity with dry matter of leaves and stems. However, reverse was found for inflorescence and root weight ratios.

Roy and Datta (1988a) observed variation in andrographolide content in the eight different cytotypes of *A. paniculata* collected from India and Bangladesh with the maximum contents in the cytotype obtained from Jessore (Bangladesh).

Gupta and Srivastava (1995) evaluated ten wild growing population of *A. paniculata* with a view to identify a suitable genotype for introduction into agriculture. The results indicated fresh weight/dry weight as the main yield attributing characters which is directly correlated to biological yield. High biological yield was recorded in IC-111287 at vegetative stage. Maximum andrographolide was found in IC-111288. Late harvested nursery raised plants had higher percentage of the andrographolide.

Kar and Choudhary (1994) have concluded through the evaluation of mineral content of few Ayurvedic herbs that minerals play an important role in the medicinal and nutritional properties of the herbs.

Alagesaboopathi and Balu (1995a) have observed difference in the nutrient uptake ability of the different *Andrographis* species. The leaf samples showed highest concentration of Mg (3.6-4.2%) in *A. alata*, of K (2.21%), P and N (2.55-3.17%) in *A. paniculata* and Mn (260 ppm) and Fe (1370-1570 ppm) in *A. lineata*.

Some of the *Andrographis* sp. like *A. alata* (Alagesaboopathi and Balu, 1995b), *A. lineata* (Balu and Alagesaboopathi, 1995) and *A. paniculata* (Balu and Alagesaboopathi, 1996) have been successfully attempted for mass multiplication through stem cutting with establishment of 81 per cent, 67 per cent and 75 per cent, respectively.

Alagesaboopathi and Balu (1996a) observed that though *Andrographis lineata* grows well at an altitude of about 1500 m but pot and field studies indicated that it could also be grown in the plains. Cultivation showed the plant to be perennial but the top dries after flowering leaving only a small part of stem green.

In case of *A. paniculata* average percentage of seed germination has been observed to be 80 per cent with plumule emergence after eight days (Alagesaboopathi and Balu, 1996b).

Chen and Jiang (1980) observed difference in the anatomical features of *A. paniculata* in different stages of the plant development.

Banerjee and Datta (1991) have shown effect of age, phase, season and hormones on the accumulation of medicinal component in case of *Catharanthus roseus*, *A. paniculata*, *Rauvolfia*, *Azadirachta* and *Eravalamia* in order to get standard drug in maximum quantity.

Tissue culture experiments have shown that andrographolide (found in high concentration in intact plants) is not synthesized in the tissue cultures of *A. paniculata* but relatively large quantities of three sesquiterpene lactones (paniculides A, B and C) are formed in the medium (Allison *et al.*, 1968; Butcher and Connolly, 1971).

Use of different synthetic auxins and replacing coconut milk with kinetin and casein hydrolysate for modifying media have been found to be ineffective in the production of paniculides by hypocotyl cultures. However, significant quantities of paniculides were synthesized by established root isolate cultured for nine months on simple synthetic medium without auxin, meso-inositol or coconut milk (Butcher and Connolly, 1971).

Roy and Datta (1988b) selected a single plant of *A. paniculata* from a total of 50 with about 19-fold increase in andrographolide content. Tissue culturing in MS-Medium supplemented with kinetin produced good amount of callus. Whereas, for production of complete plantlets, the addition of IBA was found essential.

2.5 VARIATION IN CONTENTS OF ACTIVE PRINCIPLES WITH PLANT GROWTH AND DEVELOPMENT

In case of *Andrographis paniculata* and other species of this genus, no specific studies have been carried out on the variation of active constituents with plant growth and development. However, relevant studies carried out on the variation of active constituents in some other medicinally important plants are reviewed as under:

Solanum khasianum Clarke (Syn. *S. viarum* Dunal.) is one of the commercial source for solasodine (Steroid alkaloid) whose berries form the economical part. Systematic studies (Saini *et al.*, 1965; Chaudhury and Hazarika, 1966; Chandra *et al.*, 1970; Khanna and Murthy, 1972; Telek, 1977; Sharma *et al.*, 1979; Gupta and Basu, 1981) carried out to find out the appropriate berry development stage with maximum solasodine contents have shown progressive accumulation of solasodine, in the form of its glycosides, with berry development till the contents become maximum in yellow coloured berries. Varghese *et al.* (1979), in the same plant, found maximum solasodine content in the walls of young berries which fastly declined in mature ones, however, exactly a reverse trend was observed in case of fruit pulp in which solasodine content after increasing with age of berries to its maximum in pale yellow ones, rapidly decreased in over matured golden yellow coloured berries.

Solanum laciniatum is another commercial crop for solasodine extraction whose all the aerial parts contain the glycoalkaloids of solasodine but its leaves and berries are more important due to high contents of solasodine. However, leaves in particular have been considered to be more economical because of its more contribution towards herbage yield and also due to almost stable solasodine contents throughout the development cycle of leaves. Fully expanded leaves have been found to possess highest solasodine contents with decrease in the latter leaf development

period (Lancaster *et al.*, 1977; Chand *et al.*, 1991). Lancaster and Mann (1975) found maximum solasodine content in the immature berries. Chand *et al.* (1991) observed solasodine content to decrease from 4.24 per cent in dark green coloured berries to 0.76 per cent in over ripe ones.

Valepotriates (also called as irridoids), the active constituents of *Valeriana* species used as sedatives, have been reported to vary with plant growth and development. Chapelle (1972) observed maximum contents of valepotriates in *Valeriana procurrens* towards the end of May which then dropped off rapidly. Sobotkma (1972) and Violin *et al.* (1983a, 1983b) found dependence of valtrate on the year of harvesting. Hölzl and Jurcic (1975) observed maximum concentration (more than 1%) of total valepotriates in the leaves of *Valeriana jatamansi* in the months of May and June of the first year of plant growth with decrease in the subsequent months. In the second year of plant growth, the valepotriates were found to decrease to as low as 0.2 per cent.

Many plant species belonging to *Gentiana* and *Swertia* genera of Gentianaceae family, containing bitter constituents, are oftenly used in formulations based on indigenous systems of medicine. The bitter and other active constituents in these species have been found to undergo changes in their accumulation with plant growth and development. Evaluation of bitter constituents from the roots of one and two year old crops of *Gentiana lutea* has shown amarogentin as the principle constituent with its contents being higher in two year old crop than in one year old one (Bricot, 1974).

The contents of sennosides in *Cassia angustifolia* were found to be maximum when the bulk of leaves started appearing mature which was judged by the leaves becoming fully grown, thick and bluish in colour (Aiyer and Yegnanarayan, 1958).

Verzar-Petri (1966), examining different *Datura* species at different growth stages, observed not only variation in their alkaloid contents in the different organs at a particular stage but contents also varied significantly with growth of the plant. During complete growth cycle two or three maxima were observed, one during or immediately before flowering, another when fruits were starting to mature, with a possible third either at the beginning or end of growth.

Ilieva *et al.* (1967) observed increase in santonin content in *Artemisia maritima* from flower bud formation stage to flowering stage with decrease in the latter plant growth phase.

Jusupov *et al.* (1967) reported maximum colchicine content in *Colchicum luteum* during flowering and beginning of fruit ripening. With seed ripening, alkaloids were found accumulated mainly in the seeds.

Bradu and Atal (1970) found contents of xanthotoxin (a furo-coumarin) in *Ammi majus* to vary with plant maturity, being 0.4 per cent in mature brown fruits, 0.72 per cent in mature but green fruits and 1.0 per cent in immature green fruits.

Shah *et al.* (1976) noticed variation of diosgenin content with the fruit development in *Balanites roxburghii*. Diosgenin contents were found low at flowering stage, which steadily increased as fruits matured and reached maximum in fully mature (ripe) fruits.

In *Costus speciosus* rhizomes, maximum diosgenin contents have been reported (Sarin *et al.*, 1982) during flowering time which sharply declined in full bloom when fruit formation started taking place in the lower parts of the floral heads. The contents continued to decline up to full maturity of fruits which afterwards started rising till dormancy of rhizomes.

Ajit and Atal (1982) have suggested harvesting of *Fagopyrum* (Buck wheat) crop during beginning of blooming phase (35-45 days after sowing) to get maximum rutin content. Delay in harvesting beyond this period was noted with decline in rutin content from 5.14 to 3.83 per cent.

Bruhn and Nyman (1982) observed rapid increase in contents of thebain (an alkaloid) in *Papaver bracteatum* from 8 to 15 per cent in fresh latex during the first two weeks after flowering and then to 18 per cent after four weeks of flowering.



Plate 3. Aerial part of *Andrographis paniculata* Nees



Plate 4. *Andrographis paniculata* Nees. growing in field

MATERIALS AND METHODS

The present investigations entitled, "Studies on bitter principles from *Andrographis paniculata* Nees." were carried out in the experimental field of the Department of Forest Products, College of Forestry, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP) during 1999-2000. The analytical work was carried out in the laboratory of the Department of Forest Products. The material and methods used during the course of investigations have been detailed under the following heads:

- 3.1 Extraction and estimation of bitter principle.
- 3.2 Studies on variation in bitter principle in different plant parts (leaves, stems and roots) at different growth and development stages of the plant.
- 3.3 Studies on the nature of solvent and extraction time on extraction of the bitter principle.
- 3.4 Statistical analysis.

3.1 EXTRACTION AND ESTIMATION OF BITTER PRINCIPLE

3.1.1 Extraction, separation and purification of the bitter principle

Solvents: Benzene, chloroform, ethyl acetate, methanol and ethanol (95%)

Adsorbents: Silica gel G and Silica gel GF₂₅₄ were used for thin layer chromatography (TLC). Silica gel (60-120 mesh) was used for column chromatography.

Solvent systems for thin layer chromatography (TLC)

- i) Mixture of Benzene and methanol (88:12)
- ii) Mixture of chloroform and methanol (92:8)

Solvent systems for column chromatography

Chloroform and chloroform: methanol (98:2 to 95:5)

All the above solvents and chemicals were of Merck grade.

TLC Developing agents

1. TLC plates prepared in silica gel G were visualized with iodine.
2. TLC plates prepared in silica gel GF₂₅₄ were visualized under UV-light.

In order to separate the bitter principle from *A. paniculata*, shade dried and finely powdered plant material (250 g) was refluxed (3 hrs) on a boiling water bath with ethanol (1 l). The contents were then filtered and residue was further extracted by refluxion (2x3 hrs.) with ethanol. All the filtrates were combined and solvent was distilled off under vacuum. In order to remove colours, the residue was refluxed (1 hr) with benzene (250 ml) and the contents were then cooled and filtered. The benzene soluble part after evaporation was found to be non-bitter. The benzene insoluble part containing bitter compound was mixed with alcohol (20 ml) and then water (100 ml) was added to it. The contents were shaken and filtered through vacuum pump and the filtrate was then completely freed from alcohol by warming on a water bath. The filtrate was then extracted with ethyl acetate (5 x 50 ml) in a separating funnel. All the ethyl acetate portions were combined and solvent was distilled off completely to give light green coloured residue (7.5 g). Solution of the

residue was run on TLC plates (Silica gel G; Benzene : Methanol ::88:12). The different zones of the developed plate were tasted which showed only one bitter zone. A parallel TLC plate developed in the same solvent system and afterwards kept in iodine jar also showed only one spot ($R_f=0.54$) corresponding to bitter zone. The bitter compound was separated through column chromatography (Silica gel, 60-120 mesh) using pure chloroform and chloroform : methanol (98:2 to 95:5) as the solvent systems for elution. All the fractions were run on silica gel GF₂₅₄ coated TLC plates. The fractions showing bitter compound ($R_f=0.54$), showing quenching of fluorescence in UV light, were all combined and then solvent was evaporated. The residue was crystallized as white crystalline compound with methanol to give the bitter compound andrographolide (m.p. 228°C). Its identity was further confirmed by m.m.p. and co-TLC using reference sample of andrographolide. The UV spectrum of the compound in methanol recorded on (Shimadzu-1601 model) UV-Visible Spectrophotometer showed an absorption peak at 224 nm corresponding to andrographolide.

3.1.2 Preparation of standard curve

Solution of andrographolide was prepared by dissolving 10 mg of andrographolide in 100 ml methanol. 10 ml of the above solution was then diluted to 50 ml with methanol. Then volumes of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 ml of the above standard solution were pipetted in different test tubes and diluted with methanol to make the final volume of each to 10.0 ml. Finally absorbance of each was recorded on UV visible-spectrophotometer at 224 nm. A graph was then constructed between concentration of andrographolide and absorbance values.

3.2 STUDIES ON VARIATION IN BITTER PRINCIPLE IN DIFFERENT PLANT PARTS (leaves, stems and roots) AT DIFFERENT GROWTH AND DEVELOPMENT STAGES OF THE PLANT

3.2.1 Material

The plant material for studying the variation of bitter principles in different plant parts at different growth and development stages of the plant was collected from the experimental area of the Department of Forest Products, College of Forestry, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP).

3.2.2 Methodology of the experiment

Seeds of *A. paniculata* were sown in the nursery on 15th June, 1999. Seedlings of uniform size from the nursery were then transplanted on 1st July, 1999 in the plots of 1.2 x 1.5 m size keeping the distance between adjacent plants in a row and between adjacent rows as 15 cm. Fertilizer and farm yard manure were used only at the time of preparing the field plots. Irrigation and weeding operations were regularly carried out. For each replications ten plants were collected at an interval of 15 days starting from one month after transplanting. For studying the variation of bitter contents in the different plant parts at their different growth and development stages, the experiment was carried out under Randomized Block Design (RBD). After collecting plants from the field at each growth and development stages, the roots were washed with tap water to remove soil particles. Then leaves, stems and roots of the plants were separated and allowed to dry under shade at room temperature for three days and then further dried in oven at 50°C till constancy of dry weights was observed. After complete drying the samples were crushed and finely powdered for extraction and estimation of andrographolide.

3.2.3 Estimation of bitter principle (Andrographolide)

Oven dried and finely powdered material of each plant part (0.5 g for each of first stage of stem; first and second stage of roots and 1.0 g for all other stages of leaves, stems and roots) of different growth and development stages of the plant was refluxed (5 hrs) with methanol (25 ml) on a boiling water bath. The contents were then filtered under vacuum and residue was thoroughly washed with hot methanol till it was completely free from bitterness. The washings were combined with the main filtrate and then solvent was distilled off and the residue dried under vacuum. The dried residue was refluxed with benzene (20 ml) on a water bath to remove colours. The contents were then cooled and filtered. The benzene insoluble residue was dried under vacuum, dissolved in methanol and total volume made to 10 ml with methanol. The bitter principle was then separated from the above solution through preparative TLC plates of sizes 10x20 cm (Silica gel GF₂₅₄; Chloroform : Methanol :: 92:8). In each case 0.2 ml of the extract was applied 2 cm above the base of the TLC plates as narrow streaks with the help of fine nozzled pipette. Alongside the extract, reference compound of andrographolide was also applied on the TLC plates. When the solvent system moved to about 15 cm, the plates were taken out and allowed to dry in open. The dried plates were then viewed under UV light. The zones containing andrographolide (indicated by quenching of fluorescence) were carefully scraped off with a blade and taken in methanol (25 ml). The contents were thoroughly shaken and then filtered under vacuum after 20 minutes. The residues in the funnel were repeatedly washed with methanol till free from bitterness. In each case methanol was distilled off from the filtrate and the residue made to 25.0 ml with methanol. In case of leaves, 5 ml of the above solution was further diluted to 10 ml with methanol for recording absorbance whereas in case of stems and roots absorbance was directly recorded without further dilution.

Absorbance of these solutions were recorded on UV visible spectrophotometer at 224 nm and then concentrations of andrographolide were

calculated for each sample from the standard curve. Finally andrographolide content was calculated by the below given formula:

$$\text{Andrographolide content (\%)} = \frac{\text{Wt. of andrographolide (mg)}}{\text{Wt. of dried powder plant material (mg)}} \times 100$$

3.2.4 Treatments

1. Plant parts = 3 (leaves, stems and roots)
2. Growth and development stages = 10

Each plant part was collected at 15 days interval after one month of transplanting

Dates of collection of samples from the field

- | | |
|-------------------|-------------------|
| 1. 1st August | 2. 16th August |
| 3. 31st August | 4. 15th September |
| 5. 30th September | 6. 15th October |
| 7. 30th October | 8. 14th November |
| 9. 29th November | 10. 14th December |

Treatment combinations = 30

Replications = 3

Observation recorded

The following observations were recorded to find out variation in the contents of bitter principle in different plant parts at different growth and development stages of each plant part.

i) Fresh weight (g) of leaves, stems and roots

Fresh weights (g) of each sample of leaves, stems and roots were recorded separately just after collecting the plants from the field at each growth and development stage of the plants.

ii) Dry weight (g) of leaves, stems and roots

Dry weights (g) of each sample of leaves, stems and roots were recorded separately after drying the sample initially at room temperature under shade (3 days) and then in the oven (50°C, 24 hour).

iii) Bitter principle content (%) of leaves, stems and roots

Bitter principle contents (%) in each sample of leaves, stems and roots were recorded separately in terms of andrographolide content which was estimated by TLC spectrophotometric method as described earlier.

3.3 STUDIES ON THE NATURE OF SOLVENT AND EXTRACTION TIME ON EXTRACTION OF THE BITTER PRINCIPLE**3.3.1 Material**

The material for studying the effect of the nature of solvent and extraction time on extraction of the bitter principle was procured from Dr. N.S. Chauhan, Principal Investigator of Central Sector Scheme for the Development of Medicinal and Aromatic Plants, Department of Forest Products, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP). The crop of *A. paniculata* used for this experiment was grown in the experimental field of the Central Sector Scheme.

3.3.2 Methodology of the experiment

The results of the experiment were statistically analysed using CRD (factorial) design with three replication. About 2.0 kg of plant material (whole herb consisting of leaves, stems and roots) was crushed and finely powdered. The powdered material was then passed through a sieve. The coarse material retained on the sieve was again powdered and passed through a sieve. The powdered material thus obtained was then thoroughly mixed to afford a homogenous material. The above material was then further dried in the oven (50°C, 48 hrs). 10.0 g of the above oven dried material was taken for each time period of extraction under each solvent (100 ml) used for extraction. For each time period of extraction, the experiment was replicated three times. All the extractions were carried out through refluxion on a boiling water bath.

3.3.3 Extraction and estimation of the bitter principle

The extracts obtained with each solvent, for every time period of extraction, were freed from the solvent under vacuum on a water bath. The residue thus obtained after being completely dried under vacuum were weighed as extractives for each time period of extraction under each solvent. The residues were then refluxed with benzene (50 ml, 1 hr) and the contents after being cooled were then filtered. The residues were again dried and then dissolved in methanol to make a final volume of 10 ml. 0.1 ml of the above solutions (containing andrographolide) were applied as a fine streak, 2.0 cm above the bottom of the TLC plates (Silica gel GF₂₅₄) using chloroform : methanol (92:8) as a solvent system. When solvent system ascended to about 15.0 cm, the plates were taken out dried in the open and viewed under UV light. The zones containing andrographolide (indicated by quenching of fluorescence) were carefully scrapped off and taken in methanol (25 ml). The contents were thoroughly shaken and then vacuum filtered after 20 minutes. The residues were repeatedly washed with methanol till all bitterness disappeared from the residues. All the washings were combined with the main filtrate. Methanol was

then distilled off from the filtrate and residue was then again dissolved in methanol and total volume made to 25.0 ml with methanol. 2.0 ml of the above solution was diluted to 10 ml with methanol and the absorbance was recorded on UV-visible spectrophotometer at 224 nm. For each solution concentration of andrographolide was calculated from the standard curve.

Treatments

- a) Solvents = 3
 Ethyl alcohol
 Chloroform
 Water
- b) Extraction periods = 5
 One hour
 Two hours
 Three hours
 Four hours
 Five hours
- Treatment combinations = 15
- Replications = 3

Observation recorded

Andrographolide content (%) in each sample was estimated by the method described earlier and then expressed as percentage on dry weight basis.

3.4 STATISTICAL ANALYSIS

The data regarding the studies on variation of bitter principle in different plant parts at different growth and development stages was subjected to statistical analysis under Randomized Block Design.

The data regarding the “Studies on the nature of solvent and extraction time on extraction of the bitter principle” was subjected to statistical analysis under completely randomized design (factorial).

EXPERIMENTAL RESULTS

The results obtained during the present course of investigations on “Studies on bitter principles from *Andrographis paniculata* Nees” are presented in this chapter under the following heads:

- 4.1 Studies on variation in andrographolide content at different growth and developmental stages of *A. paniculata* Nees.
- 4.2 Studies on the effect of type of solvent and extraction time on andrographolide content and extractives

4.1 STUDIES ON VARIATION IN ANDROGRAPHOLIDE CONTENT AT DIFFERENT GROWTH AND DEVELOPMENT STAGES OF *A. paniculata*

In this investigation, the study was carried out under the four experiments as follows:

4.1.1 Variation in andrographolide content and yield with growth and development of leaves of *A. paniculata*

The observations recorded for fresh weight of leaves per plant (g), dry weight of leaves per plant (g), andrographolide content (%) and andrographolide yield per plant leaves (mg) for all the growth and development stages were significant and are presented in Table 1. Maximum fresh weight of leaves per plant was recorded as 27.650 g from 4.5 months old plant, whereas, 1.5 month old plants registered minimum fresh weight (1.389 g). The dry weight of leaves per plant was found maximum as 10.672 g from 6 months old plants which was at par with dry weight (10.642 g) of 5.5 months old plants and minimum (0.261 g) was in 1.5 months old plants.. Andrographolide content was maximum ^(2.84%) in 5.5 months old plants which was

Table 1 Variation in different characters with growth and development of leaves of *Andrographis paniculata* Nees.

Sr. No.	Plant age (Months)	Date of collection (1999)	Phase	Fresh weight/plant leaves (g)	Dry weight/plant leaves (g)	Andrographolide content (%)	Andrographolide yield/ plant leaves (mg)
1.	1.5	1 st August	Vegetative	1.389	0.261	0.56 (0.748)	1.46
2.	2.0	16 th August	Vegetative	3.649	0.790	0.53 (0.730)	4.21
3.	2.5	31 st August	Vegetative	8.607	2.185	0.58 (0.762)	12.68
4.	3.0	15 th September	Flower initiation	13.086	3.667	1.02 (1.008)	37.29
5.	3.5	30 th September	Peak flowering	13.550	3.775	1.31 (1.143)	49.32
6.	4.0	15 th October	Fruit initiation	23.330	8.434	1.40 (1.185)	118.34
7.	4.5	30 th October	Fruiting	27.650	10.088	1.59 (1.261)	160.40
8.	5.0	14 th November	Fruiting	27.070	10.480	1.68 (1.296)	176.08
9.	5.5	29 th November	Fruiting	23.650	10.642	2.04 (1.426)	216.70
10.	6.0	14 th December	Fruit dehiscence	23.040	10.672	1.97 (1.405)	210.60
CD _{0.05}				0.058	0.055	0.036	0.010

Values in parenthesis are transformed values (square root)

also at par with that recorded in 6.0 months old plant (1.97%). Andrographolide content in plants of 4.5 months (1.59%) and 5 months (1.68%) age were though lower, but were at par to each other. Minimum andrographolide content (0.53%) recorded in leaves from 2.0 months old plants was found statistically at par with that recorded in 1.5 months old plants (0.56%) as well as with ~~2.5~~ 3.5 months old plant (0.58%) and the later was also at par with andrographolide content of leaves in 1.5 months old plants. Andrographolide yield in leaves per plant was recorded maximum (216.70 mg) in 5.5 months old plants and minimum (1.46 mg) in 1.5 months old plants.

4.1.2 Variation in andrographolide content and yield with growth and development of stems of *Andrographis paniculata*

Under this experiment the observations were recorded on fresh weight of stems per plant (g), dry weight of stems per plant (g), andrographolide content (%) and andrographolide yield in stems per plant (mg) and are presented in Table 2. The results were significant for all the growth and development stages.

Maximum fresh weight of stems per plant was registered from 5.0 months old plants (53.970 gm) followed by those of 5.5 months old plants (52.070 g). The minimum fresh weight (0.306 g) was found in stems of 1.5 months old plants. Six months old plants recorded maximum dry weight of stems (22.970 g) per plant followed by stems of 5.5 months old plants (22.850 g) and 5.0 months old plants (22.750 g), whereas, minimum dry weight (0.047 g) was found in 1.5 months old plants.

Stems of 6 months old plants recorded maximum andrographolide content (0.65%) which was statistically at par with that recorded in stems of 5.5 months old plants (0.64%). Minimum andrographolide content (0.29%) was recorded in stems of 1.5 months old plants. In stems of 3.5 months and 4.0 months old plants same andrographolide content (0.52%) was recorded which was at par with the value in

Table 2 Variation in different characters with growth and development of stems of *Andrographis paniculata* Nees.

Sr. No.	Plant age (Months)	Date of collection (1999)	Phase	Fresh weight/plant stems (g)	Dry weight/plant stems (g)	Andrographolide content (%)	Andrographolide yield/ plant stems (mg)
1.	1.5	1 st August	Vegetative	0.306	0.047	0.29 (0.538)	0.14
2.	2.0	16 th August	Vegetative	1.244	0.189	0.32 (0.568)	0.61
3.	2.5	31 st August	Vegetative	3.627	0.761	0.43 (0.656)	3.27
4.	3.0	15 th September	Flower initiation	10.796	2.444	0.50 (0.705)	12.14
5.	3.5	30 th September	Peak flowering	10.720	3.865	0.52 (0.721)	20.09
6.	4.0	15 th October	Fruit initiation	40.560	14.844	0.52 (0.723)	77.69
7.	4.5	30 th October	Fruiting	51.620	20.460	0.53 (0.726)	107.80
8.	5.0	14 th November	Fruiting	53.970	22.750	0.54 (0.737)	123.58
9.	5.5	29 th November	Fruiting	52.070	22.850	0.64 (0.800)	146.26
10.	6.0	14 th December	Fruit dehiscence	51.270	22.970	0.65 (0.806)	149.32
CD _{0.05}				0.039	0.031	0.019	0.004

Values in parenthesis are transformed values (square root)

4.5 months old plants (0.53%) and the later was also at par with andrographolide content in 5.0 months old plants (0.54%). Andrographolide yield in stems per plant was found maximum in 6 months old plants (149.32 mg) and minimum was observed in 1.5 month old plants (0.14 mg). Both andrographolide content and andrographolide yield significantly increased with age of the plants.

4.1.3 Variation in andrographolide content and yield with growth and development of roots of *A. paniculata*

The observations recorded on fresh weight of roots per plant (g), dry weight of roots per plant (g), andrographolide content (%) and andrographolide yield in roots per plant (mg) were significant for the plants of different ages and are presented in Table 3.

The maximum values for all the characters studied i.e. fresh weight of roots per plant (3.588 g), dry weight of roots per plant (1.947 g), andrographolide content (0.53%) and andrographolide yield in roots per plant (10.32 mg) were found in 6 months old plants, whereas, minimum values for fresh weight (0.204 g), dry weight (0.054 g), andrographolide content (0.26%) and andrographolide yield (0.14 mg) were observed in 1.5 months old plants i.e. at the first stage of collection of plants from the field. The minimum andrographolide content in roots observed in 1.5 months old plants (0.26%) was at par with that of 2 months (0.27%) and 2.5 months (0.28%) old plants. Andrographolide content which was found same (0.29%) in roots of 3.0 months and 3.5 months old plants were at par with contents in 4.0 months old plants (0.30%) as well as with andrographolide content in roots of 2.0 months (0.27%) and 2.5 months (0.28%) old plants. The values for all the parameters studied significantly increased with age of the plant.

Contribution of leaves, stems and roots for total andrographolide yield per plant is given in Table 4. The maximum andrographolide yield per whole plant was recorded at 371.40 mg in 6 months old plant which was closely followed by 370.20

Table 3 Variation in different characters with growth and development of roots of *Andrographis paniculata* Nees.

Sr. No.	Plant age (Months)	Date of collection	Phase	Fresh weight/plant roots (g)	Dry weight/plant roots (g)	Andrographolide content (%)	Andrographolide yield/ plant roots (mg)
1.	1.5	1 st August	Vegetative	0.204	0.054	0.26 (0.510)	0.14
2.	2.0	16 th August	Vegetative	0.250	0.080	0.27 (0.520)	0.22
3.	2.5	31 st August	Vegetative	0.791	0.272	0.28 (0.529)	0.76
4.	3.0	15 th September	Flower initiation	0.993	0.421	0.29 (0.539)	1.22
5.	3.5	30 th September	Peak flowering	1.451	0.621	0.29 (0.541)	1.82
6.	4.0	15 th October	Fruit initiation	1.728	0.896	0.30 (0.551)	2.72
7.	4.5	30 th October	Fruiting	2.850	1.535	0.35 (0.594)	5.42
8.	5.0	14 th November	Fruiting	3.534	1.909	0.40 (0.632)	7.63
9.	5.5	29 th November	Fruiting	3.568	1.935	0.43 (0.658)	8.39
10.	6.0	14 th December	Fruit dehiscence	3.588	1.947	0.53 (0.728)	10.32
CD _{0.05}				0.011	0.006	0.021	0.0001

Values in parenthesis are transformed values (square root)

Table 4 Variation on mean performance of andrographolide yield in *Andrographis paniculata* Nees.

Sr. No.	Plant age (Months)	Date of collection	Phase	Andrographolide yield/ plant (mg)
1.	1.5	1 st August	Vegetative	1.74
2.	2.0	16 th August	Vegetative	5.04
3.	2.5	31 st August	Vegetative	16.71
4.	3.0	15 th September	Flower initiation	50.65
5.	3.5	30 th September	Peak flowering	71.24
6.	4.0	15 th October	Fruit initiation	198.70
7.	4.5	30 th October	Fruiting	273.60
8.	5.0	14 th November	Fruiting	307.30
9.	5.5	29 th November	Fruiting	370.20
10.	6.0	14 th December	Fruit dehiscence	371.40
C.D. _{0.05}				0.012

Table 4 Variation on mean performance of andrographolide yield in *Andrographis paniculata* Nees.

Sr. No.	Plant age (Months)	Date of collection	Phase	Andrographolide yield/ plant (mg)
1.	1.5	1 st August	Vegetative	1.74
2.	2.0	16 th August	Vegetative	5.04
3.	2.5	31 st August	Vegetative	16.71
4.	3.0	15 th September	Flower initiation	50.65
5.	3.5	30 th September	Peak flowering	71.24
6.	4.0	15 th October	Fruit initiation	198.70
7.	4.5	30 th October	Fruiting	273.60
8.	5.0	14 th November	Fruiting	307.30
9.	5.5	29 th November	Fruiting	370.20
10.	6.0	14 th December	Fruit dehiscence	371.40
C.D. _{0.05}				0.012

mg in 5.5 months old plants, whereas, minimum was found as 1.74 mg in 1.5 months old plants. The andrographolide yield in whole plants significantly increased with age of the plant.

4.2 STUDIES ON THE EFFECT OF TYPE OF SOLVENT AND EXTRACTION TIME ON ANDROGRAPHOLIDE CONTENT AND EXTRACTIVES

The results obtained on the effect of different solvents and extraction times for extraction of andrographolide content and total extractives were found significant and are presented in Tables 5 and 6. It is evident from the results that with each solvent, andrographolide content (%) in the extracts and total extractives (g) increased with increase in extraction duration.

Maximum andrographolide content in the extracts in each solvent was obtained when extraction was allowed for five hours with the values of 1.12 per cent in ethanol, 0.93 per cent in chloroform and 0.35 per cent in water. Extraction of the powdered herb (whole plant) of *A. paniculata* for one hour registered the minimum values of andrographolide contents under each solvent (0.25% in water 0.53 per cent in chloroform and 0.64 per cent in ethanol). Andrographolide contents analyzed in extracts were found statistically at par for ethanol (1 hour, 0.64%) with chloroform (2 hour, 0.63%); ethanol (2 hour, 0.77%) with chloroform (3 hour, 0.74%); ethanol (3 hour, 0.85%) with chloroform (4 hours, 0.83%); with increase in extraction duration of four hours (0.34%) and five hours (0.35%) in case of water as the solvent.

Extraction of the powdered herb (whole plants) of *A. paniculata* with ethanol gave maximum mean value (taken all the five time durations) of andrographolide content (0.89%), whereas, minimum mean value of andrographolide content was found on extraction with water (0.30%). Extraction for five hours duration recorded the highest mean value (taken for all the three solvents) of andrographolide content

Table 5 Effect of type of solvent and extraction time on the andrographolide content (%)

Solvents	Extraction time (T) in hours					Mean
	1	2	3	4	5	
Ethanol	0.64 (0.798)	0.77 (0.876)	0.85 (0.922)	1.07 (1.036)	1.12 (1.057)	0.89 (0.94)
Chloroform	0.53 (0.730)	0.63 (0.794)	0.74 (0.860)	0.83 (0.911)	0.93 (0.964)	0.73 (0.85)
Water	0.25 (0.500)	0.28 (0.526)	0.30 (0.548)	0.34 (0.586)	0.35 (0.594)	0.30 (0.55)
Mean	0.47 (0.68)	0.56 (0.73)	0.63 (0.78)	0.75 (0.84)	0.80 (0.87)	

$$\text{C.D. (solvent)}_{0.05} = 0.00815$$

$$\text{C.D. (time)}_{0.05} = 0.01052$$

$$\text{C.D. (Solvent x time)}_{0.05} = 0.01822$$

Values in parenthesis are transformed values (square root)

Table 6 Effect of type of solvent and extraction time on the total extractives (g)/ 10 g of plant material

Solvents	Extraction time (T) in hours					Mean
	1	2	3	4	5	
Ethanol	0.481	0.535	0.561	0.605	0.675	0.571
Chloroform	0.117	0.133	0.153	0.200	0.217	0.164
Water	1.330	1.843	2.283	2.333	2.367	2.031
Mean	0.643	0.837	0.999	1.045	1.087	

$$\text{C.D. (solvent)}_{0.05} = 0.0391$$

$$\text{C.D. (time)}_{0.05} = 0.0302$$

$$\text{C.D. (Solvent x time)}_{0.05} = 0.0068$$

Values in parenthesis are transformed values (square root)

(0.80%) closely followed by extraction for four hours (0.75%). Extraction for one hour showed minimum extraction of andrographolide (0.47%).

It is evident from Table 6 that with each solvent, total extractives increase with increase in extraction duration. Maximum total extractives per 10 g herb extracted in each solvent were obtained when extraction was allowed for five hours with the value of 2.367 g in water, 0.675 g in ethanol and 0.217 g in chloroform. Extraction with chloroform for one hour registered the minimum value of total extractives (0.117 g). Extraction of whole herb with water recorded maximum mean value (taken for all the time durations) of total extractives (2.031 g), whereas, minimum mean values of total extractives was obtained on extraction with chloroform (0.164 g). Five hour extraction gave maximum mean value (taken for all the three solvents) of total extractives (1.087 g) followed by four hour extraction (1.045 g). Extraction for one hour duration recorded minimum total extractives (0.643 g). Total extractives were at par with ethanol solvent for extraction period of two hours (0.535 g) and three hours (0.561 g). In case of chloroform solvent extractives obtained after extraction period of two hours (0.133 g) was at par with one hour (0.117 g) as well as with three hours (0.153 g) extraction periods. Similarly total extractives were at par when extraction was carried out for four hours (0.200 g) and five hours (0.217 g) in case of chloroform.

DISCUSSION

The results obtained during the present investigation “Studies on bitter principles from *Andrographis paniculata* Nees.” have been discussed in this chapter as under:

5.1 VARIATION IN ANDROGRAHOLIDE CONTENT AND YIELD WITH GROWTH AND DEVELOPMENT OF PLANT

Secondary metabolites with diverse structures and classes are oftenly found in varying concentrations in different plant parts viz., leaves, stems, roots, flowers, fruit and seeds. The concentrations of these compounds in plant tissues are the net result of rates of biosynthesis and catabolism which vary with plant growth, environmental changes and diurnal variations.

These secondary metabolites, though present in small amounts, not only play important role in safeguarding the plants from various types of diseases but some of them have proved to be extremely useful for the well being of mankind as well. In fact many of the pharmaceutical and perfumery industries are based on the utilization of these secondary metabolites either in their natural or chemically modified (semi-synthetic) forms. One can learn a lot from the knowledge of the primitive societies who used medicinal plants for protecting themselves from different diseases. They used to collect the plants at specific times of the season and at a particular time of day or night. The idea behind this was that they had the knowledge from their age old experiences about the appropriate growth stage(s) of the plant when contents of these active constituents attained their maximum limits.

Along with having knowledge of the contents of active principles in different plant parts at different growth and development stages, it also becomes equally essential to know the total quantity (yield) of active constituents in whole of the plant part to ascertain its economic viability. As long as biosynthesis of active principles continues, may be at decreased rates than of dry weight production, the total yield of active principle(s) keeps on increasing. In the light of the above facts, the results obtained in this part of the study have been discussed here.

The present studies show increase in fresh weight of leaves per plant from 1.389 g in 1.5 months old plants to 27.650 g in 4.5 months old plants which afterwards decreases to 23.040 g in 6.0 months old plants. Dry weight of leaves per plant on the other hand continuously builds up from 0.261 g in 1.5 months old plants to 10.672 g in 6.0 months old plants. However, the growth period from 4.5 months to 6.0 months shows only marginal increase in dry weight (10.088 g to 10.672 g). Low and almost stable andrographolide content (0.53% to 0.58%) in the initial phase of plant growth from 1.5 months to 2.5 months show that andrographolide is biosynthesized at a slow and stable rate in comparison to latter plant growth and development phase (Fig. 1). During this initial phase rate of andrographolide formation almost keeps pace with dry weight accumulation. However, fast increase in andrographolide content (becomes more than double) from 0.58 per cent in leaves of 2.5 months old plants to 1.31 per cent in 3.5 months old plants, when dry weight increases only by little more than 1.5 times (2.185 g to 3.775 g) suggests that rate of andrographolide formation exceeds rate of dry weight accumulation. During the growth and development of plants between 3.5 months and 5.0 months, though andrographolide contents still show rising trend but at a slow rate because this period is accompanied by fast growth of the plant (dry weight increases by about 2.75 times from 3.775 g in leaves of 3.5 months old plants to 10.480 g in leaves of 5.0 months old plants). After fifth month when plant shows very small further growth, andrographolide content rises to 2.04 per cent in leaves of plants of 5.5 months age and then afterwards the content marginally decreases to 1.97 per cent when plants become 6.0 months old. The marginal decrease in the andrographolide content along

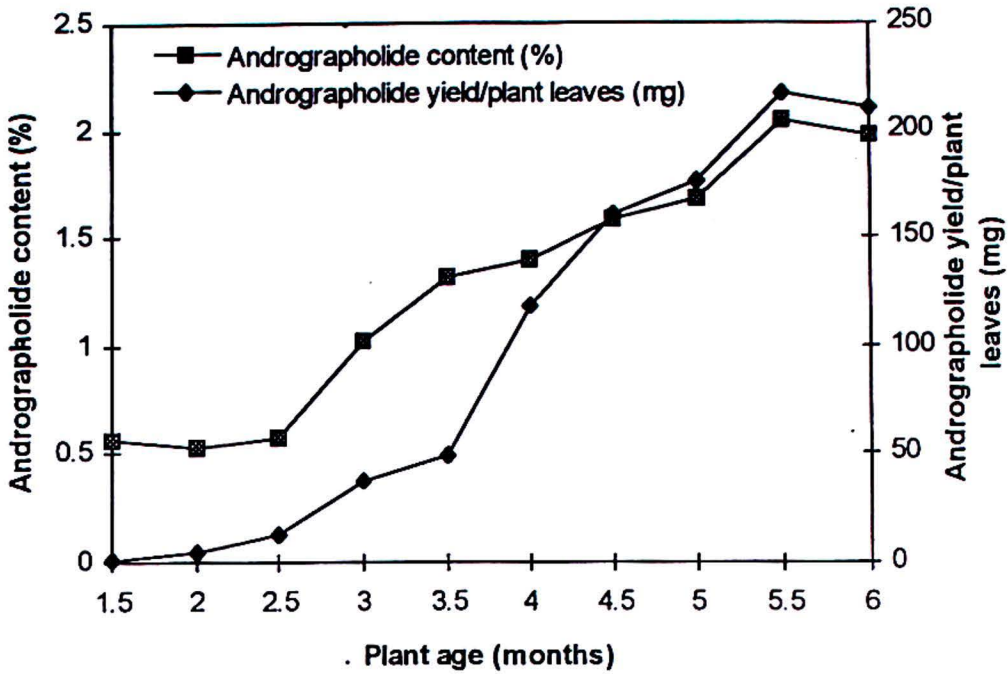


Fig. 1. Variation of andrographolide yield and andrographolide content with growth and development stages of *Andrographis paniculata* leaves

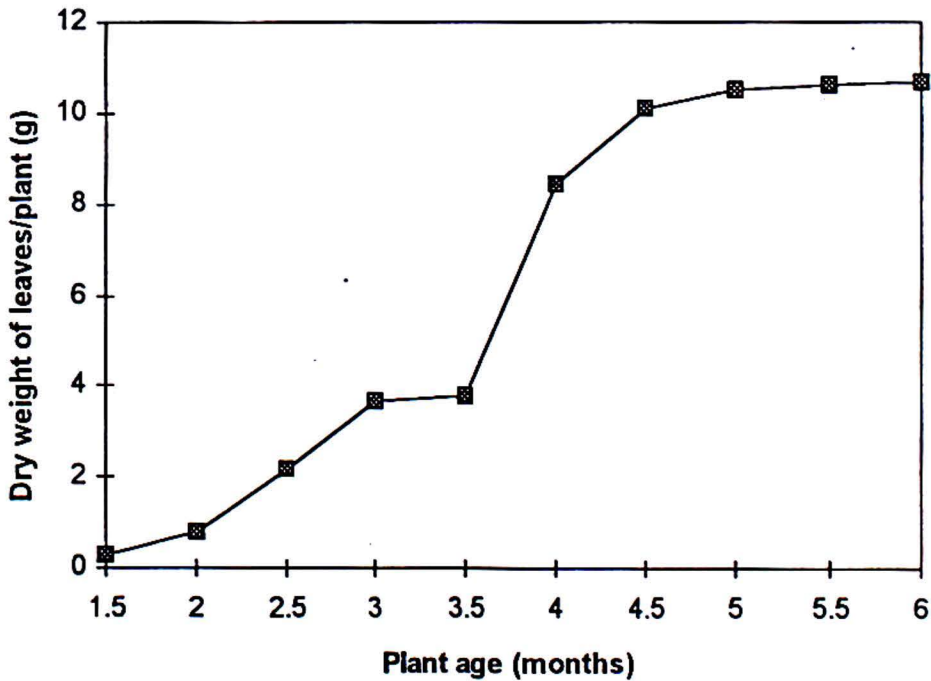


Fig. 2. Variation of dry weight with different growth and developmental stages of *Andrographis paniculata* leaves

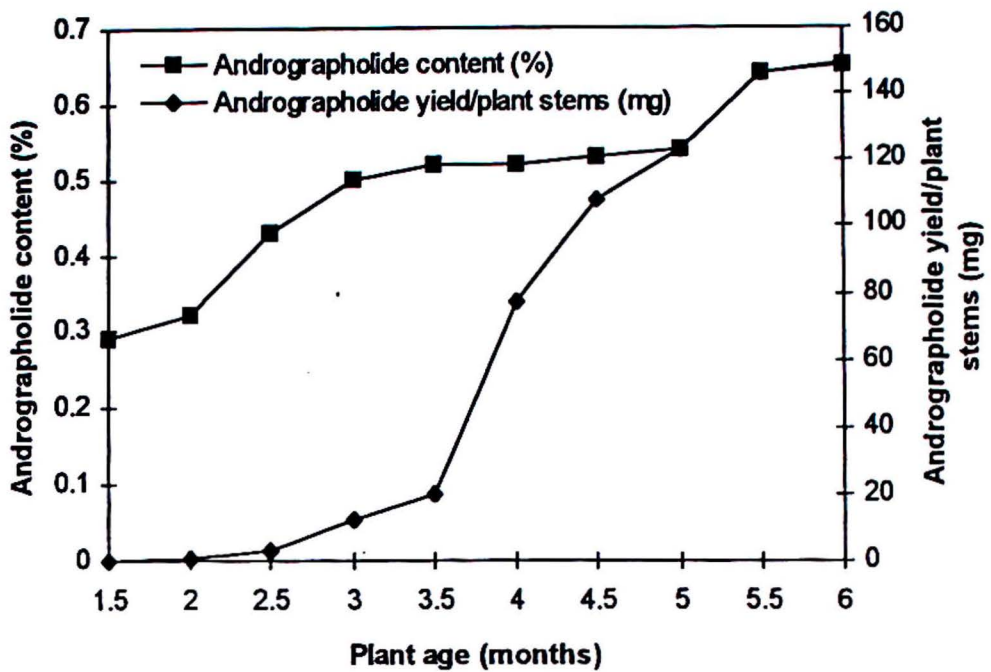


Fig. 3. Variation of andrographolide yield and andrographolide content with growth and development stages of *Andrographis paniculata* stems

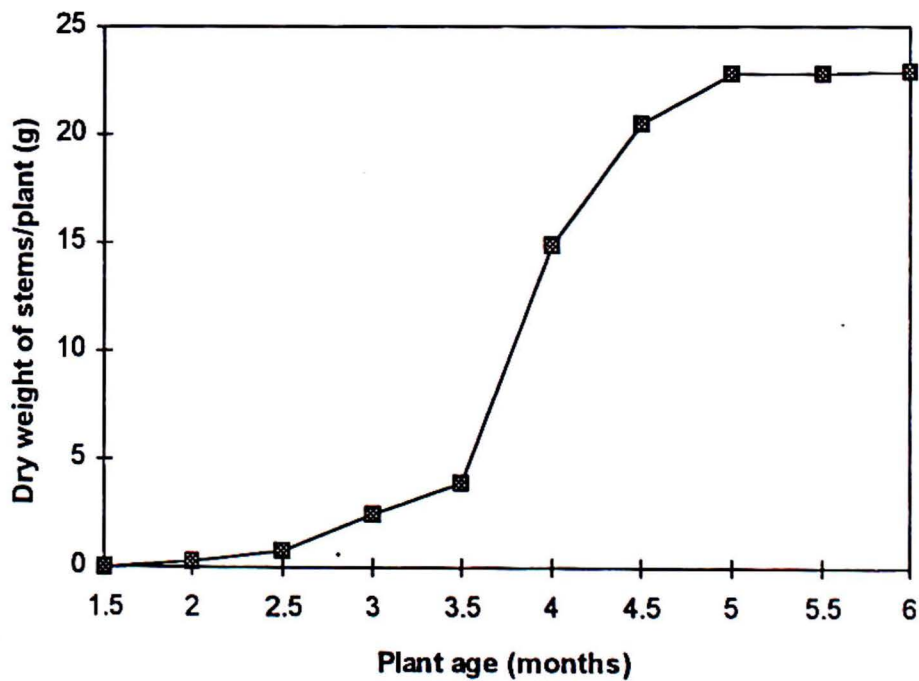


Fig. 4. Variation of dry weight with different growth and developmental stages of *Andrographis paniculata* stems

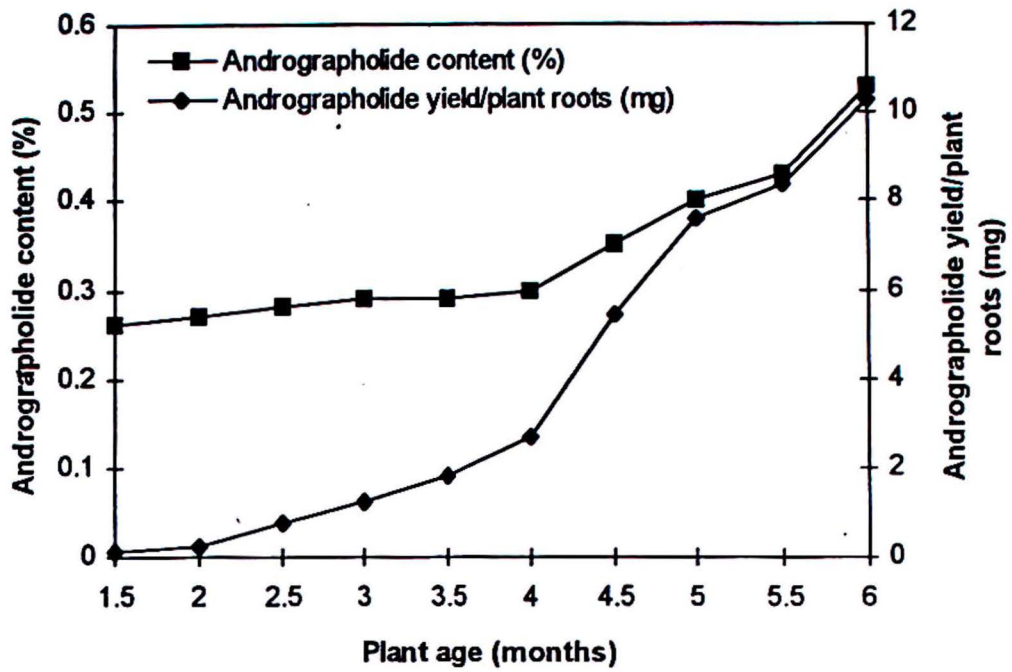


Fig. 5. Variation of andrographolide yield and andrographolide content with growth and development stages of *Andrographis paniculata* roots

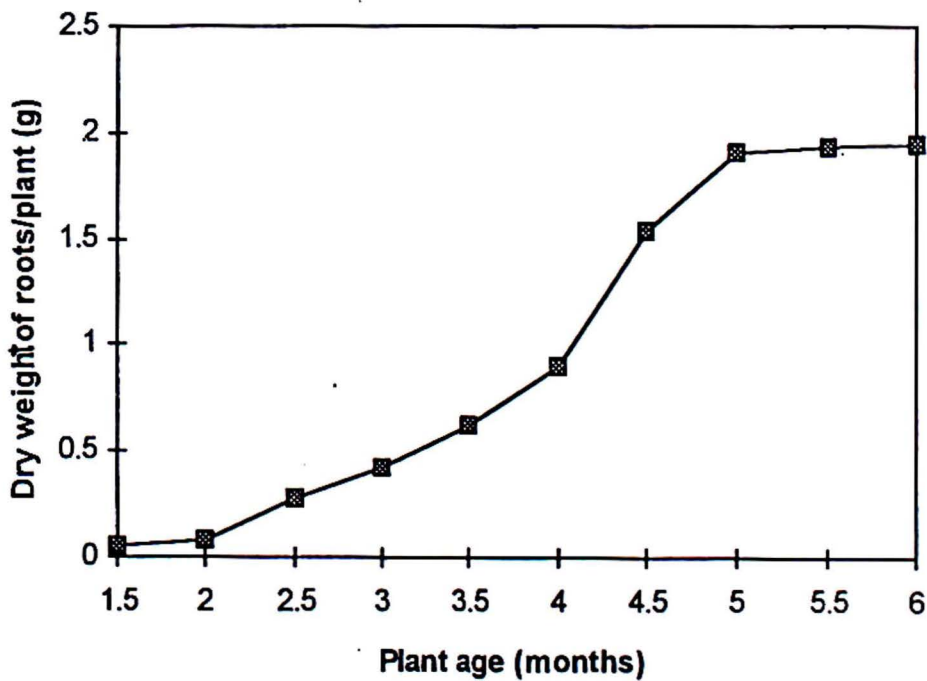


Fig. 6. Variation of dry weight with different growth and developmental stages of *Andrographis paniculata* roots

with small decrease in andrographolide yield (216.70 mg to 210.60 mg) in the last phase may be either due to degradation of andrographolide or its transportation to other plant parts.

As in case of leaves, the maximum fresh weight (53.970 g) of stems in plants of 5.0 months age marginally decreases with further advancement of plant age. Dry weight contribution of stems per plant is little more than twice than that of the leaves particularly in plants which are older than 4.0 months (20.460 g in stems of plants of 4.5 months and 22.970 g in stems of plants of 6.0 months). Stem shows low andrographolide content (in comparison to leaves) throughout the growth period which increases from 0.29 per cent in plants of 1.5 months age to 0.65 per cent in those of 6.0 months age. The andrographolide content in stems increases only marginally from 0.50 per cent in plants of 3.0 months age to 0.54 per cent in 5.0 months old plants, whereas, during the same period dry weight increases by about ten times (2.444 g to 22.750 g). This shows that during this growth period andrographolide is produced only at a slightly higher rate than the rate at which dry weight is accumulated. On the other hand, in young plants with age upto 3.0 months, andrographolide is produced at a faster rate than dry weight is accumulated and so is the case in plants of age group between 5.0 and 5.5 months.

Throughout the growth and development period of the plants of *A. paniculata*, roots show low values of fresh weight, dry weight and andrographolide yield (each per plant) which increase from their minimum values of 0.204 g, 0.054 g and 0.14 mg in 1.5 months old plants to their maximum values of 3.588 g, 1.947 g and 10.32 mg, respectively in 6.0 months old plants. Comparison of data for roots (Table 3) with those of leaves (Table 1) and stems (Table 2) shows that roots contribute not only less dry biomass but also show low andrographolide content throughout the growth period (andrographolide contents range between 0.26% in 1.5 months old plants and 0.53% in 6.0 month old plants). Low dry biomass with low andrographolide content is responsible for low andrographolide yield. In the roots, during initial growth phase up to 4.0 months, andrographolide is

accumulated/biosynthesized at marginally higher rate than increase in root dry weight (andrographolide content in this period increases only marginally from 0.26% to 0.30%). However, beyond 4.5 months, when dry biomass remains almost stable (Fig. 6), andrographolide content still gradually increases which shows that in this phase andrographolide is accumulated at a faster rate and this can be due to migration from other plant parts.

Out of the three plant parts studied for andrographolide content at different time intervals, the highest amount of andrographolide content was observed in leaves followed by stem and roots. There was observed much difference in andrographolide content in leaves, stem and roots (maximum values being 2.04, 0.65 and 0.53% in leaves, stems and roots, respectively). This indicates that the site of synthesis of andrographolide is the leaf and part of the andrographolide gets percolated to stem and root with root accumulating the least. During vegetative phase of the plant non-significant increase in andrographolide content in leaves takes place which however abruptly increases with the onset of flowering phase (1.02% from 0.58%). As the flowering phase continues and the fruit setting takes place, there is slow but gradual increase in the andrographolide content which reaches its peak value of 2.04 per cent at the fruit dehiscent stage. This indicates that there is strong correlation between sexual phase and the synthesis of andrographolide content in *A. paniculata*. No such correlation can be drawn about the andrographolide content in stem and roots since there is gradual increase in andrographolide content starting from vegetative stage to the seed dispersal stage.

As far as total yield of andrographolide per plant was concerned (Table 4), significant increase in andrographolide yield has been observed for all the stages studied. However, an abrupt increase (from 71.24 mg to 198.70 mg) was observed immediately after peak flowering stage. Since photosynthetic rate has to increase to support the metabolic processes associated with reproductive phase, the peak flowering stage is accompanied by vigorous increase in leaf biomass and hence abrupt increase in andrographolide yield.

Little systematic study appears to have been carried out to know the variation of andrographolide in *A. paniculata* with plant growth and development. Chakravarty and Chakravarty (1952) observed considerable seasonal variation in the andrographolide content of the leaves. Gupta and Srivastava (1995) observed higher contents of andrographolide in late harvested nursery raised plants.

The trend in the present studies on the variation of andrographolide content and andrographolide yield in the different plant parts is supported by some of the following studies related to other plants. Evaluation of roots of one and two year old crops of *Gentiana lutea* has shown the contents of amarogentin (bitter compound) to be higher in two year old crop than in one year old crop (Bricot, 1974). Ghosal *et al.* (1978) observed that Xanthone-o-glucosides remained absent in *Swertia angustifolia* till plants were four to six weeks old but the same were found to increase with further plant development. Further the aerial parts were observed with higher contents of these compounds than roots. During analysis of fluid extract of *Gentiana lutea* roots for bitter constituents, free amino acids and carbohydrates at various vegetative stages, the gentiopicroside content was found to vary between 1.09 to 1.59 per cent at various vegetative stages (Sancin *et al.*, 1981). Aiyer and Yegnanarayan (1958) observed maximum contents of sennosides (active constituents) in *Cassia angustifolia* when the bulk of leaves started appearing mature which was judged by the leaves becoming fully grown, thick and bluish in colour.

5.2 EFFECT OF NATURE OF SOLVENT AND EXTRACTION TIME ON EXTRACTION OF ANDROGRAPHOLIDE

Herbs are used either in the form of crude extracts enriched with active principles or in many cases the phyto-pharmaceuticals are isolated in pure forms and then used in different formulations either as such or in their chemically modified forms.

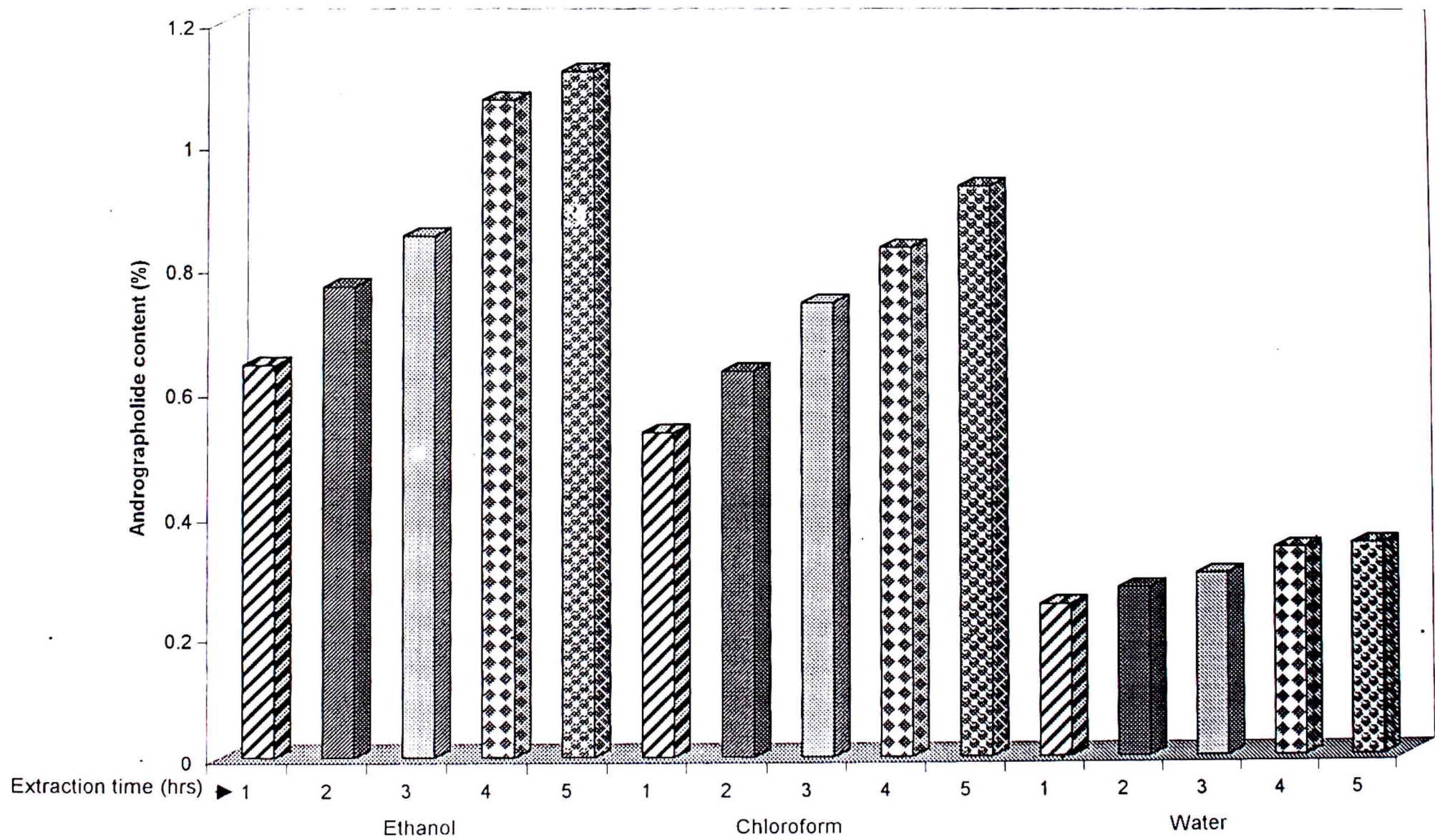


Fig. 7. Effect of type of solvent and extraction time on the andrographolide content

While designing strategy for extraction of active principle(s) one has to make a choice of proper solvent/solvent mixture for extraction and the time given for extraction in order to ensure complete extraction of active principles. For deciding the solvent one must have complete knowledge of the structure(s) of the active principle(s) and the solvent polarities. Less polar solvents are generally suitable for extraction of less polar substances, whereas, highly polar compounds like glycosides, flavonoids or other compounds having polar hydroxy groups are best extracted with polar solvents. During extraction, compounds other than the active principles either should not get extracted or if so then these should be easily removable from the active compounds in the extracts during further processing steps.

Though both extractions allowed for longer time durations as well as repeated extractions using solvents of appropriate polarities, which match the polarities of the active constituents to be extracted, lead to almost complete extraction of active principles but un-necessary wastage of time, labour and chemicals should also be taken into consideration.

High temperatures employed during extractions though lead to quick extraction of active principles, but in some cases extractions are advised to be carried out at low temperatures when compounds containing unstable group(s) are liable to get degraded at high temperatures. In case of light sensitive compounds, the whole extraction and processing steps should be carried out in absence of light.

The data (Table 5) on the present studies show increase in the andrographolide contents in the extracts with increase in extraction time for every solvent (0.64 to 1.12% for ethanol, 0.53 to 0.93% for chloroform and 0.25 to 0.35% for water). It is also clear from the results (Table 5, Fig. 7) that ethanol is most effective solvent for extraction of andrographolide and water is least effective. In chloroform, andrographolide is though extracted to some less extent than in ethanol but for every time period of extraction it is more than two times efficient than water (as the andrographolide contents appear more than two times than in water for every

time period of extraction). The small difference in the andrographolide contents in the extracts obtained after extraction period of four hours (1.07%) and five hours (1.12%) with ethanol solvent than in the previous time periods of extractions suggests that allowing extraction for five hours with ethanol almost completely extracts andrographolide. However, in case of both chloroform and water extraction of andrographolide remains incomplete (particularly in case of water) even after five hours. The results suggest that diffusion of andrographolide from herb particles into the solvent becomes almost complete after five hours of extraction period when ethanol is used as the solvent for extraction, however, this is not so in case of chloroform and particularly in water.

Andrographolide is a bicyclic α , β -unsaturated -lactone, along with having three hydroxyl groups and one additional $>C=C<$ bond. This imparts polar character to the andrographolide molecule, however, still it remains comparatively less polar than the glycosides. This is why andrographolide is more efficiently extracted in ethanol than in water because polarity of ethanol rightly matches the polarity of andrographolide. Results suggest that with chloroform complete extraction can be ensured only after longer period of extraction. In case, if water is to be used for extracting andrographolide then still much longer period should be allowed for extraction.

The data on the total extractives obtained for extraction periods from one to five hours (Table 6) shows maximum extractives in water (1.330 to 2.367 g) and least in chloroform (0.117 g to 0.217 g), whereas, in ethanol total extractives (0.481 to 0.675 g) are found three to four times less than in water. In addition to active compounds, many other types of different compounds having different chemical structures are also found in the plants. While using any solvent for extraction of active constituents, some of these other chemical compounds also get extracted in the solvent used along with the active constituents. Presence of more extractives in water extraction suggests that water extracts more of these polar compounds (like glycosides, flavonoids, etc., present in *A. paniculata*) than andrographolide, whereas,

unwanted polar compounds are not extracted in chloroform. However, in ethanol some of these compounds, which dissolve in it also get extracted along with andrographolide which gets almost completely extracted after five hours.

Less extraction of andrographolide in water has also been pointed out by Talukdar *et al.* (1968) who based on their findings, suggested use of alcohol for complete extraction of andrographolide. Valepotriates which belong to an irridoid class of compounds are moderately polar compounds present in *Valeriana jatamansi* and other *Valeriana* species. These are moderately polar compounds and are used as sedatives. In order to extract these compounds completely from the underground parts of the plant, moderately polar solvents like dichloromethane, diethyl ether, benzene and ethyl acetate have been suggested (Danielak, 1971; Gupta *et al.*, 1986). Handa and Prabhakar (1952) have suggested use of alcohol for better extraction of bitters from *Swertia chirayita*. Not only alcohol extraction afforded higher bitter contents in the extracts than in water but at the same time extraction was achieved in less time and also it was easy to separate alcohol from the extracts.

Chapter 6

SUMMARY

The present investigations entitled, "Studies on bitter principles from *Andrographis paniculata* Nees." was carried out in the experimental field of the Department of Forest Products, College of Forestry, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni-Solan (HP) during 1999-2000. The analytical work was carried out in the laboratory of the Department of Forest Products.

Observations on fresh weight (g) and dry weight (g) of each plant part (leaves, stems and roots) were recorded at the different growth and development stages of the plant for studying the variation of andrographolide content (%). Andrographolide yield (mg) per plant parts was calculated by using the above observations. In the second experiment involving different solvents for different time durations for extracting the bitter principle (andrographolide^{content}) from each extract was recorded to find out the effect of different solvents and extraction durations.

Isolation and purification of andrographolide was done by using TLC and column chromatography. Quantitative estimation of andrographolide was carried out by using preperative TLC-spectrophotometric method.

In the first part of the study variation of bitter principle (andrographolide) in leaves, stems and roots was studied with growth and development of the plant. Leaves showed maximum value (per plant) for fresh weight (27.650 g) and dry weight (10.672 g) in 4.5 and 6 months old plants, respectively. Maximum andrographolide yield (216.70 mg) per plant leaves was found in 5.5 months old plants, while andrographolide content was maximum (2.04%) in 5.5 months old plants. In the growth and development cycle of stems, maximum values (per plant)

for fresh weight (53.970 g) and dry weight (22.970 g) were recorded from stems of 5.0 months and 6.0 months old plants, respectively. Andrographolide content (%) and andrographolide yield per plant stems were recorded maximum 0.65 per cent and 149.32 mg, respectively in 6.0 months old plants. Andrographolide content and andrographolide yield per plant stem increased with every growth and development stage of plants. Throughout the growth period of stems both andrographolide content and andrographolide yield were much less than the leaves. Total dry biomass contribution of roots towards the whole dry biomass of plant was observed much less than that of both leaves and stems. In case of roots maximum fresh weight (3.588 g), dry weight (1.947 g) and andrographolide yield (10.32 mg) each per plant were found in 6 months old plants, whereas, minimum fresh weight (0.204 g), dry weight (0.054 g), and andrographolide yield (0.14 mg) were observed in 1.5 months old plants. Andrographolide content varied from 0.26 per cent in 1.5 months old plants to 0.53 per cent in 6.0 months old plants. In the initial phase of plant growth from 1.5 months old plants to 4.0 months old plant, andrographolide content increased slowly from 0.26 per cent to 0.30 per cent.

The second part of the investigation was carried out to study the efficiency of the different solvents using different extraction periods for the extraction of andrographolide. Under each solvent and for each extraction period, total extractives and andrographolide contents in the extracts were estimated. Maximum andrographolide contents in each solvent was obtained when extraction was allowed for five hours with values of 1.12 per cent in ethanol, 0.93 per cent in chloroform and 0.35 per cent in water. Total extractives increased in each solvent with extraction durations. Maximum total extractives (per 10 g of herb) in each solvent were obtained when extraction was allowed for five hours with values of 2.367 g in water, 0.675 g in ethanol and 0.217 g in chloroform. Andrographolide contents (analyzed in the extract) increased with each extraction period.

Based on the results obtained from the present studies following conclusions were drawn:

- i) *Andrographis paniculata* should be harvested when plants are about 5.5 months old, because at this stage maximum andrographolide content and yield are obtained in all the plant parts.
- ii) Ethanol should be used as solvent for maximum extraction of andrographolide.
- iii) Water should be the least preferred solvent for extraction of andrographolide.
- iv) Extraction period should be about five hours for complete extraction of andrographolide from the herb when alcohol is used as the solvent for extraction of andrographolide.
- v) If chloroform is to be used, then extraction should be carried out for more longer period to ensure complete extraction of andrographolide.

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