Acta Chimica & Pharmaceutica Indica

Acta Chim. Pharm. Indica: 3(1), 2013, 52-64
ISSN 2277-288X

STUDY OF PHYSICO-CHEMICAL PROPERTIES OF DRUG AND PHYSIOLOGICAL VARIATION IN LEAVES OF *ANDROGRAPHIS PANICULATA (BURM. F.) NEES*

MEENU SHARMA[*], R. G. SHARMA and AAKANKSHA SHARMA[a]

Department of Chemistry, Graphic Era University, DEHRADUN – 248001 (Uttarakhand) INDIA
[a]Department of Biotechnology, Graphic Era University, DEHRADUN – 248001 (Uttarakhand) INDIA

(*Received : 30.01.2013; Revised : 03.02.2013; Accepted : 04.02.2013*)

ABSTRACT

Samples of *Andrographis paniculata (Burm. f.) Nees* of different age i.e. after 30, 60, 90, 110 and 130 days of plantation with samples of different location were studied. Results of extractive value determination show that alcohol soluble extractive value was maximum and Pet. ether soluble extractive value was least. Among all samples studied alcohol soluble extractive value of L_L sample was highest (23.42%) followed by Q_L sample. Results of physiological variation clearly indicate that both Chlorophyll a and Chlorophyll b were more in young plants than matured ones. Results also show that as Chlorophyll content decreases Carotenoids and Anthocyanin content increases.

Key words: Andrographis paniculata, Kalmegh, Chlorophyll, Physico-chemical, Physiological variation.

INTRODUCTION

Andrographis paniculata (Burm. f.) Nees belongs to the family of Acanthaceae and commonly called as Kalmegh. It is found in wild through out of plains of India especially in Tamil Nadu, Karnataka, Maharashtra, Orissa, Uttar Pradesh and Uttarakhand. *Andrographis paniculata (Burm. f.) Nees* is one of the important herbs among 17,000 higher plant species occurring in India, out of which more than 1000 species are used over several centuries in the traditional systems of medicine viz. Ayurveda, Siddha and Unani. According to Pushpangadan et al.[1], the villagers and tribal folks spread across the length and breadth of the country make use of more than 7000 plant species through oral traditions. Nearly 3/4 of the herbal drugs and perfumery products used in the world are available naturally in India. Therefore, the rich and varied plant diversity, especially the genetic diversity of medicinal and aromatic plants, is one of India's important strengths. In a significant study from China showed that not only plants growing in different geographical areas with different morphological characteristics could have different chemical constituents and physiological variation but also plants with similar morphological features and growing on the same site may have different contents of chemical constituents and physiological variation[2]. *Andrographis paniculata* or Kalmegh is one of the most widely used plants in ayurvedic formulations[3]. Study of physico-chemical properties of the drug, change in chlorophyll, carotenoids and anthocyanins amount with the increasing days of plantation of plant is very important because it directly relates with effectiveness and the quality of medicinal plant.

Available online at www.sadgurupublications.com
[*]Author for correspondence; E-mail: meenu7dec@gmail.com, meenus1968@gmail.com

Two types of chlorophyll are found in plants - chlorophyll a and chlorophyll b. Both chlorophylls absorb light most strongly in the red and violet parts of the spectrum. Chlorophyll is responsible for photosynthesis i.e. for the food of plant hence for the chemical constituents found in plants, which directly affects the medicinal values of plant.

Carotenoids are organic pigments that are found in the chloroplasts and chromoplasts of plants. Carotenoids in general absorb blue light. They serve two key roles in plants: they absorb light energy for use in photosynthesis, and they protect chlorophyll from photodamage[4]. Carotenoids have many physiological functions. Given their structure, carotenoids are efficient free-radical scavengers, and they enhance the vertebrate immune system. There are several dozen carotenoids in foods people consume, and most carotenoids have antioxidant activity[5].

Anthocyanins are water-soluble vacuolar pigments that may appear red, purple, or blue depending on the pH. They are odorless and nearly flavorless. Anthocyanins are derivatives of anthocyanidins, which include pendant sugars[6]. Anthocyanins can be used as pH indicators because their color changes with pH; they are pink in acidic solutions (pH < 7), purple in neutral solutions (pH ~ 7), greenish-yellow in alkaline solutions (pH > 7), and colourless in very alkaline solutions, where the pigment is completely reduced[7]. Anthocyanins also act as powerful antioxidants. Richly concentrated as pigments in berries, anthocyanins were the topics of research presented at a 2007 symposium on health benefits that may result from berry consumption[8]. Laboratory-based evidence was provided to demonstrate potential health effects against: cancer, aging and neurological diseases, inflammation, diabetes, bacterial infections and fibrocystic disease[9].

Therefore, in order to assure the effectiveness and the quality of the medicinal plants, it is necessary to monitor availability of the bioactive constituents both in terms of quantitative and qualitative aspects. Hence study of location variations of a particular species is an important issue with respect to physico-chemical properties and chemical constituents present, in finding pharmaceutical potential.

EXPERIMENTAL

Plant material

The whole plant material, only leaves, samples of whole plant material at different stages of life cycle and **s**amples of different location variation were used. Samples after 30, 60, 90, 110 and 130 days of plantation were collected.

Samples of whole plant material at different stages of life cycle were-

A-30 days, B-60 days, C-90 days, D-110 days (after 110 days of plantation i.e., just before flowering), E-130 days (At maturity of the crop i.e., bearing flowers, fully matured seed capsules etc.).

Leaves and whole plant material of *Andrographis paniculata* grown in natural environment (wild) from different locations were used as samples with self cultivated *Andrographis paniculata*. Samples of different location variation were named as –

L_W - Dehradun self growned whole plant material

L_L - Dehradun self growned leaves

M_W - FRI, -Dehradun (Uttarakhand) whole plant material

M_L - FRI, -Dehradun (Uttarakhand) Leaves

N_W - Patanjali Ayurved, Haridwar (Uttarakhand) whole plant material

N_L - Patanjali Ayurved, Haridwar (Uttarakhand) Leaves

O_W - Selaqui Dehradun (Uttarakhand) whole plant material

O_L - Selaqui Dehradun (Uttarakhand) Leaves

P_W - Balawala (Uttarakhand) whole plant material

P_L - Balawala (Uttarakhand) Leaves

Q_W - Himalaya Drug (Uttarakhand) whole plant material

Q_L - Himalaya Drug (Uttarakhand) Leaves

Study of the powder materials (drug)

The whole plant materials of *Andrographis paniculata* were dried under shade and were powdered using a homogenizer and whole powder was considered as drug. Various chemical tests were performed according to conventional methods given in Lab manuals of Pharmacognosy by Lala[10], Wallis[11] and Kokate[12].

Organolaptic properties

Organolaptic Properties of drug were examined according to conventional methods given by Kokate[12]. The green powder was treated with some acidic, basic and neutral routinely used reagents and characteristic changes were observed. Results are given in Table 1.

Fluorescence behaviors

The fluorescence behavior of the powder of *Andrographis paniculata* as such and after treating with some chemical reagents was determined according to the methods of Chase and Pratt[13]. The dried powder sample was treated with various chemical reagents like H_2SO_4, HCl, HNO_3 etc. The mixture was mixed well, allowed to stand for few minutes and filtered. The filtrate was examined under both U.V. and visible light. Fluorescence characteristics of the powder were observed in day light as well as in ultraviolet radiation at 254 nm and 366 nm. The solvents used for the extraction procedure were Acetone, chloroform, ethanol, hexane, methanol, petroleum ether and water. After extraction all extracts were observed in day light as well as in ultraviolet (UV) light at 254 nm and 366 nm and colour was noted. Results are given in Table 2 and 3.

Physico-chemical properties of the drug

Physico-chemical constants like ash value, water soluble extracts, alcoholic extracts, loss on drying and pH values were determined as per method described in Indian Pharmacopoeia[14].

Melting point of the drug

The melting point of the drug was determined by melting point apparatus. The shade dried coarse powder of the leaves and whole plant material of *Andrographis paniculata* was examined and it matches with the value as given in Indian Pharmacopoeia.

pH values

The shade dried coarse powder of the leaves and whole plant material of *Andrographis paniculata* was examined. pH value of drug was determined by pH meter (QC/Micro/pH 01Sr No. 391505).

Results are given in Table 4.

Determinations of ash values

Ash is the inorganic residue left after ignition at 650-700°C. The ash content is an approximate measure of the mineral content and other inorganic matter in biomass. The ash content is used in conjunction with other assays to determine the total composition of biomass samples. Methods used were according to Harborne[15].

Apparatus

Silica crucibles

Furnace - An electric furnace was used for igniting the samples. Furnace was fitted with an indicating pyrometer, so that the desired temperature can be maintained.

Analytical balance - sensitive to 0.1 mg.

Desiccator, Drying oven - with temperature control of 105 ± 2°C.

(1) Total ash (TA) value: This value was determined using a minimum of 2.0-3.0 g of material in a furnace heated gradually to the ignition temperature of 650-700°C. Accurately 2 to 3 g of air–dried samples of *Andrographis paniculata* were weighed in a tared silica dish and incinerate at a temperature not exceeding 700°C until ash free from carbon is obtained. Then it was cooled and weighed. The process was repeated until at least two consecutive constant weights were obtained. The results are expressed as range or mean value ± standard deviation. The percentage of ash was calculated with reference to the air – dried drug.

(2) Determination of acid insoluble ash (AIA): Above obtained ash was boiled with 25 mL of 2 M hydrochloric acid for 5 min, the insoluble matter was collected in a Gooch crucible or on an ash less filter paper, washed with hot water, ignited and cooled in desiccators. Then it was weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

(3) Determination of water soluble ash (WSA): Ash was boiled for 5 min. with 25 mL of water; the insoluble matter was collected in a Gooch crucible or on an ash less filter paper, washed with hot water, and ignited for 15 min at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of the ash, the difference in weight represent the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

Formula used for the calculation of percentage of all three types of Ash Values is -

$$\text{Ash \%} = \frac{\text{Loss in weight}}{\text{W}} \text{ x } 100$$

W = Weight of air – dried drug. Results are given in Table 5.

Loss on drying (LOD)

Methods used were according to Brain[16]. Loss on drying of the air-dried samples of *Andrographis paniculata was* analyzed. This was carried out using a minimum of 0.5-1.0 g of material. Accurately weighed quantity of sample was taken in a tared glass bottle and initial weight was taken. The sample was heated in a Lindberg/Blue M gravity-convention oven maintained at 105-110°C, for 3 h, after which the sample was allowed to cool to room temperature in desiccators, and subsequently weighed. The time interval

from the oven to point of weighing was usually about 30 minutes. This procedure was repeated until a constant weight was obtained.

$$\text{Loss on drying (\%)} = \frac{\text{Loss in weight}}{\text{W}} \text{ x } 100$$

Where W = weight of the sample powder in gms.

The results are expressed as a range or as mean ± standard deviation. Results are given in Table 6.

Determination of extractive values

Methods used were according to Kokate et al.[12] The extractive values for various solvents of air-dried sample were evaluated.

(i) Water-soluble extractives

(ii) Alcohol soluble extractives

(iii) Pet ether soluble extractives

(1) Evaluation of water extractive value: About 5 g of accurately weighed coarsely powdered, air-dried sample was transferred into a glass-stoppered, 250 mL reflux conical flask, followed by the addition of 50 mL of boiled water. The flask was well shaken, and allowed to stand for 10 minutes. It was cooled and filtered. Filtrate was transferred to an evaporating dish, which was 7.5 cm in diameter; the solvent was evaporated on water bath, allowed to dry for 30 minutes, finally dried in an oven and residue was weighed. Percentage of water-soluble extractives was calculated with reference to the air-dried drug.

(2) Alcohol soluble extractive value: 5 g of dried samples of *Andrographis paniculata* were macerated with 100 mL of alcohol in a closed flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours separately. Thereafter, it was filtered rapidly taking precaution to minimize the loss of methanol. Evaporated 25 mL of filtrate to dryness in a tared flat bottom shallow dish dried at 105ºC and weighed. Percentage of Alcohol soluble extractive was calculated with reference to the air-dried samples.

(3) Pet. ether soluble extractive value: 5 g of dried samples of *Andrographis paniculata* were macerated with 100 mL of pet ether (40-60^{o}C) in a conical flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours separately. Thereafter, it was filtered rapidly taking precaution against loss of pet ether. Evaporated 25 mL of filtrate to dryness in a tared flat bottom shallow dish dried at 105ºC and weighed. Percentage of pet ether soluble extractive was calculated with reference to the air-dried samples.

Results are given in Table 7.

Physiological variation

The experiment was conducted for the young and mature plants of selected populations of *Andrographis paniculata (Burm. f.) Nees*. Young (60 days old) and mature (130 days old) plants were selected for analyzing age-related changes in chlorophyll, carotenoid and anthocyanin content among different samples. Fresh leaves of different samples of *Andrographis paniculata* were used.

(1). Variation in Chlorophyll and Carotenoids: Chlorophyll and carotenoids were extracted using 80% acetone as shown by Sadasivam and Manikkam[17] and also by Sabu[18]. 5 g fresh leaves were

homogenized in 40 mL of 80% acetone and then the extract was centrifuged at 1000 rpm for 2 min in Remi (S/N0. BCBR-2182) cooling centrifuge. The supernatant was collected and used for measuring optical density at 490, 645 and 663 nm using UV10 Thermo scientific visible spectrophotometer (S/No. HEDM218008).

(2). Variations in Anthocyanin content: For estimating variation in anthocyanin content of leaves, 0.5 g leaf tissue was homogenized in 4 mL of methanol containing 1.0 M HCl and was kept at 4°C for 4 h. The homogenate was filtered and centrifugedat 10,000 rpm for 30 min and the absorbance of the supernatant was measured at 530 nm[19], after suitable dilution.

Results are given in Table 8.

Green leaves of young plant.

Coloured leaves of matured plant.

RESULTS AND DISCUSSION

Organolaptic Properties: Coarse Powder

Colour-Green

Foreign matter- < 1.0%

Sand & Silica- Absent

Odour- Slight characteristic

Taste- Intensely bitter

Insect infestation- Nil

Rodent contamination- Nil

Powder pressed between two filter papers for 24 hours-Stained

Table 1: Behavior of *Andrographis paniculata* (drug) with different reagents

S. No.	Chemical treatment	Observation
1	Drug Powder treated with HCI	Powder settles down slowly Colour: Light brown
2	Drug green Powder treated with Conc. HNO_3	Powder float on the surface. Colour: Yellowish brown
3	Drug green Powder treated with Conc. H_2SO_4	Powder settles down slowly. Colour: Brownish black
4	Drug green Powder treated with 5% aqueous NaOH	Powder settles down slowly Colour: Brownish
5	Drug green Powder treated with iodine solution	Powder settles down immediately. Colour: Brownish
6	Drug green Powder treated with 5% aqueous KOH	Powder settles down slowly. Colour: Dark brown
7	Drug green Powder treated with Glacial Acetic Acid	Powder settles down immediately. Colour: Light Brown
8	Drug green Powder treated with 5% aqueous $FeCI_3$	Powder settles down immediately. Colour: Greenish

Fluorescence behavior of *Andrographis paniculata* (drug powder) without any extraction

Drug powder of *Andrographis paniculata* **(**whole plant material) without any extraction was observed in day light as well as in ultraviolet (UV) light at 254 nm and 366 nm and colour was noted.

Table 2: Fluorescence behavior of *Andrographis paniculata* (drug powder)

S. No.	Chemical treatment	Under ordinary light	Under UV light 254 nm	Under UV light 366 nm
1	Drug green powder as such	Green	Green	Silver green
2	Drug green powder treated with 1N NaOH in methanol	Dull green	Greenish white	Cream
3	Drug green powder treated with 1 N NaOH in water	Yellowish	Green	Dark greenish white
4	Drug green powder treated with 50% HNO_3	Dark yellow	Green	Greenish yellow
5	Drug green powder treated with 50% H_2S0_4	Yellowish brown	Yellowish green	Greenish white

Fluorescence behavior of the drug powder after extraction with chemical reagents

Whole plant material of *Andrographis paniculata* was extracted. Then extracts were observed in day light as well as in ultraviolet (UV) light at 254 nm and 366 nm and colour was noted.

Table 3: Fluorescence behavior of *Andrographis paniculata* extracts

S. No.	Extractives	Under ordinary light	Under UV light (254 nm)	Under UV light (366 nm)
1	Hexane	Light green	Colourless	Colourless
2	Pet. ether	Yellowish	Light green	Colourless
3	Chloroform	Yellowish	Light green	Colourless
4	Acetone	Green	Light green	Umber
5	Ethyl alcohol	Green	Dark green	Umber
6	Methyl alcohol	Green	Dark green	Umber
7	Dist. water	Green	Pale green	Umber

Physico-chemical properties of the drug: 1. Melting point of the drug: The melting point of the drug, determined by melting point apparatus. Average value of triplicates is given in the table. It is between 226-229°C, which matches with the standard melting point value (229°C) of drug as given in Indian Pharmacopoeia.

pH values of the drug: pH value of shade dried coarse powder of the leaves and whole plant material of *Andrographis paniculata* was examined. pH of 1% aqueous solution and pH of 10% aqueous solution was measured. Average value of triplicates is given in the table.

Table 4: Melting point and pH values of Different samples of *Andrographis paniculata*

S. No.	Samples of *Andrographis paniculata*	Melting point	pH values 1% aq. sol.	pH values 10% aq.sol.
1	Whole plant material	228.5^{o}C	7.94	8.22
2	Leaves	226^{o}C	7.82	7.64

Determination of ash values

Three ash values were calculated. They are-

1) Total Ash (TA) Value

2) Determination of Acid Insoluble Ash (AIA)

3) Determination of Water Soluble Ash (WSA)

Samples of whole plant material at different stages of life cycle and Samples of different location variation were used. The results are expressed as range or mean value ± standard deviation. The percentage of ash was calculated with reference to the air – dried drug. Result is reported up to two decimal places, as a percentage of the samples.

Table 5: Ash values of different samples of *Andrographis paniculata.*

S. No.	Samples of *Andrographis paniculata*	Total ash (% w/w)	Acid insoluble Ash (% w/w)	Water soluble Ash (% w/w)
1	**A**	10.12%	1.22%	1.13%
2	**B**	10.14%	1.24%	1.14%

Cont…

S. No.	Samples of *Andrographis paniculata*	Total ash (% w/w)	Acid insoluble Ash (% w/w)	Water soluble Ash (% w/w)
3	**C**	9.84%	1.18%	1.23%
4	**D**	9.24%	0.98%	1.02%
5	**E**	12.82%	1.97%	1.42%
6	**L_W**	11.79%	1.62%	1.65%.
7	**L_L**	10.02%	1.11%	1.16%
8	**M_W**	11.84%	1.99%	1.74%
9	**M_L**	11.01%	1.84%	1.66%
10	**N_W**	11.65%	2.03%	1.53%
11	**N_L**	11.78%	1.74%	1.25%
12	**O_W**	12.65%	1.64%	1.51%
13	**O_L**	11.08%	1.82%	1.08%
14	**P_W**	10.52%	1.97%	1.21%
15	**P_L**	11.24%	2.01%	1.60%
16	**Q_W**	12.14%	1.88%	1.32%
17	**Q_L**	11.99%	1.74%	1.66%

Loss on drying (LOD)

Loss on drying of the air-dried samples of. *Andrographis paniculata* was carried out using Lindberg/Blue M gravity-convention oven maintained at 105-110°C. The results are expressed as a range or as mean ± standard deviation.

Table 6: Loss on drying (LOD) (% w/w) of Different samples of *Andrographis paniculata*

S. No.	Samples of *Andrographis paniculata*	Loss on drying (LOD) (% w/w)
1	**A**	8.40%
2	**B**	8.62%
3	**C**	9.45%
4	**D**	9.12%
5	**E**	10.45%
6	**L_W**	10.20%
7	**L_L**	10.24%
8	**M_W**	11.22%
9	**M_L**	11.26%
10	**N_W**	10.98%
11	**N_L**	11.02%
12	**O_W**	11.43%
13	**O_L**	11.08%
14	**P_W**	9.99%
15	**P_L**	10.03%

Deterioration time of the plant material depends upon the amount of water present in plant material. If the water content is high, the plant can be easily deteriorated due to fungal attack. To determine chemical constituents correctly it is necessary to calculate LOD.

Determination of extractive values

Three extractive values were calculated. They are-

(1) Evaluation of water extractive value

(2) Alcohol soluble extractive value

(3) Pet. ether soluble extractive value

Table 7: **Extractive values of different samples of *Andrographis paniculata***

S. No.	Samples of *Andrographis paniculata*	Water extractive value	Alcohol soluble extractive value	Pet. Ether soluble extractive value
1	**A**	12.24%	18.45%	10.96%
2	**B**	12.25%	18.99%	11.23%
3	**C**	14.82%	19.82%	11.16%
4	**D**	16.24%	20.88%	10.88%
5	**E**	13.98%	20.42%	11.45%
6	**L_W**	16.13%	21.12%	10.99%
7	**L_L**	17.02%	23.42%	11.02%
8	**M_W**	15.98%	20.45%	11.43%
9	**M_L**	16.79%	21.23%	11.62%
10	**N_W**	14.97%	21.22%	11.03%
11	**N_L**	16.82%	22.15%	11.32%
12	**O_W**	14.85%	21.17%	11.23%
13	**O_L**	16.05%	21.65%	11.14%
14	**P_W**	16.49%	20.43%	10.89%
15	**P_L**	16.89%	19.69%	11.21%
16	**Q_W**	15.73%	21.48%	11.14%
17	**Q_L**	16.11%	22.88%	11.29%

A-30 days, B-60 days, C-90 days, D-110 days, E-130 days of plantation

L_W - Dehradun self grown whole plant material

L_L -Dehradun self grown Leaves

M_W - FRI, -Dehradun (Uttarakhand) whole plant material

M_L - FRI, -Dehradun (Uttarakhand) Leaves

N_W - Patanjali Ayurved, Haridwar (Uttarakhand) whole plant material

N_L - Patanjali Ayurved, Haridwar (Uttarakhand) Leaves

O_W - Selaqui Dehradun (Uttarakhand) whole plant material

O_L - Selaqui Dehradun (Uttarakhand) Leaves

P_W - Balawala (Uttarakhand) whole plant material

P_L - Balawala (Uttarakhand) Leaves

Q_W - Himalaya Drug (Uttarakhand) whole plant material

Q_L - Himalaya Drug (Uttarakhand) Leaves

Results show that alcohol soluble extractive value is maximum and Pet. ether soluble extractive value is least. Among all samples studied alcohol soluble extractive value of L_L sample is highest (23.42%) followed by Q_L sample.

Physiological variation

There are a bunch of different equations for calculating amounts of chlorophyll in plants. Arnon's[20] equations for calculation of chlorophyll extracted in 80% acetone were proven by Hiscox & Israelstam[21]. These equations are used for calculating amounts of chlorophyll in different samples of *Andrographis paniculata.*

Arnon[20] reported the following equations for quantification of the total chlorophyll, chlorophyll a and chlorophyll b content in an 80% acetone extract:

Total chlorophyll (µg/mL) = 20.2 (A_{645}) + 8.02 (A_{663})

Chlorophyll a (µg/mL) = 12.7 (A_{663}) – 2.69 (A_{645})

Chlorophyll b (µg/mL) = 22.9 (A_{645}) – 4.68 (A_{663})

Where A_{663} is the solution absorbance at 663 nm andA_{645} is the absorption at 645.

Optical density of different samples of *Andrographis paniculata* was measure at 490, 645 and 663nm using UV10 Thermo scientific visible spectrophotometer (S/No. HEDM218008).

Table 8: **Chlorophyll, Carotenoids and Anthocyanin content of Different samples of *Andrographis paniculata***

S. No.	Samples of *Andrographis paniculata*	Total chlorophyll mg/plant tissue	Chlorophyll a mg/plant tissue	Chlorophyll b mg/plant tissue	Carotenoids mg/plant tissue	Anthocyanin mg/plant tissue
1	**YL_1**	0.067	0.049	0.018	3.423	0.892
2	**YL_2**	0.055	0.042	0.013	4.121	1.213
3	**ML_1**	0.048	0.036	0.012	5.627	1.567
4	**ML_2**	0.045	0.034	0.011	4.891	1.712

YL_1, $YL_{2 = Young}$ (60 days old) Leaves, ML_1, ML_2=mature (130 days old) Leaves of *Andrographis paniculata*

Results clearly indicate that both Chlorophyll a and Chlorophyll b are more in young plants than matured ones. Results also show that as Chlorophyll content decreases, Carotenoids and Anthocyanin content increases.

CONCLUSION

Study of physico-chemical properties of the drug of different locations and study of physiological variation at different stages of life cycle of *Andrographis paniculata* will serve as the pioneering project to assess this medicinally important native plant and future research on other medicinal native plants of Uttarakhand based on the fact that plants from different sources may have different phytochemical profiles and pharmacological actions.

ACKNOWLEDGMENT

The authors are extremely grateful to Mr. Ashok Windlass, M.D. of Windlass biotech Ltd., Dehradun, Uttarakhand, India and Dept. of Biotechnology, Graphic Era University, Dehradun, Uttarakhand, India for providing all research facilities to accomplish this study.

REFERENCES

1. P. Pushpangadan, K. N. Nair and V. Santhosh, Biodiversity- An Overview, in the Natural Resources of Kerala, Eds. K. B. Thampi, N. M. Nayar and C. S. Nair, World Wide Fund for Nature-India, Thiruvananthapuram (1997) pp. 130-139.
2. S. A. He and N. Sheng. Utilization and Conservation of Medicinal Plants in China with Special Reference to Atractylodes Lancea, in, Medicinal Plants for Forest Conservation and Health Care, G.C. Bodeker (Ed.) FAO, Rome (1997).
3. J. D. Hooker, Flora of British India, L. Reeve & Co. Ltd. Ashford, Kent, **Vol. IV** (1885).
4. G. A. Armstrong and J. E. Hearst Carotenoids 2: Genetics and Molecular Biology of Carotenoid Pigment Biosynthesis, FASEB J.**, 10(2)**, 228-237 (1996).
5. β-Carotene and other Carotenoids as Antioxidants, From U.S. National Library of Medicine, November (2008).
6. ØM Andersen and M. Jordheim, "The anthocyanins" In: Andersen ØM and Markham KR (Eds.) Flavonoids: Chemistry, Biochemistry and Applications, Boca Raton: CRC Press (2006) pp. 471–553.
7. Michaelis Leonor, M. P. Schubert and C. V. Smythe (1 December 1936), Potentiometric Study of the Flavins, J. Biol. Chem., **116(2)**, 587-607.
8. Seeram, P. Navindra, Berry Fruits: Compositional Elements, Biochemical Activities, and the Impact of Their Intake on Human Health, Performance, and Disease, J. Agri. Food Chem., **56(3)**, 627-9 (2008).
9. M. Leonardi, Treatment of Fibrocystic Disease of the Breast with Myrtillus Anthocyanins, Our Experience, Minerva Ginecologica, **45(12)**, 617-621 (1993).
10. P. K. Lala, Lab Manuals of Pharmacognosy, CSI Publishers and Distributors, Calcutta (1993).
11. T. E. Wallis, Editor, Text Book of Pharmacognosy (Ed. V) CBS, Publisher and Distributor, Delhi, 104-119 (1985).
12. C. K. Kokate, A. P. Purohit and S. B. Gokhale, Text Book of Pharmacognosy, 18th Ed. Pune: Nirali Prakashan (2002).
13. C. R.Chase and R. Pratt, Florescence of Powdered Vegetable Drugs with Particular Reference to Development of System of Identification, Am. Pharm. Assoc., **38**, 324-331 (1949).
14. Indian Pharmacopoeia, Government of India Ministry of Health & Family Welfare Published by The Indian Pharmacopoeia Commission, Ghaziabad, **Vol. III** (2010).
15. J. B. Harbome, Phytochemical Methods, London, Chapman and Hill Ltd., 49-188 (1973).
16. K. R. Brain T. D. Turner, A Practical Evaluation of Phytopharmaceuticals, Bristol: Wright Scientechnica (1975).
17. S. Sadasivam and A. Manikkam, Biochemical Methods, 2nd Ed. New Age International (P) Ltd., Publishers, New Delhi (1996).

18. K. K. Sabu, Intraspecific Variations in Andrographis Paniculata Nees., Ph.D. Thesis, Kerala University, Thiruvananthapuram, India (2002).

19. T. Janda, G. Szalai and E. Paldi, Chlorophyll Fluorescence and Anthocyanin Content in Chilled Maize Plants After Return to a Non-Chilling Temperature Under Various Irradiances, Biologia Plantarum, **38**, 625-627 (1996).

20. D. Arnon, Plant Physiology, **24**, 1-15 (1949).

21. J. D. Hiscox and G. F. Israelstam, Different Methods of Chlorophyll Extraction, Can. J. Bot., **57**, 1332-1332 (1979).