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# Phytochemical composition and andrographolide content of Kalmegh (*Andrographis paniculata*) grown in Andaman Islands

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**Abstract**
*Kalmegh (Andrographis paniculata Nees.)* is known as king of bitterness, Kalmegh is used as traditional medicine for curing different ailments and is known for its wide medicinal properties. The whole herb is used for herbal cure at Andaman Islands and is grown in homestead gardens, An attempt was made to study the phytochemical composition and andrographolide content of both leaves and stem extracts. Methanolic extract yielded maximum total phenols in both leaf (24.18 mg/g) and stem extract (27.35 mg/g). Flavonoid content was also maximum in methanolic extract of leaf (52.57 mg/g) and stem (71.82 mg/g) of *A. paniculata.* Highest DPPH activity was found in methanolic extract in both leaf (7.08 mg BHT/g) and stem samples (7.15 mg BHT/g). The NO activity was highest in ethanolic extract in both leaf (13.85 mg vit C/g) and stem (12.86 mg Vit C/g). The methanol extract of leaf (14.65 mg EDTA/g) and stem (12.85 mg EDTA/g) showed higher chelating activity. The acetone extract of stem (3.16 mg vit c/g) and leaf (2.97 mg vit c/g) of Kalmegh showed effective reduction in the activity of hydrogen peroxide. Andrographolide content was maximum in leaf (3.49%) followed by stem (1.59%).

**Keywords:** Kalmegh, Andaman, phytochemicals, andrographolide, flavonoids

## Introduction
Medicinal plants constitute a major segment of the flora, which provides raw materials for use in the pharmaceuticals, cosmetics and drug industries. The majority of population in the developing countries rely on the medicinal plants for their primary health care. Modern pharmacopoeias still contain at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants. Demand for medicinal plant is increasing in both developing and developed countries due to growing recognition of natural products, being non-narcotic, having no side-effects, easily available at affordable prices and sometime the only source of health care available to the poor (Karunamoorthi *et al.*, 2013) [13]. There are many evidences worldwide to show the extensive use of medicinal herbs by all primitive people (Gilani and Rahman, 2005) [9]. The traditional medicine all over the world is revalued by extensive research on their therapeutic values (Scartezzini and Speroni, 2000) [25]. The widespread drug resistance emerged for common diseases have paved a way for search of naturally available medicinal herbs (Okeke *et al.*, 2005) [17]. The medicinal plants are rich in phytochemicals which are non-nutritive but have protective or disease preventive properties. Based on the presence of phytochemicals, the medicinal plant is correlated for their pharmacological activity (Kumar *et al.*, 2009) [15]. In addition to this, medicinal plants are also rich in their bioactive constituents which play a major role in their therapeutic properties. Andaman and Nicobar Islands is a paradise of rich biological diversity and houses a large number of endemic medicinal plants. The tribal population and settlers of the Island use different medicinal plants that are native and introduced for curing many ailments. *Andrographis paniculata* Nees (Burm. f.) Wall. Ex Nees is one of the important medicinal plants found common in Andaman Islands and is known as King of bitters, Maha-tita, Bhunimba or Hara Chirayata belonging to family Acanthaceae. It is an erect herb extremely bitter in taste and used as a medicinal herb for centuries in several traditional systems of medicine all over the world. The fresh and dried leaves and juice extracted from this herb is official drug in Indian pharmacopeia and is widely used as a bitter tonic and as a febrifuge (Pharmacopoeia, 1955) [18]. Since Ancient times, *A. paniculata* is used

as a wonder drug in traditional siddha and ayurvedic systems of medicine as well as in tribal medicine in India and other countries like Bangladesh, China, Hongkong, Pakistan, Philippines, Malaysia, Indonesia and Thailand (Akbar, 2011; Kabir *et al.*, 2014) [1, 12] for multiple clinical applications (Raina *et al.*, 2013) [20]. *A. paniculata* is official in Indian Pharmacopoeia as a predominant constituent of at least 26 Ayurvedic formulations used to treat liver disorders. It is one of the herbs, which can be used to treat neoplasm as mentioned in ancient Ayurvedic literature. It is reported as a cold property herb in Traditional Chinese medicine (TCM) and is used to get rid of body heat and to expel toxins. The aerial part of the plant, used medicinally, contains a large number of chemical constituents, mainly lactones, diterpenoids, diterpene glycosides, flavonoids, and flavonoid glycosides. Controlled clinical trials report its safe and effective use for reducing symptoms of uncomplicated upper respiratory tract infections (Sivananthan and Elamaran, 2013) [32]. Andrographolide, a bitter principle obtained from *Andrographis paniculata* is a diterpene lactone, responsible for various pharmacological activities (Saxena *et al.*, 1998) [24]. This is a well-known phytoconstituent from Indian System of Medicine, used in the management of different diseases since time immemorial. Andrographolide is reported to possess anti-cancer (Sheeja and Kuttan 2007) [29], anti-HIV (Calabrese *et al.* 2000) [6], cardioprotective (Yoopan *et al.* 2007) [37] and hepatoprotective (Trivedi *et al.* 2007) [34] properties. The other active components include 14-deoxy-11,12-dihydroandrographolide (andrographolide D), homoandrographolide, andrographosterin and stigasterol (Siripong *et al.* 1992) [31]. The Andaman and Nicobar Island is known for its biodiversity and species richness. The ethnomedicinal knowledge of the tribals of the Island is well known and many endemic species exists in the Island. Kalmegh is a common medicinal plant distributed in different parts of the Andaman Island and is used by the inhabitants for curing different ailments. The crop is not commercially is grown but restricted to home garden cultivation. There are reports that phytochemical variability occurs in kalmegh due to genotypic differences and also based on their geographical location of cultivated area (Sharma *et al.*, 2009; Archana *et al.*, 2016; Hiremath *et al.*, 2020) [28, 2, 11]. Studies are limited in quantitative estimation of phytochemicals and andrographolide in kalmegh grown in Andaman Island. Hence, we have attempted to estimate the phytochemicals and andrographolide content of kalmegh which will help in commercial exploitation of the species in the island by potential increase in area under its cultivation.

## Materials and Methods

### Plant materials

Seeds of kalmegh (*Androgrphis paniculata*) are collected from wild and they are raised at our experimental farm of Garacharma, ICAR-CIARI. The crop was grown by standard cultivation practices. The herbs are harvested at flowering stage and they are washed with Millipore water and leaves and stem bits were separated.

### Sample preparation

The separated stem and leaves of kalmegh were dried in tray drier at temperature of 45 ºC for 48 hours. The dried leaves and stem bits were grounded to powder form using high speed blender. One gram of leaf and stem sample were extracted in 80% solution of ethanol, methanol and acetone separately. The slurry were kept for 1day followed by centrifugation at 8000rpm for 10 min. This procedure was repeated till the powder was devoid of colour and filtered through Whatman No. 1 filter paper. The extract was concentrated by rotary evaporator and kept in a refrigerator for analysis.

### Estimation of phytochemicals

#### Total phenolic content

The Folin-Ciocalteu colorimetric method was used to measure the total phenolic content (Singleton *et al.*, 1999) [30] with little modification. Briefly, 200 µl of the extractions were oxidized with 1 ml of 0.5 N Folin-Ciocalteu reagent and then the reaction was neutralized with 1 ml of the saturated sodium carbonate (75 g/L). The absorbance of the resulting blue color was measured at 760 nm with a UV-2600 spectrophotometer (Simadzu, Japan) after incubation for 2 h at room temperature. Quantification was done on the basis of the standard curve prepared using solutions of gallic acid. The total phenolic content was expressed in GA equivalents (mg GA/g of sample weight).

#### Total flavonoid content

Total flavonoid content was estimated by colorimetric method (Bao *et al.*, 2005) [4]. Briefly, 0.5 ml extracts were added to 15 ml polypropylene conical tubes containing 2 ml distilled water mixed with 0.15 ml 5% sodium nitrate. After 5 min, 0.15 ml 10% Aluminum chloride solution was added. After another 5 min, 1 ml 1 M sodium hydroxide was added. The reaction solution was well mixed, kept for 15 min and the absorbance was determined at 415 nm. Qualification was done using the Rutin as standard and the results was expressed as milligrams of rutin equivalent (mg RE) per 100 g of dry weight. Using the Rutin as standard and the result was expressed as milligrams of quercetin equivalent (mg QE) per 100 g of dry weight

### Estimation of free radical scavenging activity

#### DPPH radical scavenging activity

It was measured with the procedure described by Rattanachitthawat *et al.*, 2010 [21] with some modification. The working solution of DPPH was freshly prepared by diluting 3.9 mg of DPPH with 95% ethanol to get with an absorbance of 0.856±0.05 at 517nm. The different concentration of extract was mixed with 1.5 ml of working DPPH and the absorbance of the mixture immediatelymeasured spectrophotometrically after 10 min. Total antioxidant activity of the extracted sample extract was expressed as mg BHA/ g sample equivalent, obtained from the calibration curve.

$$\% \text{ inhibition of DPPH radical} = (A_{control} - A_{sample}/A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of the control (without extract) and $A_{sample}$ is the absorbance in the presence of the extract/standard.

#### ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) activity

The total antioxidant capacity was determined by a colorimetric method (Re *et al.*, 1999) [22] with a little modification. The ABTS radical cation was generated by oxidation of 7 mM ABTS with $K_2S_2O_8$ (2.45 mM) in 10 mL of deionzed water and kept in darkness at 4 °C during 16 hours. Once the radical cation was obtained, the $ABTS^+$cation solution was diluted with 80% ethanol to an absorbance of 0.784±0.01 at 734 nm. Then, 3.9 ml $ABTS^+$cation solution was added to 1 ml of extracts and mixed thoroughly. The mixture incubated for 6 min at room temperature and tested

the absorbance at 734 nm. Results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC, mM Trolox equivalents per 100 g dry weight).

% inhibition of ABTS radical = ($A_{control}$- $A_{sample}$/$A_{control}$) x100

**Nitric oxide scavenging activity**

Nitric oxide scavenging activity was measured using the procedure described by Marcocci *et al.*, 1994. SNP (10 mM) in phosphate buffer saline (PBS) was mixed with different concentrations of the extract and incubated at 25°C for 150 min. The samples were added to Greiss reagent (1% sulphanilamide, 2% H3PO4 and 0.1% napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm and referred to the absorbance of standard solutions of ascorbic acid treated in the same way with Griess reagent as a positive control. The percentage of inhibition was measured by the following formula:

% inhibition of NO radical = (Acontrol- A sample/A control) x100

**Metal chelating activity (MCA)**

The chelating of ferrous ion was measured using the method of Dinis *et al.*, 1994 [7]. The crude extracts were reacted with 0.05 mL of 2.0 mM Ferric chloride. The mixture was then added with 0.2 mL of 5.0 mM ferrozine. After which, the reaction was shaken and incubated at room temperature for 10 min and the absorbance of the red color was measured at 562 nm. EDTA was used as a positive control. The percentage of metal chelating activity was calculated by the following equation:

% Metal chelating activity = (Ac – As)/Ac ×100 Where, Ac is the absorbance of the control and As is the absorbance of the extract/standard.

**Hydrogen peroxide Radical scavenging activity**

The ability of the Kalmegh extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.* (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/mL) in different solvents were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both kalmegh extracts and standard compounds were calculated: % Scavenged [$H_2O_2$] = [(AC – AS)/AC] x 100 Where AC is the absorbance of the control and AS is the absorbance in the presence of the sample of kalmegh extracts or standards.

**Estimation of andrographolide**

The method suggested by Sharma *et al.* (2012) [26] was used to estimate the andrographolide content of leaf and stem of kalmegh with minor modifications. A modular Ultra high-performance liquid chromatography (UHPLC) system (Shimadzu corporation, Kyoto, Japan) was used with the following chromatographic conditions. The Pump mode: Low pressure gradient, Pump flow: 1ml/min, Solvents: Buffer (0.136g of anhydro potassium dihydrogen orthrophoaphate + 0.5 ml of phosphoric acid in 1000 ml of HPLC grade water)-55%, Acetonitrile-45%, Oven temperature: 35 C Detector: PDA @223nm, Process time: 8 min, Column: C18, Weight of the sample: 10g and Methanol; 250ml. Percentage of andrographolide was calculated from the peak response using the formula as follows.

$$\frac{\text{Area of sample}}{\text{Area of standard}} \times \frac{\text{Standard weight}}{\text{Standard dilution}} \times \frac{\text{Sample Solution}}{\text{Sample weight}} \times \frac{\text{Purity of Standard}}{100} \times 100$$

**Results and Discussion**

In our study acetone, methanolic and ethanolic extracts of leaf and stem of *Andrographis paniculata* were estimated for phytochemicals, antioxidant activities and the andrographolide content.

**Phenolic and flavonoid content**

Total phenolic and flavonoid content of different extracts of leaf and stem of *A. paniculata* used in the study are presented in Table 1. The total phenolic content was maximum in stem extract of *A. paniculata* when compared with leaf extract whereas, the flavonoid content was maximum in leaf extract than the stem extract (Table 1). Among the different solvents used, the methanolic extract yielded maximum total phenols in both leaf (24.18 mg/g) and stem extract (27.35 mg/g). Flavonoid content was also maximum in methanolic extract of leaf (52.57 mg/g) and stem (71.82 mg/g) of *A. paniculata*. Swain *et al.*, 2020 [33] reported highest phenolic and flavonoid content in methanolic extract when compared with ethanol and aqueous extracts in *Alpinia calcarata*. Similarly, variations were observed in phenol and flavonoid content in *A. paniculata* when grown in different organic based media (Upadhyaya *et al.*, 2011) [35]. Variations were observed in the quantification of phenols and flavonoids due to different growth period, geographical location, climatic condition, genetic variability, etc. The higher values for methanolic extract may be due to the extraction power of solvent (80% methanol) to the flavonoid compounds (Swain *et al.*, 2020) [33] in the dilution media. The presence of appreciable amounts of phenols and flavonoids are important as they play an important role in quenching free radicals and may help in exploration of antioxidants.

**Table 1:** Total phenolic and flavonoid content of stem and leaf extracts of *Andrographis paniculata*

| Solvents | Total phenols (mg GAE/g) | | Flavonoids (mg rutin/100g) | |
|---|---|---|---|---|
| | ***A. paniculata* (Leaf)** | ***A. paniculata* (Stem)** | ***A. paniculata* (Leaf)** | ***A. paniculata* (Stem)** |
| Acetone | 19.84 | 21.97 | 51.28 | 63.95 |
| Ethanol | 17.63 | 19.82 | 60.71 | 68.27 |
| Methanol | 24.18 | 27.35 | 52.57 | 71.82 |

***In-vitro* radical scavenging activity**

**ABTS radical scavenging activity**

The ABTS assay is based on the inhibition by antioxidants of the absorbance of the ABTS radical cation (ABTS•+) (Sanchez-Moreno, 2002). The ABTS activity was found to be highest in methanolic extract in both leaf (9.43 mg trolox/g) and stem (8.61 mg trolox/g) samples (Table 2). The highest activity in methanolic extract may be due to high phenolic

compound when compared with other two extracts. The IC50 value was also computed (Table 4). Different radical scavenging capacity of the of the different extracts may be due to the different mechanisms involved in the radical-antioxidant reactions. Some compounds, though possessed ABTS•+ scavenging activity, did not exhibit DPPH scavenging activity (Wang *et al.*, 1998) [36]. Thus, comparison of assays is difficult and ranking of antioxidant activity is strongly dependent on the test system and on the substrate to be protected by the antioxidants (Frankel and Meyer, 2000).

**Table 2:** Free radical scavenging activity (ABTS, DPPH and NO) of stem and leaf extracts of *Andrographis paniculata*

| Solvents | ABTS (mg trolox/g) | | DPPH (mg BHT/g) | | NO (mg vit c/g) | |
|---|---|---|---|---|---|---|
| | *A. paniculata* (Leaf) | *A. paniculata* (Stem) | *A. paniculata* (Leaf) | *A. paniculata* (Stem) | *A. paniculata* (Leaf) | *A. paniculata* (Stem) |
| Acetone | 6.85 | 6.17 | 6.73 | 6.85 | 11.82 | 11.75 |
| Ethanol | 7.51 | 7.23 | 6.97 | 6.76 | 13.85 | 12.86 |
| Methanol | 9.43 | 8.61 | 7.08 | 7.15 | 12.13 | 12.15 |

**DPPH radical scavenging activity**

This is based on the reduction of DPPH- solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by the reaction. The extracts were able to reduce the stable radical DPPH- to the yellow-coloured diphenyl picryl hydrazine. Results from present study indicated highest DPPH activity in methanolic extract in both leaf (7.08 mg BHT/g) and stem samples (7.15 mg BHT/g). IC50 (concentration required for 50% reduction of scavenging activity) is shown in table 4. Smaller IC50 value correlated with higher DPPH scavenging activity. Highest IC50 value was found in methanolic extract in leaf (58.34 µg/ml) and stem (61.65 µg/ml) followed by ethanolic extract of leaf (58.34 µg/ml) and stem (61.65 µg/ml) samples. The higher scavenging activity was due to high level of phenolic content in methanolic extract of both leaf and stem samples. Phenolic compounds are high level antioxidants (Hall and Cuppett, 1997) because they possess the ability to adsorb and neutralize free radicals, quench active oxygen species and decompose superoxide and hydroxyl radicals (Duh *et al.*, 1999) [8].

**Table 3:** Free radical scavenging activity (MCA and $H_2O_2$)) of stem and leaf extracts of *Andrographis paniculata*

| Solvents | MCA (mg EDTA/g) | | $H_2O_2$) (mg vit c/g) | |
|---|---|---|---|---|
| | *A. paniculata* (Leaf) | *A. paniculata* (Stem) | *A. paniculata* (Leaf) | *A. paniculata* (Stem) |
| Acetone | 13.67 | 11.69 | 3.16 | 2.97 |
| Ethanol | 13.82 | 12.18 | 3.92 | 3.18 |
| Methanol | 14.65 | 12.85 | 4.76 | 3.75 |

**Table 4:** IC 50 values (µg/ml) of stem and leaf extracts of *Andrographis paniculata* with different antioxidant methods

| Solvents | ABTS | | DPPH | | $H_2O_2$ | |
|---|---|---|---|---|---|---|
| | *A. paniculata* (Leaf) | *A. paniculata* (Stem) | *A. paniculata* (Leaf) | *A. paniculata* (Stem) | *A. paniculata* (Leaf) | *A. paniculata* (Stem) |
| Acetone | 198.31 | 158.14 | 192.95 | 190.64 | 1258.75 | 1245.56 |
| Ethanol | 192.48 | 169.33 | 58.34 | 61.65 | 713.79 | 623.66 |
| Methanol | 168.51 | 138.79 | 45.67 | 48.69 | 535.35 | 449.36 |

**Nitric oxide scavenging activity**

Nitric oxide (NO) is generated by endothelial cells, macrophages, neurons, *etc* and is involved in the regulation of various physiological processes (Lata *et al.*). Concentration of NO in excess may cause several diseases (Ialenti *et al.*). The toxicity caused by NO and O2 multiplies as they react to produce reactive peroxynitrite, which leads to serious toxic reactions with biomolecules such as protein, lipids and nucleic acids (Swain, *et al.*, 2020) [33]. In present study, kalmegh extracted in different extracts effectively reduced the generation of NO. The activity was highest in ethanolic extract in both leaf (13.85 mg vit C/g) and stem (12.86 mg Vit C/g). The NO activity was found lowest in acetone extract of both stem (11.82 mg vit C/g) and leaf of kalmegh (11.75 mg vit C/mg). The ethanolic extract of leaf of kalmegh has better nitric oxide radical scavenging activity in competing with oxygen to react with nitric oxide and thus the inhibition of generation of anions. Similar results were reported by Bakakrishnan and Kokilavani, 2011 and Swain *et al.*, 2020 [3, 33]. Thus, the ethanolic extract of both stem and leaf of kalmegh is very effective in scavenging the nitric oxide free radical.

**Metal chelating activity**

In antioxidant mechanisms, metal chelating activity (MCA) plays a significant role as it reduces the concentration of the catalyzing transition metal in LPO (Duh *et al.*, 1999) [8]. The chelating agents recues redox potential and thus stabilizes the oxidized form of the metal ion (Kumaran and Karunakaran, 2006) [2]. In present study the metal chelating activity was more in leaf extract when compared with stem extract. The methanol extract of leaf (14.65 mg EDTA/g) and stem (12.85 mg EDTA/g) showed higher chelating activity. The higher metal chelating activity in both leaf and stem extract of kalmegh shows its effectiveness to control oxidative damage to cell as reported in other crops like *Alpinia calcarata* (Swain *et al.*, 2020) [33].

**Hydrogen peroxide radical scavenging activity**

Hydrogen peroxide is mostly non-reactive but may become toxic rarely as it is converted to free radical (OH) that react with biomolecules and cause tissue damage and cell death (Khan *et al.*, 2012) [14]. The acetone extract of stem (3.16 mg vit c/g) and leaf (2.97 mg vit c/g) of kalmegh showed effective reduction in the activity of hydrogen peroxide. The scavenging of hydrogen peroxide by the extract may be attributed to active secondary metabolites, phenolics which neutralize hydrogen peroxide by donating electrons thereby neutralizing it to water (Mathew and Abraham, 2006) [16].

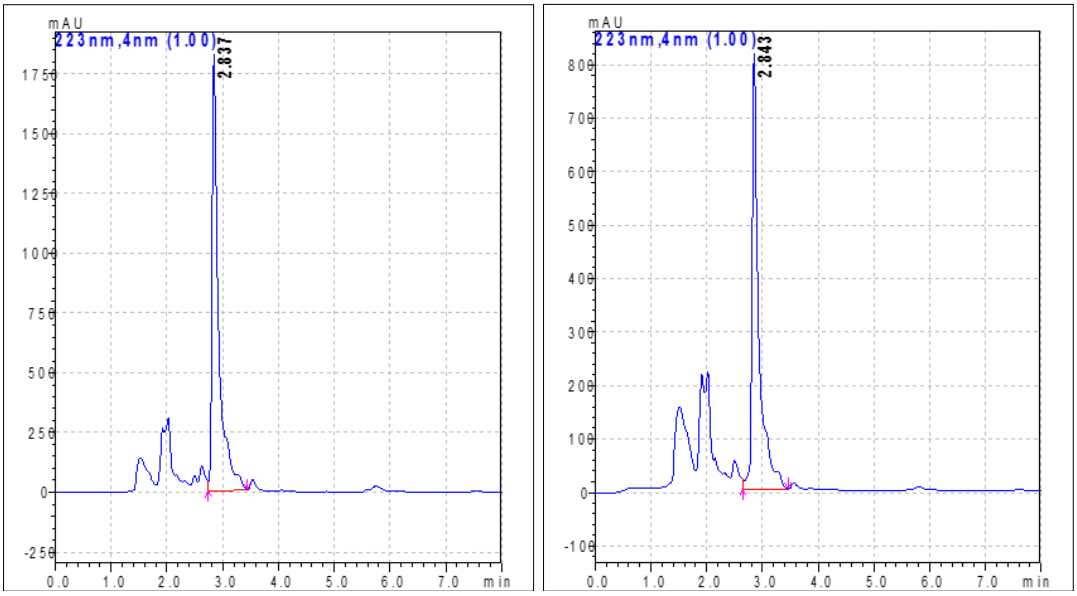
**Andrographolide content**

The study on the estimation of andrographolide content in stem and leaf extracts of kalmegh showed (Table 5) maximum content in leaf (3.49%) followed by stem (1.59%). Earlier studies showed the presence of andrographolide content from 1.14% to 2.60% (Raina *et al.*, 2007) [19]. However, higher concentration in leaves from 6.23% to 6.96% is also reported based on seasonal influence (Bhan *et al.*, 2006) [5]. The variation in the andrographolide content is due to the influence of environmental, seasonal and other edaphic factors. The geographical location of the species also plays a vital role in composition of the secondary metabolite.

The Andaman and Nicobar Island witnesses luxurious growth of kalmegh and found growing wild. Good concentration of andrographolide and other phytochemical compounds in both leaves and stems show wide scope for commercial exploitation of this crop under different cropping system.

**Table 5:** Andrographolide content in leaf and stem extracts of *Andrographis paniculata*

| Sample | RT | Area | Andrographolide (%) |
|---|---|---|---|
| *Andrographis paniculata* Stem | 2.843 | 6833115 | 1.59 |
| *Andrographis paniculata* Leaves | 2.837 | 14902685 | 3.49 |




Andrographolide (%) in stem Andrographolide (%) in leaf

**Fig 1:** Andrographolide (%) content in leaf and stem of *Andrographis paniculata*

**References**


1. Akbar S. *Andrographis paniculata*: A review of pharmacological activities and clinical effects. Alternative Medicine Review 2011;16(1):66-77.
2. Archana PR, Sivaraj N, Kumar A. Chemical diversity among *Andrographis Paniculata* Nees (Kalmegh) and assessing climate suitable regions for elite germplasm distribution in India. Medicinal Plants 2016;8(4):294-302. DOI: 10.5958/0975-6892.2016.00036.8
3. Balakrishnan A, Kokilavani R. *In vitro* free radical scavenging activity of+ethanolic extract of *Cucumis trigonus* Roxburxii fruit. Int. J Pharm Biol Arch 2011, 1439-1443.
4. Bao J, Cai Y, Sun M, Wang G, Corke H. Anthocyanins, flavanols and free radical scavenging activity of Chinese Bayberry (*Myrica rubra*) extracts and their colour properties and stability. Journal of Agricultural and Food Chemistry 2005;53(6):2327-2332.
5. Bhan MK, Dhar AK, Khan S, Lattoo KK, Gupta KK, Choudhary DK. Screening and optimization of *Andrographis paniculata* (Burm.f.) Nees for total andrographolide content, yield and its components. Scientia Horticulturae 2006;107(4):386-391.
6. Calabrese C, Berman SH, Babish JG, Ma X, Shinto L, Dorr M *et al.* A phase I trial of andrographolide in HIV positive patients and normal volunteers. Phytotherapy Research 2000;14:333-338.
7. Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (Acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys 1994;315:161-169.
8. Duh PD, Tu YY, Yen GC. Antioxidant activity of water extract of Harng Jyur (*Chrysanthemum morifolium* Ramat). Lebensm. Wiss. Technol 1999;32:269-277.
9. Gilani AH, Rahman AU. Trends in Ethnopharmacology. Journal of Ethnopharmacology 2005;100:43-49.
10. Hall CA, Cuppett SL. Structure Activities of Natural Antioxidants. In: Antioxidant Methodology *in vivo* and *in vitro* concept Aruoma OI, and SL Cuppett (Eds.). AOCS Press, Champaign, IL 1994. ISBN: 9780935315790, 141-170.
11. Hiremath C, Greeshma M, Namita Gupta, Baskaran KK, Shanker, Sundaresan V. Morphometric, chemotypic, and molecular diversity studies in *Andrographis paniculata*, Journal of Herbs, Spices & Medicinal Plants, DOI 2020. 10.1080/10496475.2020.1787290
12. Kabir MH, Hasan N, Rahman MM. A survey of medicinal plants used by the Deb Bar*ma clan of the Tripura tribe of Moulvibazar district, Bangladesh. Journal of Ethnobiology and Ethnomedicine 2014;10(1):19.
13. Karunamoorthi K, Jegajeevanram K, Vijayalakshmi J, Mengistie E. Traditional medicinal plants: A source of phytotherapuetic modality in resource constrained health care settings. Journal of Evidence-Based Complementary & Alternative Medicine 2013;18(1):67-74.
14. Khan RA, Khan MR, Sahreen S. Evaluation of phenolic contents and antioxidant activity of various solvent

extracts of *Sonchus asper* (L.) Hill. Chemistry Central Journal 2012;6:12. https://doi.org/10.1186/1752-153X-6-12.
15. Kumar A, Ilavarasan R, Jajachandran T, Decaraman M, Aravindhan P, Padmanaban N *et al.* Phytochemical Investigation on tropical plants. Pakistan journal of Nutrition 2009;8:83-85.
16. Mathew S, Abraham TE. Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various *in vitro* models. Food Chem 2006;94:520-528.
17. Okeke IN, Laxmaninarayan R, Bhutta ZA, Duse AG, Jenkins P. Antimicrobial resistance in developing countries. Part 1: Recent trends and current status, Lancet Infect. Dis 2005;5:481-493.
18. Pharmacopoeia of India. Government of India Press. Calcutta 1955, 355.
19. Raina AP, Kumar A, Pareek SK. HPTLC analysis of hepatoprotective diterpenoid andrographolide from *Andrographis paniculata* Nees (Kalmegh). Indian Journal of Pharmaceutical Science 2007;69:473-475.
20. Raina AP, Gupta V, Sivaraj N. *Andrographis paniculata* (Burm.f.) Wall. Ex Nees (Kalmegh), A traditional hepatoprotective drug from India. Genetic resources and Crop Evolution 2013;60:1181-1189.
21. Rattanachitthawat S, Suwannalert P, Riengrojpitak S, Chaiyasut CB, Pantuwatana S. Phenolic content and antioxidant activities in red unpolished Thai rice prevents oxidative stress in rats. Journal of Medicinal plants Research 2010;4:796-801.
22. Re R, Pellegrini N, Proteggente A, Pannala A, Yang, Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free radical biology and Medicine 1999;26:1231-1237.
23. Ruch RT, Cheng SJ, Klaunig JE. Spin trapping of superoxide and hydroxyl radicals. Methods in enzymology 1984;105:198-209.
24. Saxena S, Jain DC, Bhakuni RS, Sharma RP. Chemistry and pharmacology of Andrographis species. Indian Drugs 1998;35:458-467.
25. Scartezzini P, Speroni E. Review of some plants of Indian traditional medicine with antioxidant activity. Journal of Ethno pharmacology 2000;71:23-43.
26. Sharma M, Sharma A, Tyagi S. Quantitative HPLC analysis of andrographolide in Andrographis paniculata at different stages of life cycle of the plant. Acta Chim Pharm Indica 2012;2:1-7.
27. Sharma M, Suman J. Comparison of antioxidant activity of *Andrographis paniculata* and *Tinospora cordifolia*. Journal of current chemical and pharmaceutical sciences 2011;1(1):1-8.
28. Sharma SN, Sinha RK, Sharma DK, Jha Z. Assessment of Intra-specific Variability at Morphological, Molecular and Biochemical Level of Andrographis Paniculata (Kalmegh). Current Science 2009;96(3):402-408.
29. Sheeja K, Kuttan G. Activation of cytotoxic T lymphocyte responses and attenuation of tumor growth *in vivo* by *Andrographis paniculata* extract and andrographolide. Immuno pharmacology and Immunotoxicology 2007;29:81-93.
30. Singleton VL, Orthofer R, Lamuela-Raventos. Analysis of total phenols and other oxidation substrated and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology, Academic press 1999;299:152-178.
31. Siripong P, Kongkathip B, Preechanukool K, Picha P, Tunsuwan K, Taylor WC. Cytotoxic diterpenoid constituents from *Andrographis paniculata*, Nees leaves. J Sci Soc Thailand 1992;18:187-194.
32. Sivanandan M, Ilamaran M. Medicinal and pharmacological properties of *Andrographis paniculata.* International Journal of Biomolecules and Biomedicine 2013;3(2):1-12.
33. Swain S, Pankhuri T, Kavita G, Laxmi MNV, Subramani T. *In vitro* antioxidant and free radical scavenging activity of Alpinia calcarata: A novel underutilized crop in Tropical Island. Journal of Pharmacognosy and Phytochemistry 2020;9(5):243-248.
34. Trivedi NP, Rawal UM, Patel BP. Hepatoprotective effect of andrographolide against hexachlorocyclohexane-induced oxidative injury. Integr Cancer Ther 2007;6:271-280.
35. Upadhyaya S, Mahanta JJ, Saikia LR. Antioxidant activity, phenol and flavonoid content of a medicinal herb *Andrographis paniculata* (Burm. F.) Nees grown using different organic manures. Journal of Pharmacy Research 2011;4(3):614-616.
36. Wang MJ, Li Rangarajan M, Shao Y, LaVoie EJ, Huang TC, Ho CT. Antioxidative phenolic compounds from sage (*Salvia officinalis*). J Agric. Food Chem 1998;46:4869-4873.
37. Yoopan N, Thisoda P, Rangkadilok N, Sahasitiwat S, Pholphana N, Ruchirawat S *et al.* Cardiovascular effects of 14-deoxy-11,12-didehydroandrographolide and *Andrographis paniculata* extracts. *Planta medica* 2007;73:503-511.