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Quality Assessment Of Andrographis Paniculata (Burm. F.) Wall. Ex Nees W.S.R To Geographical Region.

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Article History

Abstract

Introduction: The place of collection of medicinal plants plays an important role in determining the extent of its therapeutic action. In Sharangadhara Samhita, *Ushna Dravya* is said to be collected from *Vindhya* and *Sheeta Dravya* from *Himalaya*. Each geographical location has a specific type of natural flora and shows similarities in properties and actions. *Kalmegh Andrographis paniculata* (Burm. F.) Wall. Ex Nees is one of the main ingredients used in formulations like *Bhunimbadi Kashaya*, etc. India`s various localities are favourable for the growth and cultivation of *Kalamegh* plant, but the geographical location and environmental factors influence the physicochemical and phytochemical characters.
Methodology: The plant along with roots were collected from Belgaum (*Sadharana desha*), Koppala (*Jangala desha*), and Kerala (*Anupa desha*) and analysed for Macroscopic features, Phytochemicals, HPTLC fingerprinting, total Phenols and total Flavonoids. The standard methods were followed.
Results: All samples showed physicochemical charaters within the API limits but showed variations in chemical strength. At UV 366 NM, the samples from Belgaum and Koppala showed the highest number of phyto-constituents. The peaks in these two samples matched in their position but the concentration appeared to be higher in the Belgaum sample. The Belgaum sample showed the highest content of both flavonoids and phenols (6.64±0.00 mg QE /gram of Hydro Alcoholic extract and 28.03±0.69mg GAE/ gram of Hydro Alcoholic extract respectively).
Conclusion: The results of the present study proved that medicinal plant quality is influenced by the geographical area from which the plant is collected w.r.t the physicochemical & chemical strength. The sample collected from Belgaum showed more potent properties compared to the Koppala and Kerala samples.

Keywords: Kalamegha, Andrographis paniculata, Phytochemicals,

 | ***HPTLC, Phenols, Flavonoids, and Geographical region.***

1. Introduction*:*

Andrographis paniculata (Burm. F.) Wall. Ex Nees (AP) also called *Kalmegh* or "King of Bitters" belongs to the family Acanthaceae. It has been used for centuries in Asia to treat gastrointestinal tract and upper respiratory infections, fever, herpes, sore throat, and a variety of other chronic and infectious diseases[1,2]. Indian Pharmacopoeia mentions that it is a predominant ingredient in many of the Ayurvedic formulations used for treating various diseases. In Traditional Chinese Medicine (TCM), *Andrographis* is considered a herb possessing an important "cold property" useful to treat the heat of the body in fevers and to dispel toxins from the body. In Scandinavian countries, it is commonly used to prevent and treat common colds[3].
Habitat: It grows abundantly in south-eastern Asia: India (and Sri Lanka), Pakistan, and Indonesia but it is cultivated extensively in China and Thailand, the East and West Indies, and Mauritius. *Andrographis paniculata* is normally grown from seeds ubiquitously in its native areas where it grows in pine, evergreen, and deciduous forest areas, along roads, and in villages[4].

2. Materials & Methods

2.1 Collection of plant parts
Roots along with the selected plant were collected as per the Guidelines of the National Medicinal Plants Board. The selected plants samples were collected in *Sharad rutu* i.e. in October and November. The roots were collected with minimum required digging with the use of suitable tools. Collected roots and other parts of the plants were thoroughly washed and cut into appropriate sizes and dried under sunlight[5,6].

2.2 Quality assessment of plant parts:
2.2.1 Identification and Authentication
Plants and collected plant parts were identified and authenticated by experts, Central Research Facility, AYUSH approved laboratory for ASU drugs, KAHER`s Shri. B.M. Kankanawadi Ayurveda Mahavidyalaya Belagavi.
Voucher no: CRF/auth/260-74/2016. Plants and their parts were identified and authenticated based on pharmacopeia standards, floras, and the Dravyaguna textbook.

2.2.2 Macroscopic study:
The macroscopic study was done w.r.t the physical evaluation of the plant materials in terms of colour, odour, shape, surface, fracture, etc.

2.2.3 Physicochemical analyses of plant parts:
Physicochemical analysis of the plant parts was done as per the parameters mentioned in Ayurvedic Pharmacopoeia of India.[7] The parameters are Foreign Matter, Total Ash, Acid insoluble ash, Water soluble extractive, and Alcohol Soluble Extractive.

2.2.4 HPTLC fingerprinting of the collected samples:
Analysis was done at Natural Remedies Bangalore. The alcohol extract of collected samples was applied band-wise using a Linomat V applicator (CAMAG, Muttenz, Switzerland) on a commercial 20 cm × 10 cm precoated HPTLC plate Silica gel 60 F254 (Merck). The application conditions were: carrier gas is nitrogen; syringe delivery speed is 10 s/µL; application volume is 10 µL; bandwidth is 8 mm; space between two bands is 20 mm; distance from bottom is 10 mm. 15 ml of mobile phase consisting of Toluene: ethyl acetate: methanol in the ratio of 7:3:1. The result was examined under UV 254 nm & 366 nm by using a UV viewer cabinet (CAMAG).[8]

2.2.5 Assay of Total Phenols & Flavonoids by UV Spectroscopy:
a) Determination of Total Phenols
The total phenols content of the plant was determined by the Folin- Ciocalteu colorimetric method and Gallic acid was used as standard.[9]

Brief protocol:
Gallic acid was used to make the calibration curve by dissolving it in methanol and then diluted to 6.25 - 100 µg/ml of serial concentrations. Stock Solution of Extracts (1mg/ml) was also prepared with methanol.
Reaction Solutions of 10ml contained: Sample extract stock solution / (Gallic acid standard) mixed with 10 fold dilute Folin- Ciocalteu reagent and 7.5% sodium carbonate. The tubes were covered with Parafilm and allowed to stand for 30 minutes at room temperature before and the absorbance was read at 760 nm. Blank was prepared similarly by replacing the sample or standard with methanol.
Sample and standard absorbance were measured at 760 nm with a Shimadzu UV-1800 spectrophotometer. A calibration curve using ABSORBANCE vs CONCENTRATION of Gallic acid standard was prepared and the concentration of total phenols in the sample was determined by using a slope equation that was obtained from the standard graph and results for total phenols were expressed as mg of Gallic acid equivalent /gm dried extract.

b) Determination of total flavonoids
The total flavonoid content of the plant was determined by the Aluminium chloride colorimetric method; quercetin was used as standard.

Brief protocol:
Quercetin was used to make the calibration curve by dissolving in methanol/Ethanol and then diluted to 6.25-200 µg/ml serial concentrations. Stock Solution of Extracts(1mg/ml) was prepared with methanol/Ethanol.
Reaction Solutions of 10 ml contained: Sample extract stock solution / (quercetin standard) to which Methanol, 10% Aluminium Chloride, 1M Potassium Acetate solution, and distilled water were added and mixed well. Sample blank was prepared similarly by replacing Aluminium chloride with distilled water.
Sample and standard absorbance were measured at 420 nm with a Shimadzu UV-1800 spectrophotometer. A calibration curve using ABSORBANCE vs CONCENTRATION of Quercetin standard was prepared and the concentration of total flavonoid in the sample was determined by using a slope equation that was obtained from the standard graph and the result for total flavonoids was expressed as mg of quercetin equivalent /gm dried extract.

3. Results:

3.1 Macroscopic study
The macroscopic/ organoleptic study results of the samples collected from different localities are depicted in the table (Table 1).

Table 1- macroscopic characters of the collected samples.

Parameter	API Standards	Belgaum	Kerala	Koppala
Part	**Whole Plant**	Whole Plant	Whole Plant	Whole Plant
Colour	**Greenish**	Greenish	Greenish	Greenish
Odor	**Characteristic**	Characteristic	Characteristic	Characteristic
Taste	**Bitter**	Bitter	Bitter	Bitter

3.2 Physicochemical Analysis
The results of physicochemical analyses of the samples collected from different localities are depicted in the table (Table 2).

Table 2- Physicochemical characteristics of the collected samples.

Parameter	API Limit	Belgaum	Kerala	Koppala
Foreign matter	**NMT 2%**	Nil	Nil	Nil
Ash value	**NMT 15%**	1.401%	1.221%	1.862%
Acid Insoluble Ash	**NMT 3%**	0.628%	0.845%	0.924%
Water Soluble Extractive	**NLT 19%**	26.114%	20.125%	23.012%
Alcohol Soluble Extractive	**NLT 12%**	15.056%	12.954%	14.145%

3.3 HPTLC Results

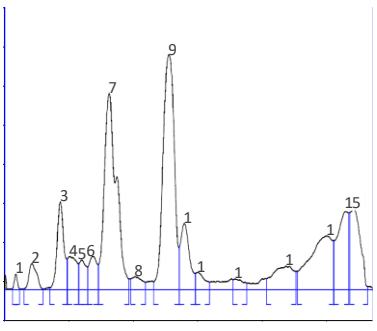
The Rf values obtained by HPTLC analysis of the samples collected from different localities visualized at UV 254 NM are depicted in Table 3. The sample collected from Belgaum showed the maximum Rf values (16). The one collected from Koppala showed 13 Rf values while the sample collected from Kerala showed 11 Rf values.

Table 3- Rf values obtained by HPTLC analysis of the collected samples at UV 254 NM.

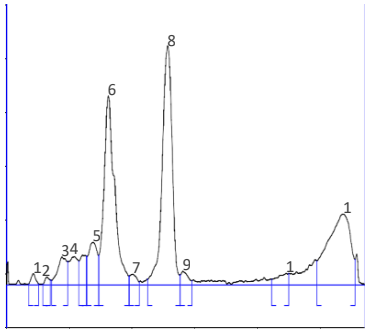
	Belgaum		Kerala		Koppala	
Peak	Rf	Area%	Rf	Area %	Rf	Area %
1	0.02	0.40	0.07	0.65	0.05	0.57
2	0.06	1.75	0.12	0.43	0.08	0.57
3	0.14	5.40	0.14	3.50	0.14	7.54
4	0.19	2.72	0.23	2.68	0.20	2.95
5	0.23	1.87	0.26	5.23	0.23	4.17
6	0.26	2.38	0.29	27.94	0.27	2.88
7	0.29	21.67	0.39	0.94	0.31	23.32
8	0.39	1.20	0.45	33.18	0.44	6.34
9	0.46	24.15	0.55	1.28	0.50	20.89
10	0.54	5.31	0.85	1.71	0.57	1.01
11	0.59	1.35	0.99	22.47	0.74	1.24
12	0.71	0.93	--	--	0.84	4.30
13	0.81	4.34	--	--	1.00	24.22
14	0.91	11.15	--	--	--	--
15	1.02	7.85	--	--	--	--
16	1.07	7.53	--	--	--	--

The HPTLC fingerprinting of the three samples collected from different localities at UV 254 NM is shown in Graph 1. A maximum number of peaks are seen in the sample collected from Belgaum. The phytoconstituents present in all the samples show similar concentrations as evident from the HPTLC fingerprint graph. However, the sample from Belgaum shows additional peaks.

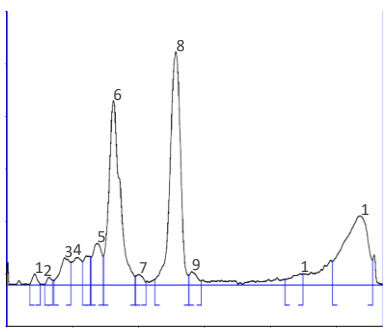
Graph 1- HPTLC fingerprinting of the samples at 254 NM



Belgaum



Kerala



Koppala

The Rf values obtained by HPTLC analysis of the samples collected from different localities visualized at UV 366 NM are depicted in the Table 4. The sample collected from Belgaum showed the maximum Rf values (13). The one collected from Koppala also showed 13 Rf values while the sample collected from Kerala showed 7 Rf values at this wavelength.

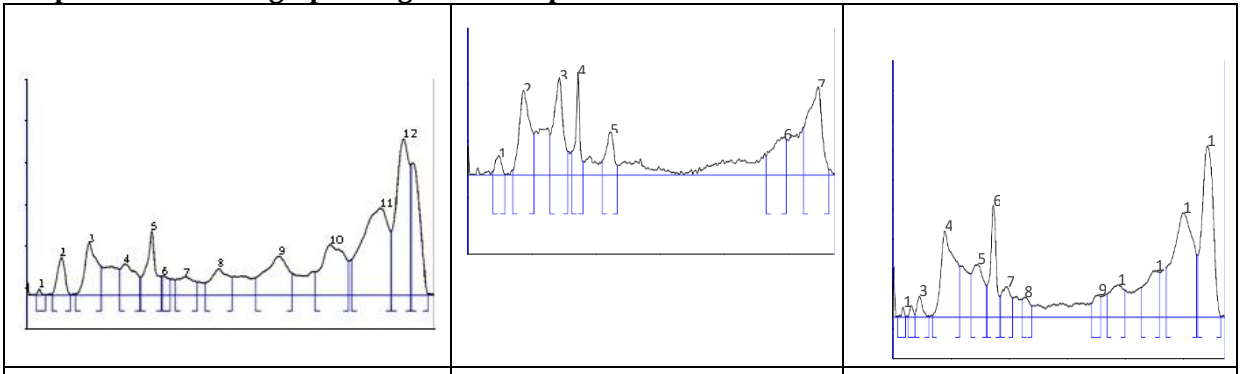
Table 4- Rf values obtained by HPTLC analysis of the collected samples at UV 366 NM.

	Belgaum		Kerala		Koppala	
Peak	Rf	Area %	Rf	Area %	Rf	Area %
1	0.02	0.14	0.08	2.55	0.02	0.26
2	0.07	2.65	0.14	20.32	0.05	0.42

3	0.13	5.97	0.26	20.71	0.08	1.26
4	0.26	4.39	0.32	9.34	0.14	14.11
5	0.32	5.45	0.42	7.72	0.27	7.52
6	0.38	1.25	0.93	12.05	0.32	7.95
7	0.41	2.90	1.05	27.31	0.37	3.27
8	0.50	4.42	--	--	0.45	1.70
9	0.64	8.58	--	--	0.68	1.91
10	0.81	11.10	--	--	0.74	5.38
11	0.91	22.11	--	--	0.86	7.67
12	1.02	20.12	--	--	0.94	25.02
13	1.08	10.93	--	--	1.05	23.54

The HPTLC fingerprinting of the three samples collected from different localities at UV 366 NM is shown in the graph 2. Maximum number of peaks are seen in the sample collected from Belgaum. As seen in the graph, the samples from Belgaum and Koppala have highest number of phytoconstituents indicating their potency and probable therapeutic efficacy. Whereas the sample from Kerala shows lowest number of peaks. The peaks in the samples from Belgaum and Koppala match in their position but the concentration appears to be more in Belgaum sample.

Graph 2- HPTLC fingerprinting of the samples at 366 NM



Belgaum	**Kerala**	**Koppala**

The HPTLC bands of the three samples are shown in images 1, 2 & 3. All the three samples show similar bands. Number and intensity of bands appears to be more in Belgaum sample.

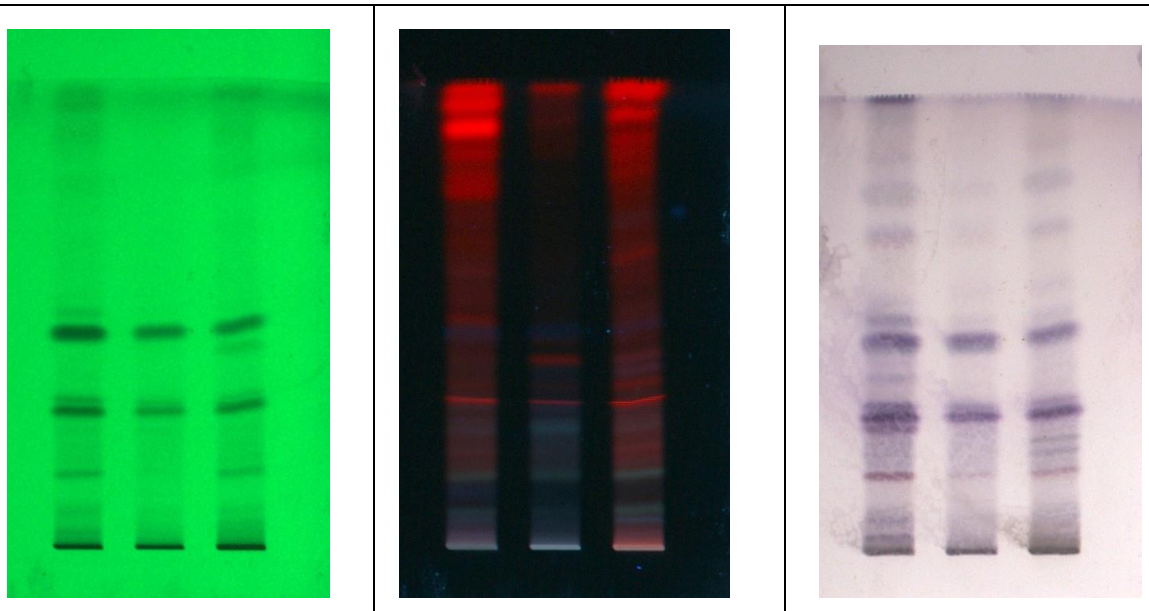


Image 1- HPTLC bands at 254 NM	**Image 2- HPTLC bands at 366 NM**	**Image 3- HPTLC bands after derivatization**

3.4Total Phenols & Flavonoids Content:

The total phenol and flavonoid content in the three samples is depicted in Table 5. Both total phenols and flavonoids are found to be highest in the sample collected from Belgaum. The next highest concentration was found in the Koppala sample while the Kerala sample showed the least concentrations of both total phenols and flavonoids.

Table 5- Total phenols and Flavonoids in the three samples

Sample	Total Phenolic	Total Flavonoid
Belgaum	28.03 ± 0.69mg GAE/ gram of Hydro Alcoholic extract	6.64±0.00 mg QE /gram of Hydro Alcoholic extract
Kerala	23.42 ± 0.27mg GAE/ gram of Hydro Alcoholic extract	4.37± 0.25 mg QE /gram of Hydro Alcoholic extract
Koppala	25.81 ± 0.51mg GAE/ gram of Hydro Alcoholic extract	5.58± 0.28 mg QE /gram of Hydro Alcoholic extract

4. Discussion:

The place of collection of medicinal plants plays an important role in determining the extent of therapeutic action of the drug. Specific place of collection for different medicinal plants is mentioned in the classical Ayurvedic literature which depends on the intended pharmacological action. Acharya Sharangadhara mentions that *Ushna virya* drugs should be collected from the Vindhya ranges whereas the *Sheeta virya* drugs should be collected from the Himalayan ranges[10]. Each geographical location has a specific type of natural flora that show similarities in properties and actions. *Jangala desha* (dry area) is said to be predominant of *Vata dosha* and is the habitat for trees like *Khadira* (*Acacia catechu*), *Asana* (*Pterocarpus marsupium*), *Badara* (*Zizyphus jujube*), *Vata* (*Ficus benghalensis*), *Amalaki* (*Emblica officinalis*) etc., *Anupa desha* is wetland with lakes and seas, full of trees and flowering plants and is the natural habitat for plants like *Narikela* (*Coconut tree*), *Kadali* (*Musa paradisiacal*), *Hintala* (Date palm) etc., *Sadharana desha* shows mixed climatic features and is the habitat for both kinds of plants and trees[11]. This division of area depending on the climatic conditions is very scientific. Today we come across tropical endemic diseases like Filariasis which shows more incidences in marshy areas (*Anupa desha*). Similarly respiratory problems like Bronchial asthma show more incidences in people dwelling in cold, hilly areas. Further, when we plan treatment for such diseases, we choose drugs having opposite qualities that come from the soil (*desha*) with specific characteristics. Eg: Bronchial asthma (*Tamaka shwasa*) is a disease that shows more incidence in *Anupa desha* and the drugs used for its treatment like *Dashamoola, Shirisha, Vasa, Amalaki, Pushkaramoola,* etc., are derived from *Jangala desha* (dry areas).

In *Ayurveda* system of Medicine, *Kalmegh Andrographis paniculata* (Burm. F.) Wall. Ex Nees is one of the main ingredients used in many formulations like *Bhunimbadi Kashaya* etc. It is used in treating fevers, hepatobiliary disorders, and different skin diseases. The drug has *Tikta rasa, and Laghu-Ruksha guna*, the set of properties that are useful in treating the diseases which are *kleda pradhana* (pathology involving increased *kleda/ snigdha guna* in the systems). So theoretically it is best to collect the drug from dry areas. In order to validate this concept of drug collection from different areas in relation to the properties of drug, *Kalmegh Andrographis paniculata* (Burm. F.) was collected from 3 different places namely Belgaum (*Sadarana desha*), Koppala (*Jangala desha*) and Kerala (*Anupa desha*) and analyzed w.r.t the physico chemical properties and chemical strength.

The present study reveals that though all three samples showed physicochemical properties within the API limits, *Kalmegh* collected from the Belgaum region has better physicochemical strength w.r.t the extractive values.

The HPTLC fingerprinting of the three samples showed that maximum number of peaks are in the sample collected from Belgaum. The phyto-constituents present in all the samples showed similar concentration as evident from the HPTLC fingerprint graph. However, the sample from Belgaum shows additional peaks. This indicates that since Belgaum is *Sadharana Desha*, it is the best place to collect the drugs for their maximum potency and therapeutic efficacy. At UV 366 NM, the samples from Belgaum and Koppala showed the highest number of phytoconstituents. The peaks in these two samples match in their position but the concentration appears to be higher in the Belgaum sample. Even the HPTLC bands were similar in these two samples. However, the Belgaum sample showed a higher number and intensity of bands. This confirms that *Jangala* and *Sadharana* localities are better for collecting this drug. When collected from such places,

Kalamegha might be showing optimum *Tikta rasa* and other properties responsible for bringing about the desired therapeutic action.

Similar study by M. Sharma and R. Sharma (2013)[12] on *Andrographis paniculata* showed that compositions of phyto-chemicals widely differ in terms of the part used, geography, season, and time of harvesting. A total of 32 bioactive compounds with seven ent-labdane diterpenoids, twelve flavonoids, and two quinic acid derivatives were isolated and characterized during this study.

In the present study both total phenols and flavonoids are found to be highest in the sample collected from Belgaum. The next highest concentration was found in the Koppala sample while the Kerala sample showed the least concentrations of both total phenols and flavonoids. During a similar study, novel flavonoid, 7, 8-dimethoxy-2′-hydroxy-5-O-β-d-glucopyranosyloxyflavone, together with 15 known flavonoids were isolated from the aerial parts of AP by Chen L. Et al., (2014)[13]. The structure was elucidated based on chemical and spectroscopic analysis. Significant antiproliferative activity of these flavonoids against human leukemia HL-60 cell was also investigated.

A. *paniculata* leaf extract was screened by Ali S.K. et al., (2023)[14] for phytoconstituents and antioxidant and hepatoprotective effects in Wistar albino rats against CCl4-induced liver dysfunction. Phytochemical analysis revealed the presence of flavonoids, alkaloids, and phenolic compounds in all extracts. The phenolic concentration ranged from 10.23 to 19.52 mg gallic acid per gram of the sample, while the highest flavonoid concentration was found in the ethanol fraction (8.27 mg rutin equivalents per gram). The results of this study conclusively demonstrated that A. *paniculata* extracts are a rich source of phytochemicals and possess significant antioxidant, free radical scavenging, and hepatoprotective properties. While in the current study, the Belgaum sample showed the highest content of both flavonoids and phenols (6.64±0.00 mg QE /gram of Hydro Alcoholic extract and 28.03 ±0.69mg GAE/ gram of Hydro Alcoholic extract respectively) which is beyond the range mentioned in the above study by Ali S.K. et al., (2023).

5. Conclusion:

The results of the present study proved that medicinal plant quality is influenced by the geographical area from which the plant is collected w.r.t the physico-chemical & chemical strength.

The sample collected from Belgaum showed more potent properties compared to the Koppala and Kerala samples. The study supports that the collection of drugs w.r.t specific geographical location should be considered along with the standard operating procedures (SOP) for the collection of medicinal plants.

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