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A Comparative Chemo-Profile Variation Study of Kalamegha (Andrographis paniculata (Burm.F.) Nees) Collected from various Bhoomi Desha of Karnataka by using HPLC Method

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Abstract: ***Background and Objectives: In Ayurveda, Desha is an important factor influencing the Guna and Karma of medicinal plants. Kalamegha (Andrographis paniculata (Burm.f.) Nees) is an important Ayurvedic drug known for its Tikta Rasa and therapeutic value. The present study was conducted to evaluate the influence of habitat on the quality, potency and andrographolide content of Kalamegha. Methods: Kalamegha Panchanga was collected from three Bhoomi Desha of Karnataka: S1 from Jangala Desha (Ballari), S2 from Anupa Desha (Mangalore) and S3 from Sadharana Desha (Belagavi). The samples were authenticated, shade-dried, powdered and subjected to pharmacognostical, physicochemical, phytochemical, quantitative inorganic, RP-HPLC and soil analysis. In-vitro antifungal activity was assessed by agar well diffusion method.***
Results: Organoleptic and microscopic characters confirmed the identity and genuineness of all samples. Physicochemical parameters were within API limits. Phytochemical screening revealed presence of saponins, tannins, alkaloids, phenols and terpenoids. Quantitative inorganic analysis showed mineral variation, with higher calcium, zinc and iron in the Anupa Desha sample and higher manganese in the Sadharana Desha sample. RP-HPLC analysis showed variation in andrographolide content, with ethanolic extracts showing better yield than aqueous extracts. Comparatively, Jangala Desha showed the highest andrographolide content due to elevated nitrogen content in the soil. Soil analysis showed variation in pH and mineral parameters among the collected samples. In-vitro antifungal activity showed concentration-dependent activity, with Jangala Desha showing the highest zone of inhibition.
Interpretation and Conclusion: Bhoomi Desha influences the chemo-profile and biological activity of Kalamegha. Comparatively, Jangala and Sadharana Desha samples showed higher percentage of Andrographolide concentration, Water and Alcohol soluble extractive values, supporting the Ayurvedic concept of superiority of Jangala and Sadharana Desha for Dravya Sangrahana.
Keywords: Kalamegha, Andrographis paniculata, Bhoomi Desha, Andrographolide, HPLC, Antifungal activity.

I. INTRODUCTION

According to WHO, almost 80% of the world's population is depending on traditional medicine and in India 60% of the people in rural areas use herbal medicines[1]. Ayurveda is based on the fundamental principle of *Trisutra*, namely *Hetu*, *Linga* and *Aushadha*.[2] Among these, *Aushadha* holds a significant place in *Chikitsa*, as it directly contributes to the restoration of *Dosha samyata* and maintenance of health. The concept of *Aushadha* is closely linked with *Dravya*, because therapeutic action is expressed through appropriately selected medicinal substances. Thus, *Dravya* forms the practical basis of *Aushadha* and plays a vital role in the successful management of disease.

Acharya Charaka explained *Dravya* (medicinal plants) grown in *Jangala Desha* possess more medicinal quality when compared to *Anupa Desha*[3]. While discussing *Bheshaja Pariksha* (examination of medicines), *Acharya* highlights the importance of evaluating the *Guna* of *Dravya* along with their *Desha*. This underscores the principle that the therapeutic efficacy of a substance is inseparably linked to the ecological conditions in which it grows.

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Kalamegha, *Andrographis paniculata* (Burm.f.) Nees, is an important Ayurvedic medicinal plant used in the management of liver-related disorders. It belongs to the family Acanthaceae and is commonly known as the "King of Bitters" due to its intensely bitter taste. In *Priya Nighantu*, *Acharya Priyavat Sharma* has described *Kalamegha* under *Shatapushpadi Varga*.[4] Botanically, it is an annual, branched, erect herb, native to India and Sri Lanka.

HPLC analysis is essential in the *Desha*-wise comparative study of *Kalamegha* as it converts the traditional concept of *Bhoomi Desha* into measurable phytochemical data. By quantifying marker compounds such as andrographolide, it helps compare samples from *Jangala*, *Anupa*, and *Sadharana Desha*, correlate chemical variation with ecological factors, and identify the most potent source for therapeutic use.

Hence, the present work aims to observe the possible changes in Chemo-profile seen in the same species of *Kalamegha* i.e. *Andrographis paniculata* (Burm.f.) Nees which is grown in different *Desha* like *Jangala Desha, Anupa Desha* and *Sadharana Desha*.

II. REVIEW OF LITERATURE

A. *Etymological derivation*

Andrographis = denotes male (*Andro* = male); *Panicula* = tuft, a loose cluster of flowers. In botanical floras, *Kalamegha* is identified as *Andrographis paniculata (Burm.f.) Nees*

1) Chronological Literary Review: During the Veda Kala, no references to *Kalamegha* are found. Similarly, in the Samhita Kala, references to *Kalamegha* are not available. In the Nighantu Kala, *Kalamegha* is mentioned in Nighantu Adarsha[5] under *Vasaadi Varga*, where its properties, actions, and therapeutic uses are explained. In Priya Nighantu, *Kalamegha* is classified under *Shatapushpadi Varga*[6] with synonyms such as *Bhunimba* and *Yavaakaraphala*, along with its properties, actions, and therapeutic uses.
2) The term '*Kalamegha*' is attributed to the plant's distant visual appearance. The word originates from Bengali vernacular nomenclature, which resembles a dark cloud against a blue sky.[7]
3) Vernacular Names of *Kalamegha*[8]*: Kalamegha* is known by different vernacular names in various languages. In English, it is called "King of Bitters." In Hindi, it is known as Charayetah, Kiryat, or Mahatitka. In Kannada, it is called Nela Bevu.
4) Rasa Panchaka of Kalamegha: According to different Ayurvedic texts, *Kalamegha* is described as having *Tikta Rasa*, *Katu Vipaka* and *Ushna Veerya*. Its main *Guna* are *Laghu* and *Ruksha*. It is considered *Kapha-Pitta hara* or *Pitta-Kapha hara* in action. In *Adarsha Nighantu*, its karma is mentioned as *Deepana*. In *Priya Nighantu*, it is described as having *Deepana* and *Swedana* actions. In *Dravya Guna Hastamalaka*, the same Rasa-panchaka is mentioned, but specific karma is not clearly stated.
5) Matra of Kalamegha[9]: The dose of *Kalamegha* varies according to the dosage form used. In *Churna* form, the recommended dose is 1–3 grams. In *Kwatha* form, it is administered in a dose of 20–40 ml. In *Swarasa* form, the dose is 5–10 ml.
6) Prayojya Anga: Panchanga of *Kalamegha* (whole plant)[10]
7) Prayoga:
    - The leaves and roots are also used as a febrifuge, stomachic, cholagogue, general debility, dyspepsia.[11]
    - The expressed juice of the leaves, together with certain spices, such as Cardamom, cloves, cinnamon etc. is dried under the sun and made into little globules, which are prescribed for infants to relieve griping irregular stools and loss of appetite, flatulence, and diarrhea.[12]
8) Morphology of *Andrographis paniculata* [Burm.f] Nees: It is an erect or sometimes procumbent, branched annual herb growing about 0.3 to 1 meter high.The branches are sharply quadrangular and the stem is dark green in colour. The stem shows longitudinal furrows, wings on younger angles, and slight enlargement at the nodes. Leaves are glabrous, lanceolate, slightly undulate or entire, and pale on the lower surface. They grow up to 8 cm long and 2.5 cm broad, with slender nerves and short petioles. Inflorescence occurs as axillary and terminal racemes or panicles. Flowers are small, distant, glandular pubescent, and arranged in lax spreading clusters. The calyx is about 3 mm long with five equal linear-lanceolate sepals. The corolla is pale, tubular at the base, and marked with brown or purple spots. Androecium consists of two stamens with flattened hairy filaments and anthers bearing white trichomes. Gynoecium has a two-celled ovary, slender style, and minutely bifid stigma.Fruits are linear-oblong capsules containing numerous yellowish-brown, rugosely pitted seeds.[13]
9) Chemical constituents: The major phytoconstituents of *Kalamegha* include flavonoids and flavones such as 2',5-dihydroxy-7,8-dimethoxyflavone-2'-O-beta-D-glucoside, 5-hydroxy-7,8-flavanone, 5-hydroxy-7,8-dimethoxyflavone, apigenin derivatives, mono-oxymethyl-wightin, and andrographidines B and D. It also contains important diterpenes and lactones, including andrographin, panicolin, panicolide, andrograpanin, andropanoside, andrographone, homoandrographolide, deoxyandrographolide-19β-D-glucoside, 14-deoxy-12-methoxyandrographolide, furanoid diterpenes, and α-β-unsaturated

lactones. The sterol and phytosterol components include α-sitosterol, β-sitosterol glucoside, and 3-hydroxy-5-stigmasta-9(11). In addition, *Kalamegha* contains acids, volatile compounds, and aliphatic constituents such as chlorogenic acid, myristic acid, carvacrol, eugenol, and hentriacontane.[14]

10) Pharmacological activities: Kalamegha is known to possess Antihepatotoxic, hepatoprotective, antimalarial, cholinergic, cardiovascular, anti-inflammatory, antiallergic, antimicrobial, antipyretic, immunostimulant, anti-HIV, antiulcer, anti-spermatogenic, antidiarrheal, filaricidal, antifertility, hypotensive, choleretic, antityphoid, anti-fungal, antibiotic, antioxidant, antihyperglycemic, analgesic.[15]

11) Desha concept: Plants growing in a particular *Desha* are influenced by the predominant *Dosha* of that region. so, consideration should be given for that also. In *Charaka Samhita, Vimanasthana*, three types of *Desha* are described. *Jangala Desha* is dry, with less water and fewer trees, and is considered the best region as it causes fewer diseases.[16] *Anupa Desha* has abundant water and trees, but has less air movement and sunlight, making it unfavorable for health.[17] *Sadharana Desha* has a moderate climate, where plants of both *Jangala* and *Anupa Desha* can grow.[18] In *Para Aparatva, Charaka* also mentions that *Jangala Desha* is more favorable than *Anupa Desha.*[19] *Anupa Desha* is generally marshy, damp, uneven, and receives insufficient sunlight. These factors may affect the medicinal quality of plants and promote the growth of microorganisms and insects. Similar references to forests and their divisions are also found in *Vriksha ayurveda, Vana Varga Sutreeya Adhyaya*

Categorization of karnataka's agroclimatic zones based on the concept of bhoomi desha[20]

SL	Desha	Agro-climatics zone of Karnataka
01	Jangala desha	North eastern dry zone, Northern dry zone, central dry zone, Eastern dry zone, southern dry zone.
02	Sadharana desha	North east transition zone, Southern transition zone, North transition zone.
03	Anoopa desha	Hilly zone, coastal zone.

III. MATERIALS AND METHODS

1) Study design: The study was conducted in two broad phases: (1) collection and preparation of Kalamegha Panchanga from different Bhoomi Desha and (2) comparative analytical evaluation of the collected samples.

2) Sample collection: Three samples were collected by handpicking during September–November. S1 was collected from Swamimalai, Sandur, Ballari representing Jangala Desha; S2 from Thalapady, Ullal, Mangaluru representing Anupa Desha; and S3 from Parishwada, Belagavi representing Sadharana Desha. The samples were authenticated and shade-dried before powdering.

3) Pharmacognostic and physicochemical evaluation: Macroscopic and powder microscopic characters were recorded. Physicochemical tests included foreign matter, loss on drying, total ash, acid-insoluble ash, water-soluble extractive value, alcohol-soluble extractive value and pH, using the procedures and standards described in the dissertation.1

4) Phytochemical and elemental evaluation: Aqueous and ethanolic extracts were screened for primary and secondary metabolites. Inorganic elemental analysis of the plant material included calcium, zinc, iron and manganese.

5) RP-HPLC analysis: Standard andrographolide and sample extracts were analysed by HPLC. The retention time of the standard andrographolide was 3.570 min. The marker peak in the sample chromatograms was used for quantitative comparison of andrographolide.

6) Soil analysis: Soil samples from the three collection sites were evaluated for pH, available nitrogen, phosphorus and potassium, together with iron, zinc, manganese, copper, boron and calcium.

7) In-vitro antifungal activity: Agar well diffusion method was used to evaluate the antifungal activity of the samples. Test volumes of 50, 100 and 150 µL were compared with clotrimazole (25 µg) as the positive control.

IV. RESULTS

Three samples of *Kalamegha* were collected from different regions and dried for further study. The *Jangala* sample (S1) was collected in a quantity of 2 kg, from which 1.05 kg was obtained after drying. The *Anupa* sample (S2) was also collected in a quantity of 2 kg, and 0.96 kg was obtained after drying. Similarly, the *Sadharana* sample (S3) was collected in a quantity of 2 kg, and 1.04 kg was obtained after drying. Thus, among the three samples, *Jangala* showed the highest dried yield, followed by *Sadharana* and *Anupa.*

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Figure 1 Swamimalai S-1



Figure 2 Thalapady sample-2



Figure 3 Parishwad sample-3

A. Pharmacognostic and Physicochemical Findings

Organoleptic and microscopic examination supported the identity and genuineness of all three samples. Powder microscopy showed features including trichomes, epidermal cells with stomata, vascular tissues, parenchymatous tissue, fibres and calcium oxalate crystals. Some microscopic features varied in prominence among the samples.

Parameter	API/Study standard	S1 Jangala	S2 Anupa	S3 Sadharana
Foreign matter	NMT 2.0%	0.8%	1.2%	0.9%
Loss on drying	NMT 12.0%	2.32%	7.18%	4.04%
Ash value	NMT 15%	5.9%	4.5%	4.6%
Acid-insoluble ash	NMT 3%	2.59%	2.34%	2.37%
Water-soluble extractive	NLT 19%	56.6%	36.4%	46.2%
Alcohol-soluble extractive	NLT 12%	30.6%	18.4%	45.8%
pH	No API standard	7.09	7.34	7.12

B. Phytochemical Screening

Carbohydrates, saponins, tannins, alkaloids, phlobatannins, phenols and terpenoids were detected in both aqueous and ethanolic extracts across the three samples. Proteins/amino acids, oils and fats, cardiac glycosides, oxalates and quinones were not detected by the respective tests. Sterols and flavonoids showed positive reactions in aqueous extracts and negative reactions in ethanolic extracts in the reported screening.

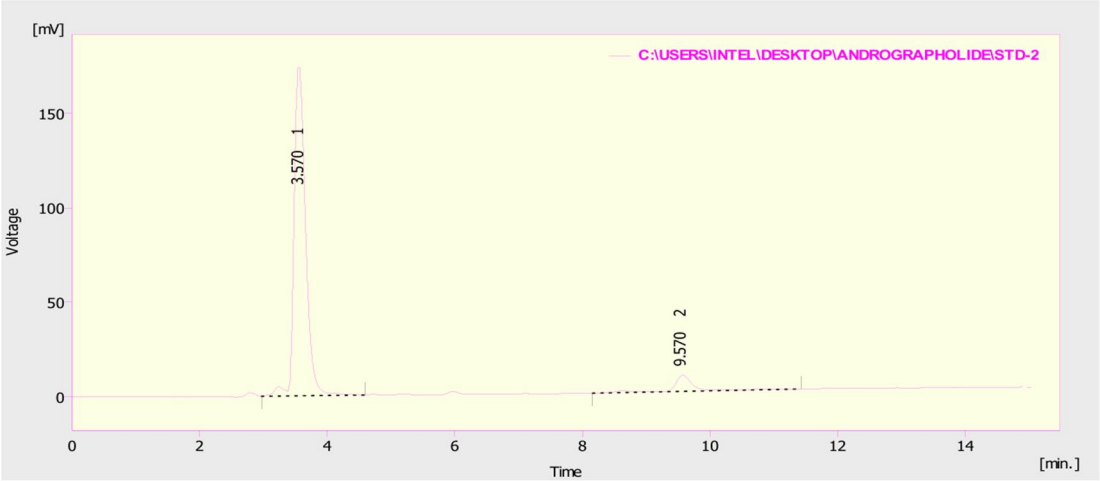
Constituent	**S1**	**S2**	**S3**
Carbohydrate	Present	Present	Present
Saponin	Present	Present	Present
Tannin	Present	Present	Present
Alkaloids	Present	Present	Present
Phlobatannins	Present	Present	Present
Phenols	Present	Present	Present
Terpenoids	Present	Present	Present

C. Inorganic Constituents of Kalamegha

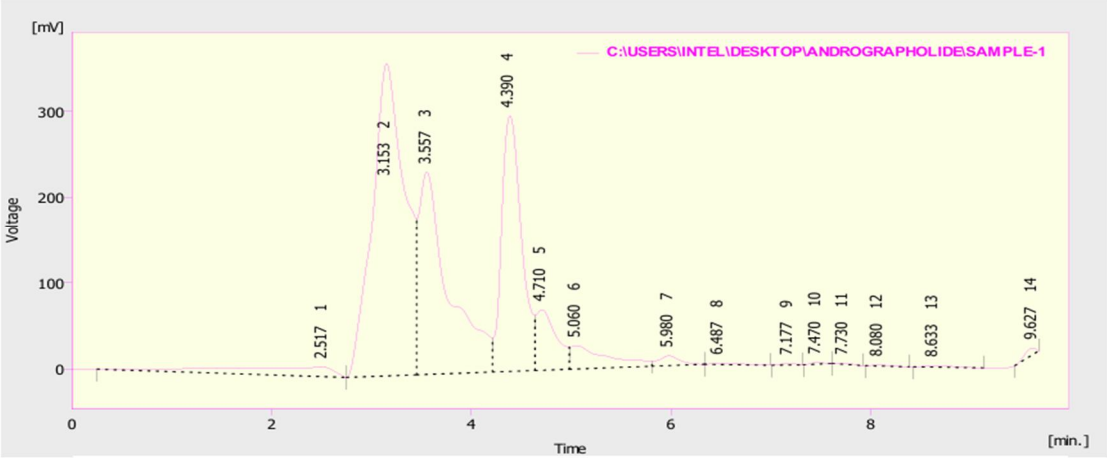
Element	**S1 Jangala**	**S2 Anupa**	**S3 Sadharana**
Calcium	4.9 mg/kg	5.6 mg/kg	4.7 mg/kg
Zinc	2.1 mg/kg	2.7 mg/kg	2.4 mg/kg
Iron	11.2 mg/kg	13.87 mg/kg	12.6 mg/kg
Manganese	1.7 mg/kg	2.1 mg/kg	2.2 mg/kg

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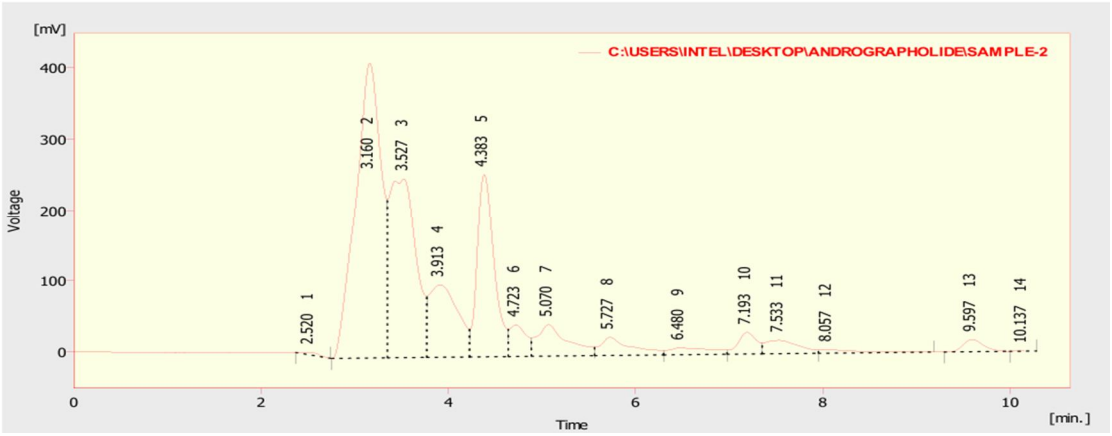
D. RP-HPLC Quantification of Andrographolide



1) Retention time of standard andrographolide is 3.570 min and area under peak is 2195.321 mVs.

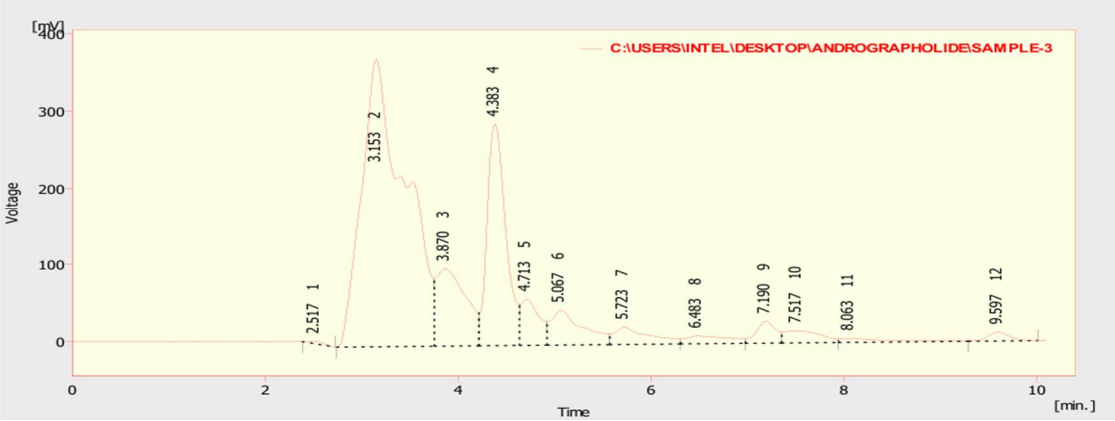


2) Retention time of Andrographolide in Ethanolic-extract of S1 is 3.557 min and area under peak is 4953.917m V. S with Andrographolide concentration 4.14mg/gm

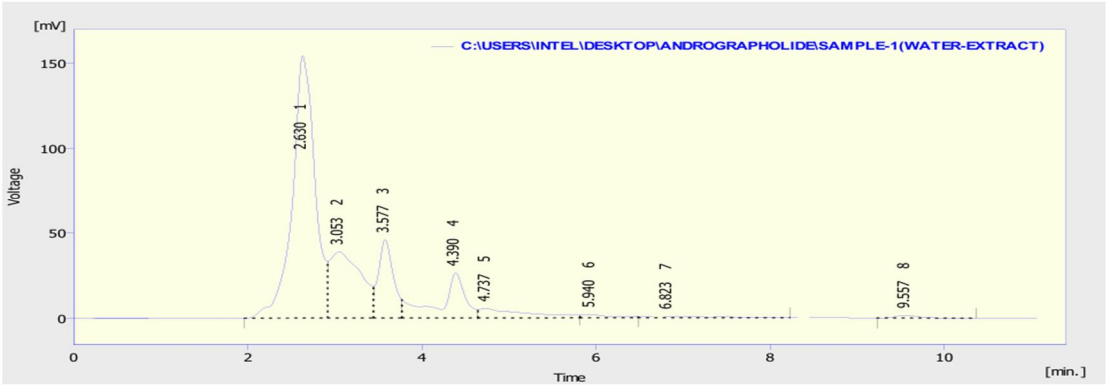


3) Retention time of Andrographolide in Ethanolic-extract of S2 is 3.527 min and area under peak is 4932.123 m V s. with Andrographolide concentration 2.99 mg/gm

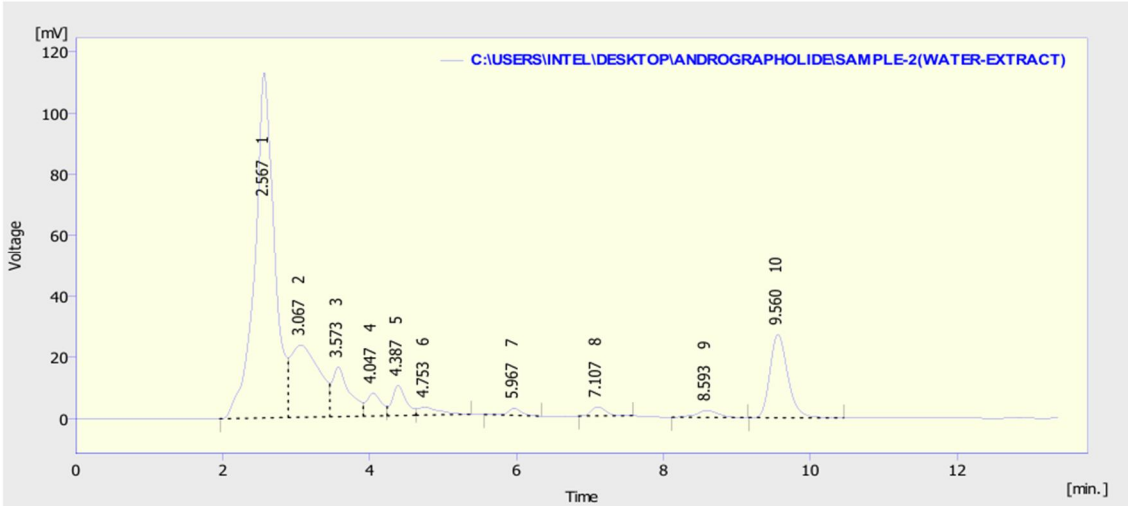
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4) Retention time of Andrographolide in Ethanolic-extract of S3 is 3.870 min and area under peak is 2165.226 m V s. with Andrographolide concentration 2.44mg/mg

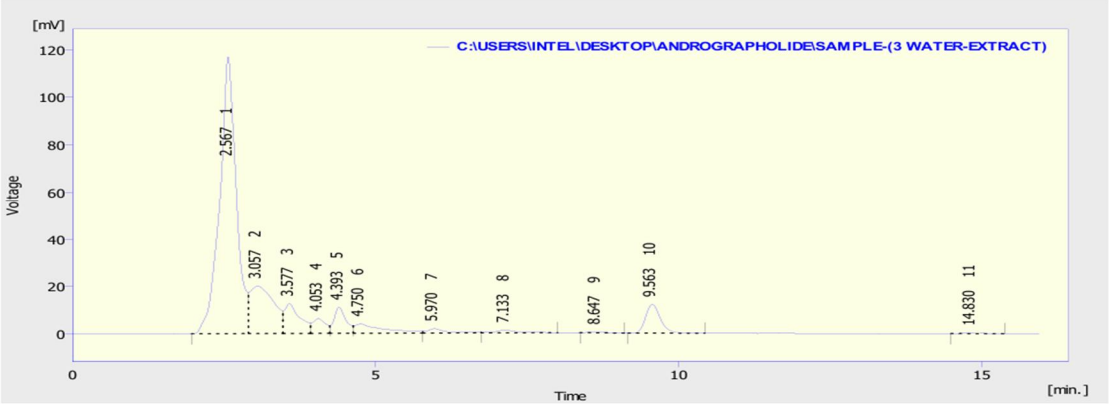


5) Retention time of Andrographolide in aqueous-extract of S1 is 3.577 min and area under peak is 553.704 m v s. with Andrographolide concentration 0.41mg/gm



6) Retention time of Andrographolide in aqueous-extract of S2 is 3.573min and area under peak is 268.431 mVs. with Andrographolide concentration 0.126mg/gm

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7) Retention time of Andrographolide in aqueous-extract of S3 is 3.577 min and area under peak is 226.859 mV. S with Andrographolide concentration 0.98mg/gm

The reported sample chromatograms showed corresponding marker peaks close to this retention time. Ethanolic extracts contained more andrographolide than aqueous extracts in all three Desha samples.

Sample	Extract	Retention time (min)	Andrographolide (mg/g)
S1 – Jangala	Ethanolic	3.557	4.14
S2 – Anupa	Ethanolic	3.527	2.99
S3 – Sadharana	Ethanolic	3.870	2.44
S3 – Sadharana	Aqueous	3.577	0.98
S1 – Jangala	Aqueous	3.577	0.41
S2 – Anupa	Aqueous	3.573	0.126

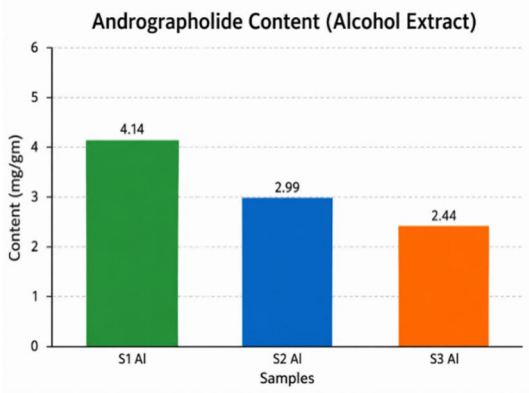


Figure 3 Andrographolide Content in the Alcoholic Extracts of Samples S1, S2 and S3

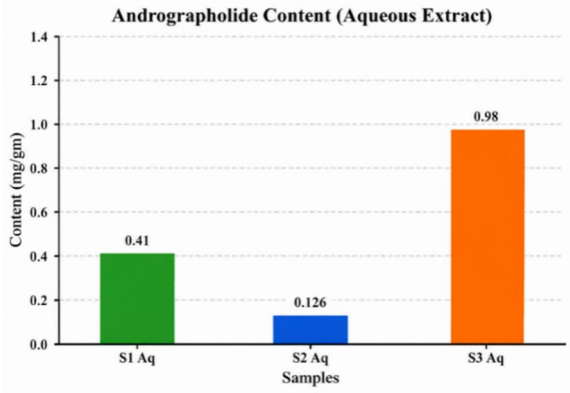


Figure 4 Andrographolide Content in the Aqueous Extracts of Samples S1, S2 and S3

E. Soil Analysis

Parameter	Unit	S1 Jangala	S2 Anupa	S3 Sadharana
pH	—	7.44	6.58	7.82
Nitrogen	kg/ha	1261.8	664.0	738.2
Phosphorus	kg/ha	78.2	104.8	279.8
Potassium	kg/ha	437.0	314.7	754.9
Iron	mg/kg	196.4	216.8	196.2
Zinc	mg/kg	8.2	7.4	8.6
Manganese	mg/kg	16.5	12.7	10.8
Copper	mg/kg	3.2	2.9	2.9
Boron	mg/kg	2.6	2.7	3.6
Calcium	meq/100 g	11.9	12.6	10.7

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F. In-vitro Antifungal Activity

Name of the organism	Vermicompost ZOI (mm)				ZOI (mm) Control (Clotrimazole)
Sample concentration	0 µL	50µL	100µL	150µL	25µg
S-01	-	9	12	14	18
S-02	-	-	7	10	18
S-03	-	-	9	12	20

The activity increased with increasing sample volume. S1 showed the highest zone of inhibition among the test samples, followed by S3 and S2. The standard clotrimazole produced larger zones than the plant samples at the tested concentrations.

V. DISCUSSION

The analytical study was carried out to scientifically evaluate and compare the quality, genuineness and potency of *Kalamegha* collected from *Jangala, Anupa* and *Sadharana Desha* of Karnataka.

A. Pharmacognostical findings

All samples showed the characteristic features of *Kalamegha*, such as dull green colour, odourless nature and intensely bitter taste. Microscopy showed calcium oxalate crystals, xylem vessels and fibres, confirming the identity and genuineness of all samples as per API standards.

B. Physicochemical findings

Foreign matter, loss on drying, total ash and acid-insoluble ash were within standard limits in all samples. This indicates that the samples were clean, properly dried and free from major impurities. Water-soluble extractive value was highest in *Jangala Desha*, while alcohol-soluble extractive value was highest in *Sadharana Desha*.

C. Phytochemical Findings

Phytochemical screening showed the presence of saponins, tannins, alkaloids, phenols, phlobatannins and terpenoids in all samples. No major qualitative difference was found among the three *Desha* samples.

D. Elemental Analysis Findings

ICP-OES analysis showed minor variation in minerals. *Anupa Desha* sample showed comparatively higher calcium, zinc and iron, probably due to coastal soil, high rainfall and lateritic soil conditions.

E. HPLC Findings

RP-HPLC analysis showed clear quantitative variation in andrographolide content. Ethanolic extract gave better andrographolide yield than aqueous extract. *Jangala Desha* sample showed the highest andrographolide content, followed by *Anupa Desha* and *Sadharana Desha*.

F. Sharad Rutu Collection Finding

Collection in *Sharad Rutu* was suitable because *Kalamegha Panchanga* becomes mature after monsoon growth, and flowering begins around this period. This supports both classical Ayurvedic collection rules and modern understanding of higher andrographolide availability.

G. Soil and Mineral Correlation Finding

Mineral accumulation in *Kalamegha* was not directly proportional to soil mineral content. It was also influenced by soil pH, moisture, rainfall, nutrient bioavailability, microbial activity and plant uptake capacity.

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H. Influence of Desha finding

The HPLC results showed marked variation in andrographolide content among the three *Desha* samples. In alcoholic extract, S1 *Jangala Desha* showed the highest andrographolide content (4.14 mg/g), followed by S2 *Anupa Desha* (2.99 mg/g) and S3 Sadharana *Desha* (2.44 mg/g).

Overall, the results suggest that *Jangala Desha* is the most favourable region for andrographolide accumulation in alcoholic extract, likely due to the combined effect of dry climate, moderate alkalinity, high nitrogen, adequate potassium and micronutrient availability. *Anupa Desha* showed moderate andrographolide, possibly due to high rainfall, high humidity, acidic coastal soil and nutrient leaching. *Sadharana Desha*, despite high P and K, showed lower alcoholic andrographolide, indicating that climate-induced stress is more important than nutrient richness alone.

I. Comparative In Vitro Antifungal Activity of Kalamegha from Different Bhoomi Desha

S1 showed the highest zone of inhibition, increasing from 9 mm at 50 µL to 14 mm at 150 µL. This suggests better antifungal activity, which may be related to the higher andrographolide content. S2, showed the lowest activity with no inhibition at 50 µL and only 10 mm at 150 µL.

S3, showed moderate activity with 12 mm inhibition at 150 µL. Compared with clotrimazole, which showed 18-20 mm inhibition, all samples showed lower but concentration-dependent antifungal activity. Thus, the *Desha* -based samples possess moderate antifungal potential with *Jangala Desha* sample showing the best activity.

J. Broader Implications of the Study

The study scientifically supports the Ayurvedic concept of *Desha* by showing that geographical habitat can influence the quality and potency of medicinal plants. It also helps in developing better standardization and quality control guidelines for *Kalamegha*. Since the *Jangala Desha* sample showed higher andrographolide content, such regions may be preferred for the cultivation and collection of high-quality *Kalamegha*. Thus, the study bridges traditional Ayurvedic knowledge and modern analytical science through HPLC-based validation.

VI.CONCLUSION

The present study on the comparative chemo-profile variation of *Kalamegha* collected from different *Bhoomi Desha* of Karnataka was carried out using HPLC and other analytical methods. Pharmacognostical and physicochemical studies confirmed the identity and genuineness of all trial drug samples as per API standards. Phytochemical screening showed the presence of important secondary metabolites in all samples, indicating no major qualitative variation among the three *Desha* samples. Inorganic elemental analysis showed minor variation in minerals such as calcium, zinc, iron and manganese, suggesting the influence of *Bhoomi Desha* on mineral accumulation. RP-HPLC analysis revealed clear quantitative variation in andrographolide content, with ethanolic extracts showing better yield than aqueous extracts. The *Jangala Desha* sample showed the highest andrographolide content and better antifungal activity compared to the other samples. This may be due to dry climatic conditions, moderate alkaline soil pH, favourable nutrients and mild environmental stress enhancing secondary metabolite production. Thus, the study scientifically supports the Ayurvedic concept of *Desha* and establishes its importance in the standardization, cultivation, collection and quality control of *Kalamegha*.

VII. FURTHER SCOPE AND RECOMMENDATIONS

The study can be extended by collecting *Kalamegha* samples from more locations under each *Desha* to obtain stronger comparative data. Advanced analytical methods such as LC-MS/MS and GC-MS may be used to evaluate other important phytochemicals besides andrographolide. Further in-vivo and clinical studies can be conducted to confirm whether higher andrographolide content produces better therapeutic activity. The findings of the present study may also help in developing standard guidelines for the cultivation, collection, and quality control of *Kalamegha*.

VIII. LIMITATIONS

The present study was limited to *Kalamegha* samples collected from only three selected regions of Karnataka representing *Jangala*, *Anupa*, and *Sadharana Desha*. The sample size was small, so the findings cannot be generalized to all geographical regions.

The study mainly focused on andrographolide as the marker compound, while other important phytoconstituents were not quantified in detail. Seasonal variation was not studied extensively, though it may influence the phytochemical profile of *Kalamegha*.

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Biological activity was assessed only through in-vitro antifungal study, without in-vivo or clinical validation. Environmental factors such as rainfall, soil type, altitude, and nutrient status were discussed, but their individual effects were not studied separately. Thus, further large-scale studies are needed to confirm and strengthen the findings.

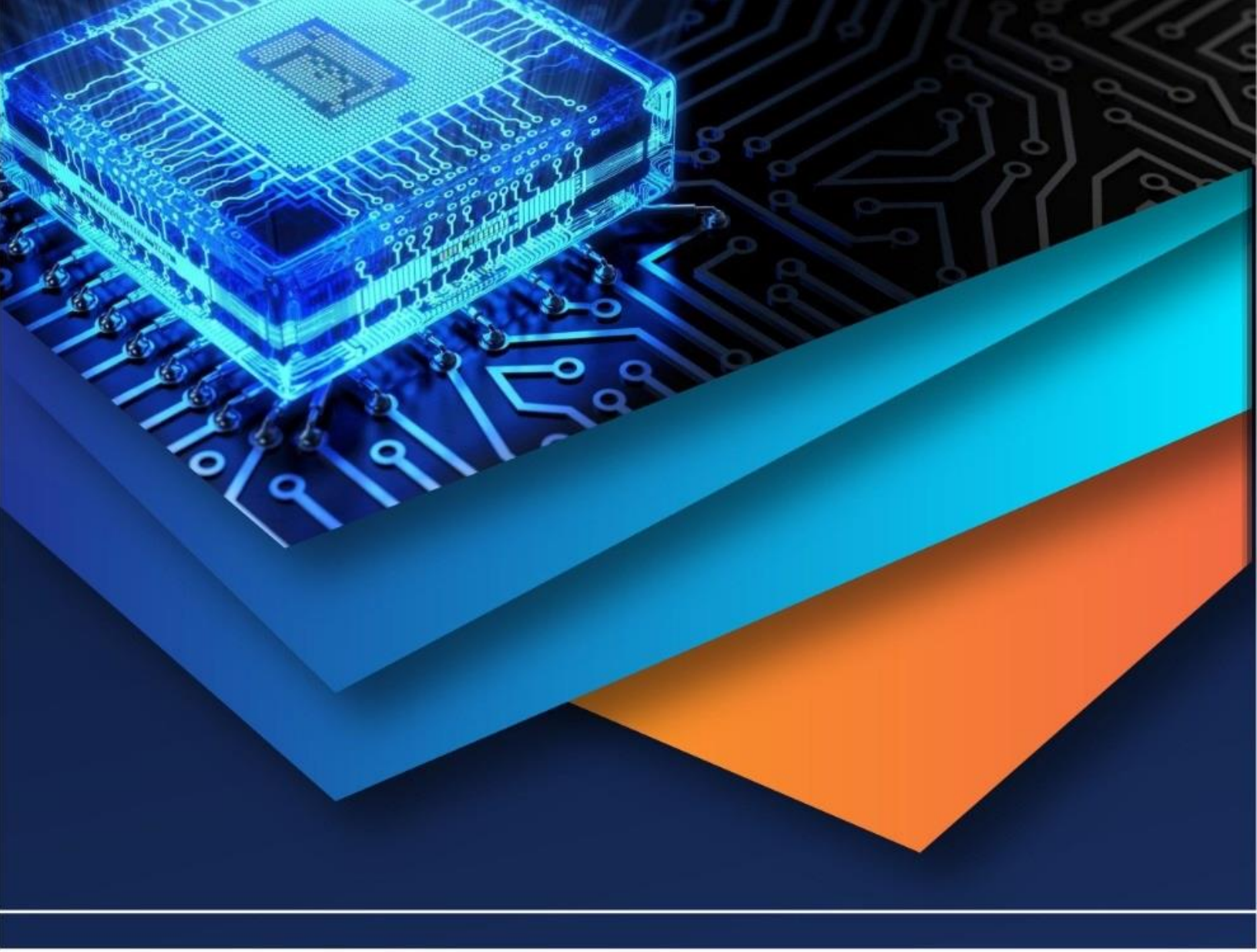
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