Int J Ayu Pharm Chem
ISSN 2350-0204
www.ijapc.com

ORIGINAL RESEARCH ARTICLE

A Comparative, Qualitative and Quantitative Analysis of *Kalamegha* (Andrographis paniculata Nees) from Different Geographical Areas of India and Srilanka

Author: Dilrukshi S M A C[1]

Co Authors: M S Veena[2]

[1,2]Department of Drawyaguna, GAMC, Banglore, KA, India

ABSTRACT

Kalamegha plant is a potent antipyretic and immuno-modulator. *Panchanga* of the plant is used in various formulations .Increased demand for this drug is leading to mass exploitation of the drug resulting in scarcity of the plant.The medicinal plants which is naturally grown or cultivated in different geographical area, indicates production within a particular area, quality and characteristics depending on natural,historical and cultural factors.Wide range of usage and increase in demand of the herb has led to broad extension of cultivation, adulteration and substitution of this drug.This may in turn lead to differences in effectiveness in management of disease. There is a need for proper identification, qualitative and quantitative analysis of *kalamegha* for better therapeutic action. It is botanically identified as *Andrographis paniculata Nees* , distributed in the Asian and African continent and is widely grown in India and Sri Lanka. Present study has been taken to compare and evaluation, qualitatively and quantitatively, phytochemically and pharmacognostically plants of naturally grown *Andrographis paniculata Nees* from different geographical areas of India and Sri Lanka.Aqueous and alcohol extracts of all the four samples were prepared and subjected to analytical evaluation. Among all four samples Chattisgarh sample is showing high anti-oxidant activity.As *Kalamegha* is rich in aqueous soluble principles, *Kashaya* is the better dosage from for therapeutic utility.Quantitative and qualitative difference in the samples signifies the role of geographical effect in enhancing the potency of *Kalamegha* which was evident sample collected from Chattisgarh natural habitat.

Key Words *Kalamegha, Andrographis paniculata,Different geographyal samples*

INTRODUCTION

Dravya being the 2nd *chikitsa chatuspada* has wider and extensive by utility. In view of the wider acceptance of herbal drug based products and fast expanding global market, the quality and genuinity of medicinal plants accounts pivotal significance for their therapeutic values. The problem in Ayush Pharmaceutical industry is, scarcity of the supply of the quality standard samples. There's always a constant need and

ORIGINAL RESEARCH ARTICLE

demand for sustainable supply of quality medicinal plants.The successful management of disease depend on quality and purity of the drug; which is based on good agriculture practices, Good Manufacturing Practices and active phytoconstitutional present.*Kalamegha* or *Kalmegh* (*Andrographis paniculata*), an herbaceous annual plant is a much esteemed medicinal plant in Ayurveda. It was used for centuries in place of or as an alternative to antibiotics before hardcore antibiotics were evolved. It has been used primarily to treat *yakruth & twak roga, agni mandya, jwara and raktha shodhana.* The plant is bitter in taste and is used to stimulate liver function, reduce inflammation, and treat worm infestations. It is one of the prominent ingredients in 28 different poly herbal patent formulations in vogue in the Ayurveda system of medicine[1].It has been used in India,Sri Lanka, China, Thailand, South East Asia and other Asian countries. Andrographis paniculata (powder and extract) is officially in Indian Pharmacopoeia as *Kalmegh* in 2007 and in 2014 edition and categorized as hepatoprotective agent.It is commonly known as “Nilavembu” in Tamil and "Heen bin kohomba" in Sinhala. *Kalamegha*, quite a name to live up to, literally it means “black cloud” or “dark cloud” perhaps attesting to its harvest time just before winter or flowering time from September to December. The plant is known in north-eastern India as *Maha-tikta* which literally means "king of bitters". Its another epithet is ‘*Bhoo-Nimba’* or *‘Bhui-Neem*’ where *bhoo* stands for earth or ground and *nimb* or *neem* refers to *Neem* tree *(Azadirachta indica).* The term therefore means *‘Neem* of the earth’ or ‘*Neem* of the ground’ referring to its *neem* like bitter taste and effects.A*ndrographoloids* and N*eo andrographoloids* are the active principals prevents oxidative damage and inhibits binding toxic metabolites to DNA.Hence used for the treatment of anti inflammatory, hepatoprotective, anti bacterial,anti viral, anti oxidant, anti parasitic, anti diarrhea and several infectious disease ranging from malaria to dysentery.

MATERIALS AND METHOD

COLLECTION OF NATURAL HABITAT SAMPLE:

Sample no 01 (S1): Collected during -April 2024 from Changalpettu, Tamilnadu, India.

Sample no 02 (S2): Collected during - April 2024 from Chatisgarh, India.

Sample no 03 (S3): Collected during - April 2024 from Mysuru, Karnataka, India.

Sample no 04 (S4): Collected during - April 2024 from Palagala,North Central province, Sri Lanka.

DRUG AUTHENTICATION:

The Natural habitat plants collected from all four places authenticated as Andrographis paniculata Nees by botanist Dr. Datchanamoorthy, Center for Herbal Garden, Institute of Ayurveda and Integrative Medicine,TDU, Yelahanka, Bangalore.

DRUG PREPARATION

PREPARATION OF EXTRACTS

ORIGINAL RESEARCH ARTICLE

Materials required:

Coarsely powdered drugs, Solvent, Soxhlet apparatus, Glass beaker, Hot plate.

The following extracts were prepared for phyto chemical study.

1. Methanolic extract:-Soxhlet extraction (Hot percolation) was done.

40grams of the coarse powder sample was moistened with methanol and filled in to the thimble.500 ml of Methanol was filled in to the round bottom flask. The equipment was setup and kept on heating mantle.Soxhlation was done for 48 hours.

2. Aqueous extract:- 20grams of the coarse powder sample was taken in round bottom flask.150ml of distilled water was added, condenser was fitted and re fluxed for 24 hours. Cooled and filtered.

The physical characters of the extracts were noted and were preserved in air tight containers for further analytical studies.

PHARMACOGNOSTIC STUDY

Macroscopic Evaluation

Microscopic evaluation

PHYSICO CHEMICAL EVALUATION

Moisture content (Loss On Drying)

Total ash

Acid insoluble ash

Alcohol insoluble ash

Alcohol soluble extractive values

Water soluble extractive values

pH Values

PHYTOCHEMICAL EVALUATION

Organic constituents

Inorganic constituents

HPLC analysis

ANALYSIS BY CHROMATOGRAPHY:

HPLC is important and simple analytical tools for qualitative & quantitative analysis of materials.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC):

Purpose:

1. To estimate the content of Andrographolide in *Kalamegha* by HPLC
2. To estimate the total bitter as Andrographolide in *Kalamegha* samples
3. To estimate the content of Gallic acid in Kalamegha samples by HPLC

1. To estimate the content of Andrographolide in *Kalamegha* by HPLC

Chromatographic system: HPLC system equipped with dual quaternary pump, manual/auto injector and photo diode array detector or UV detector supported by suitable software.

Chromatographic conditions:

- Column:RP-H PLC C-18 column
- Size: 4.6mm x 250nm
- Mobile phase: Acetonitrile and 0.1% Phosphoric acid, ratio 60:40
- Stationary phase: Silica
- Injection volume: 20 µl
- Flow rate: 1.3 ml/min
- Wave length: 230 nm
- Run time: 20 min.
- Instrument: Shimadzu SPD-10A

Mobile phase preparation: The mobile phase used here is Acetronitril and Phosphoric acid in

ORIGINAL RESEARCH ARTICLE

the ratio of 60: 40 which means 600 ml of Acetronitril and make up to 400ml by adding of HPLC grade water. This mobile phase solution is filtered by Membrane filtration method and Degas is done by sonicator for 15 minute.

Standard preparation:

The standard Andrographolide with the concentration 1mg/ml was dissolved in mobile phase.

Sample preparation:

Aqueous extract and Alcohol extract 10mg/ml were dissolved in mobile phase.

The above procedure was carried out for extraction of all the samples of *Kalamegha*.

Procedure:

- The mobile phase was forced through the packed column with flow rate of 10ml/min and under pressure gradient elution 272nm.
- The chromatograph of the standard Andrographolide with the concentration 1mg/ml is done by injecting 20µl of standard Andrographolide solution.
- The chromatograph of Aqueous extract and Alcohol extract with concentration 10mg/ml of *Kalamegha* all the samples are also done by injecting 20µl of sample solution.
- The computer recorded the peaks of Absorbance of the substance.
- The report contains the retention time(RT).
- The amount of Andrographolide present in the sample was estimated using the formula.

Andrographolide was selected as standard for HPLC since it is one among the common phytochemical found in all the samples of *Kalamegha.*

2. To estimate the content of Gallic acid in *Kalamegha* by HPLC

Chromatographic system: HPLC system equipped with dual Quaternary pump, manual/auto injector and photo diode array detector or UV detector supported by suitable software.

Chromatographic conditions:

- Column: RP-H PLC C-18 column
- Size: 4.6mm x 250nm
- Mobile phase:A common mobile phase is a mixture of water and acetonitrile (70:30 v/v) with the pH adjusted to 3.0 using orthophosphoric acid
- Stationary phase: Silica
- Injection volume: 20 µl
- Flow rate: 1.0 ml/min
- Wave length:UV 272 nm
- Run time: 20 min.
- Instrument: Shimadzu SPD-10A

Calibration Curve:

- Prepare standard solutions of Gallic acid in the concentration range of 0.5-50 µg/ml.
- Plot the peak area against the concentration to create a calibration curve.

Sample preparation:

- Aqueous extract and Alcohol extract 10mg/ml were dissolved in mobile phase.
- The above procedure was carried out for extraction of all the samples of *Kalamegha*.

ORIGINAL RESEARCH ARTICLE

Procedure:

- The mobile phase was forced through the packed column with flow rate of 10ml/min and under pressure gradient elution 272nm.
- The chromatograph of the standard Gallic acid with the concentration 1mg/ml is done by injecting 20µl of standard Gallic acid solution.
- The chromatograph of Aqueous extract extract with concentration 10mg/ml of *Kalamegha* all the samples are also done by injecting 20µl of sample solution.
- The computer recorded the peaks of Absorbance of the substance.
- The report contains the retention time(RT).
- The amount of Gallic acid present in the sample was estimated using the formula.

Calculation: The percent of Gallic acid was calculated using the formula.

$$\frac{\text{Area of Sample X Standard amount X Sample dilution X Mean weight}}{\text{Area of the Std. Sample amount Dilution of standerd}}$$

- Gallic acid was selected as standard for HPLC since it is one among the common phenolic compound which is showing anti oxidant activity found in all the samples of *Kalamegha*

INVITRO STUDY

ANTI OXIDANT ACTIVITY OF KALAMEGHA WITH ITS AQUEOUS AND ALCOHOL EXTRACTS

- **Selected Test:** ABTS Assay ***2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic) acid assay***
- **Standard :** Gallic acid

Procedure:

A. Working solution preparation

1. 10 mg Gallic acid mixed with 10ml methanol.(A solution)

2. 1ml from solution A mixed with 9ml methanol.(Working solution)

B. Preparation of ABTS solution

1. Ingredients
   - Concentrated ABTS solution
   - Diluted ABTS solution
   - 30% Methanol
2. Added 3ml methanol to the qvete and run under the 734nm in spectrophotometer .
3. Added 3ml diluted ABTS solution to the qvete and run under the 734nm in spectrometer .
4. If reading above 1, then add 3ml methanol to the diluted ABTS solution to make it more dilute.
5. If reading below 1, then add 3ml of concentrated ABTS solution to make it more concentrate.

Likewise should have to do till reading get as 1.Then that sample using in assay.Standard and Kalamegha sample making procedure in table 01 and 02:

ORIGINAL RESEARCH ARTICLE

Table 1 Standard sample making procedure

Working solution(Gallic)	Methanol	ABTS(Same concentrated)	Incubation	Observation at 734 nm
Blank	3ml	Blank	30 min in Dark	If sample showing anti oxidant activity Should be decreased order.
10µl	90µl	3ml		
20µl	80µl	3ml		
30µl	70µl	3ml		
40µl	60µl	3ml		
50µl	50µl	3ml		

Table 2 *Kalamegha* sample making procedure

Kalamegha Sample	Methanol	ABTS(Same concentrated)	Incubation	Observation at 734 nm
10µl	90µl	3ml	30 min in Dark	If sample showing anti oxidant activity Should be decreased order.
20µl	80µl	3ml		
30µl	70µl	3ml		
40µl	60µl	3ml		
50µl	50µl	3ml		

OBSERVATION AND RESULTS

Observation: Collection of genuine samples showing Table no 03.

Table 3 Observation during the collection of the drugs

Sample name	Quantity collected	Dried quantity	Height(cm)	LeafLength (cm)	Leafbreadth(cm)
Changalpet (S1)	2Kg	1.100Kg	57.38	6.88	1.79
Chatisgarh (S2)	2Kg	1.100Kg	53.40	6.74	1.73
Mysuru (S3)	2Kg	1.010Kg	61.30	7.12	1.88
Sri Lanka (S4)	2Kg	1.080Kg	57.66	6.93	1.75

Changalpettu (S1)

Chhatisgarh (S2)

Mysuru (S3)

Sri Lanka (S4)

Changalpettu (S1)

Chhatisgarh (S2)

ORIGINAL RESEARCH ARTICLE

Mysuru (S3) Sri Lanka (S4)

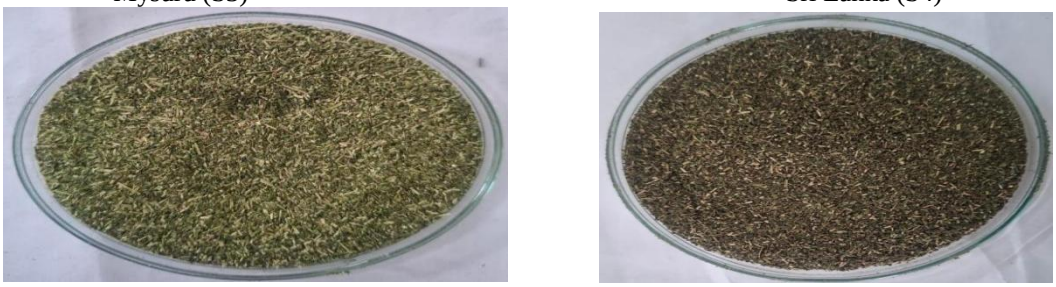
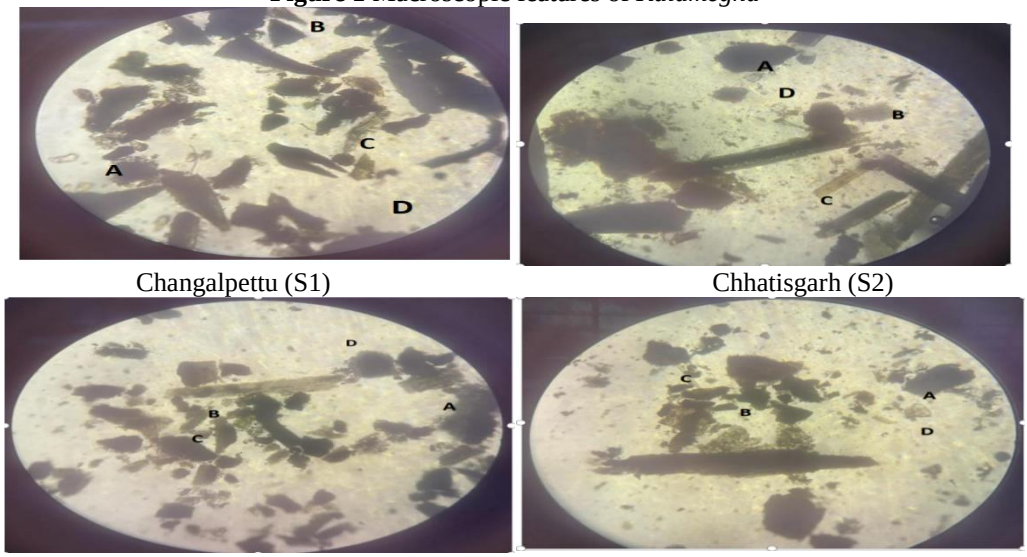


Figure 1 Macroscopic features of *Kalamegha*



Changalpettu (S1) Chhatisgarh (S2)

Mysuru (S3) Sri Lanka (S4)

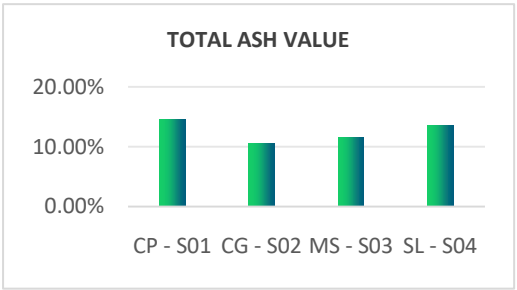
A-Lamina B-Trichoma C-Vascular bundle D-Starch grains

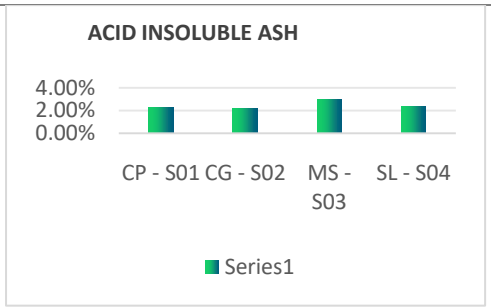
Figure 2 Powder microscopy - features of different samples of *Kalamegha*

Physical constituents of all samples of *Kalamegha showing table no 04*:

Table 4 Physical constituents of all samples of *Kalamegha*

Parameters	Standard	S1	S2	S3	S4
Total ash	NMT 15%	14.50%	10.53%	11.50%	13.50%
Acid insol: ash	NMT 3%	2.29%	2.14%	2.95%	2.37%
Alcohol sol: extr: valu	NLT 12%	16.8%	20.00%	21.36%	16.40%
Water sol: extr:value	NLT 19%	20.83%	21.92%	22.16%	20.14%
Loss on drying	NMT 12%	9.31%	8.99%	11.88%	11.42%
PH value	NoAPI stdrd	6.83	6.36	7.59	7.50





Int J Ayu Pharm Chem
ISSN 2350-0204
www.ijapc.com

ORIGINAL RESEARCH ARTICLE

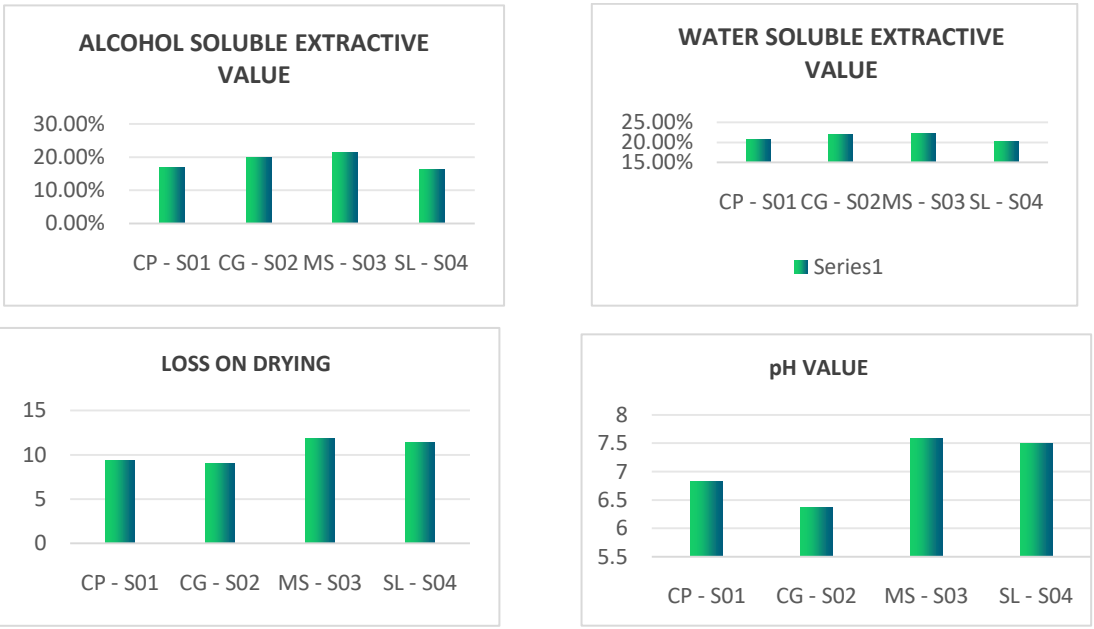


Figure 3 Physical constituents of all samples of ***Kalamegha***

Table 5 Primary metabolite

Phytoconstituents	**Tests**	**S1**		**S2**		**S3**		**S4**	
		Me	**Aq**	**Me**	**Aq**	**Me**	**Aq**	**Me**	**Aq**
Carbohydrates	Molisch'test	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Proteins	Ninhydrintest	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
Starch	Test - starch	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve

Table 6 Secondary metabolites. A:ORGANIC ANALYSIS

Phytoconstituents	**Tests**	**S1**		**S2**		**S3**		**S4**	
		Me	**Aq**	**Me**	**Aq**	**Me**	**Aq**	**Me**	**Aq**
Alkaloids	Wagner's	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Flavanoids	Ferric chloride test:	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Triterpenoids	Liberman burchard	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Steroids	Salkowski test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Saponins	Form test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Glycosides	Kellerkilli test	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
Tannin	Ferric chloride	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Phenolic compound	Lead acetate	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Resins	Acetone-water test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

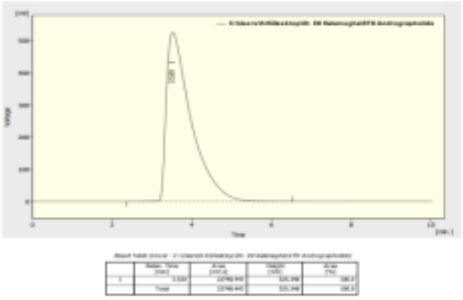
Table 7 INORGANIC ANALYSIS

SI. No	**Inorganic constituents**	**S1**		**S2**		**S3**		**S4**	
		Me	**Aq**	**Me**	**Aq**	**Me**	**Aq**	**Me**	**Aq**
1	Iron	+	+	+	+	+	+	+	+
2	Sodium	+	+	+	+	+	+	+	+
3	Calcium	+	+	+	+	+	+	+	+
4	Magnesium	-	-	-	-	-	-	-	-
5	Potassium	+	+	+	+	+	+	+	+
6	Sulphate	+	+	+	+	+	+	+	+
7	Chloride	+	+	+	+	+	+	+	+
8	Nitrate	+	+	+	+	+	+	+	+
9	Carbonate	-	-	-	-	-	-	-	-
10	Phosphate	+	+	+	+	+	+	+	+

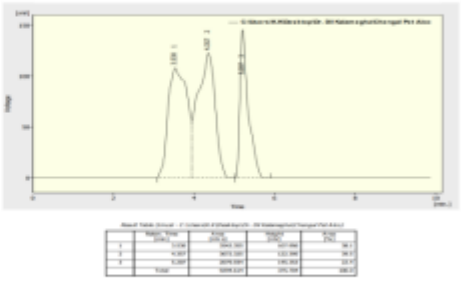
ORIGINAL RESEARCH ARTICLE

HPLC ANALYSIS - ANDROGRAPHOLIDE STANDARD

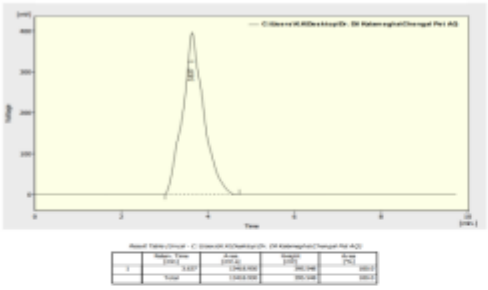
Graph 1: standard Andrographolide



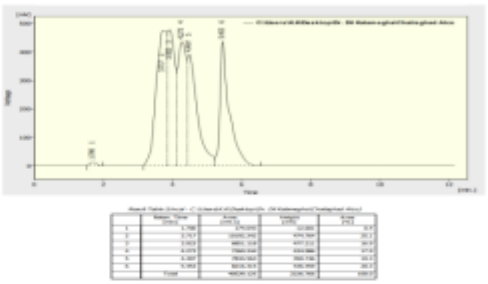
Graph 2. Changalpet sample (Al)



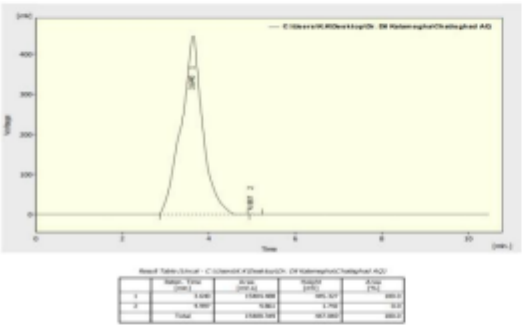
Graph 3. Changalpet sample (Aq)



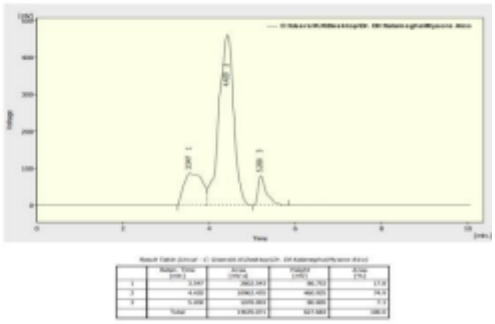
Graph 4. Chatisghad sample (Al)



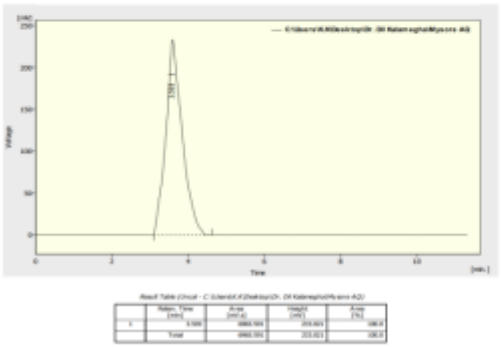
Graph 5. Chatisghad sample(Aq)



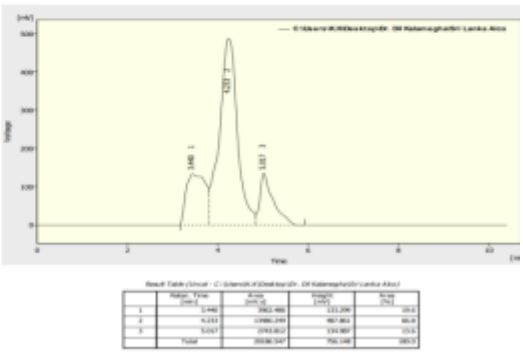
Graph 6. Mysuru sample(Al)



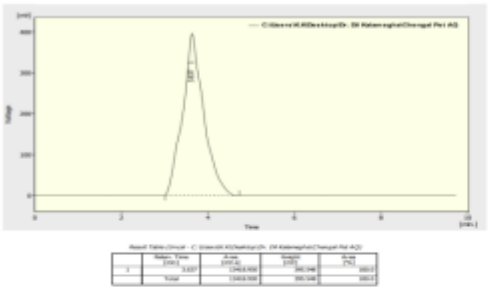
Graph 7. Mysuru sample (Aq)



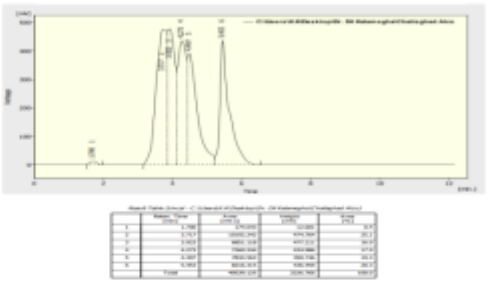
Graph 8. Sri Lanka sample (Al)



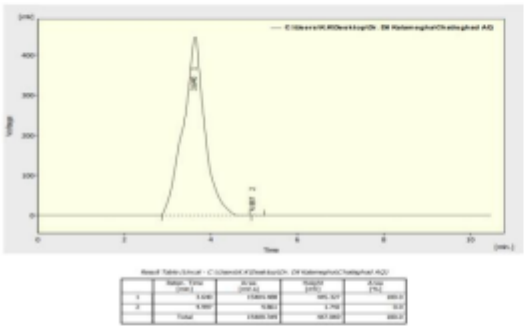
ORIGINAL RESEARCH ARTICLE



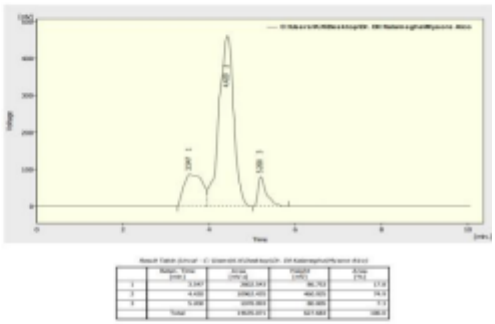
Graph 5. Chatisghad sample(Aq)



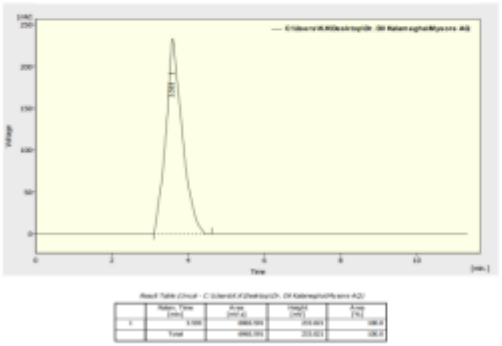
Graph 6. Mysuru sample(Al)

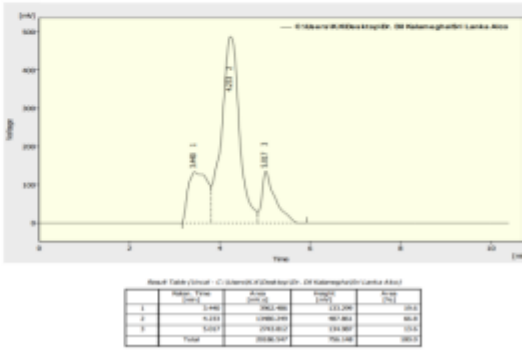


Graph 7. Mysuru sample (Aq)



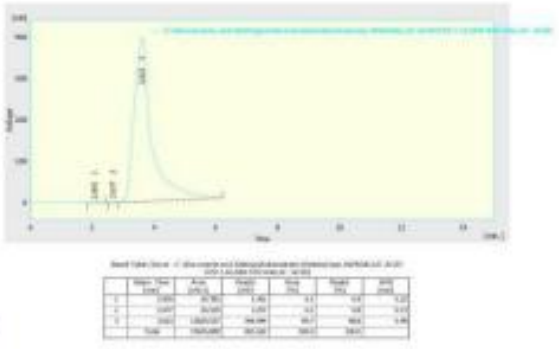
Graph 8. Sri Lanka sample (Al)



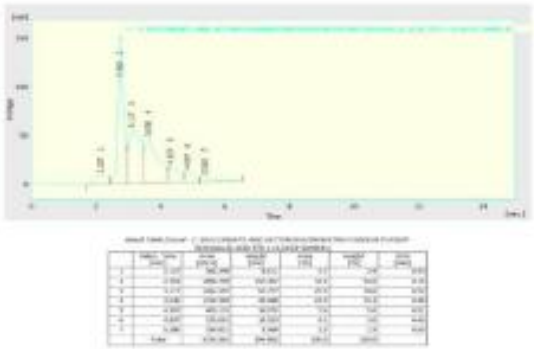


HPLC ANALYSIS - GALLIC ACID STANDARD

Graph 11: standard Gallic acid

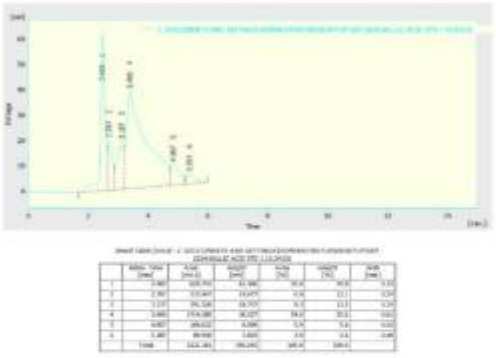


Graph 12: Changalpet sample (Aq)

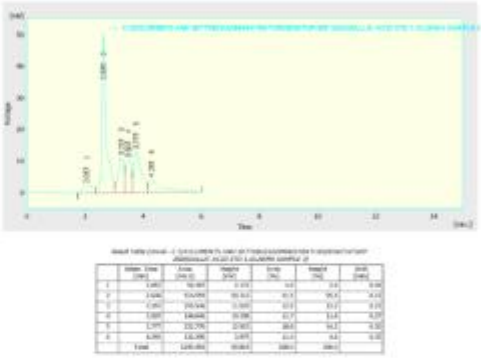


ORIGINAL RESEARCH ARTICLE

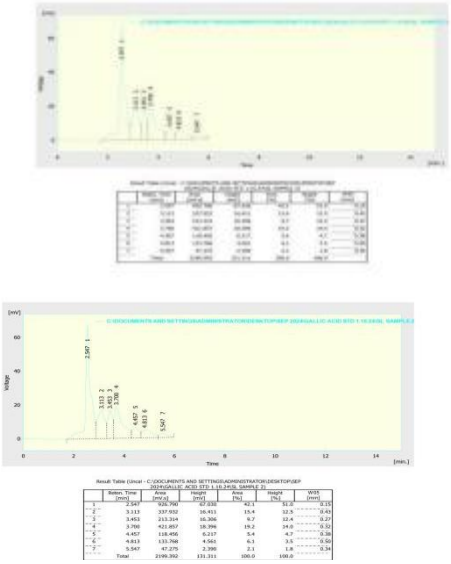
Graph 13: Chatisgarh sample (Aq)



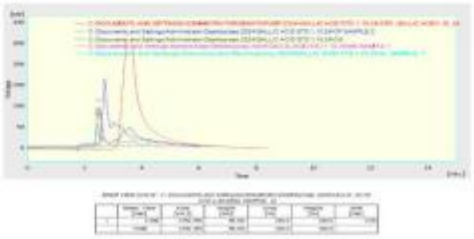
14:Mysore sample (Aq)

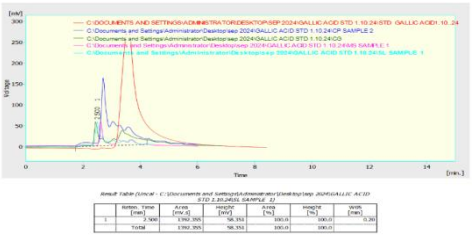


Graph 15: Sri Lanka sample (Aq)



Graph 16: Overlaid HPLC graph





INVITRO STUDY

ANTI OXIDANT ACTIVITY OF *KALAMEGHA* WITH ITS AQUEOUS AND ALCOHOL EXTRACTS

Selected Test: ABTS Assay - ***2,2-azino*-bis-*(3-ethylbenzothiazoline-6-sulphonic) acid assay,***

Standard : Gallic Acid

Calculation: The values obtained by the in vitro studies are calculated for Percentage Inhibitory Activity(%) and Half maximal inhibitory concentration.

Table 8 Sample order according to the high potential of Anti oxidant activity

S I	Sample name	IC 50 Value
01	Standard	1.91 µg/ml
02	Chatisgarh Aq	14.975 µg/ml
03	Changalpet Aq	32.158 µg/ml
04	Sri Lanka Aq	52.325 µg/ml
05	Mysuru Aq	69.127 µg/ml
06	Chatisgarh Al	112.401 µg/ml

ORIGINAL RESEARCH ARTICLE

07	Mysuru	Al	127.450 µg/ml
08	Changalpet	Al	228.195 µg/ml
09	Sri Lanka	Al	348.863 µg/ml

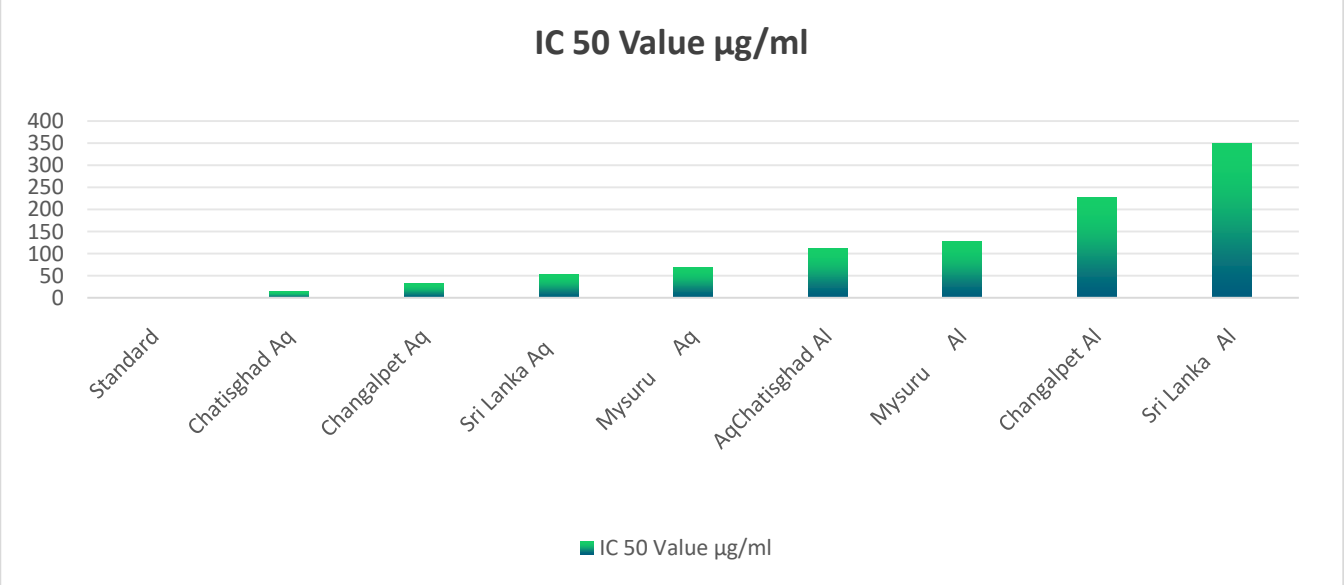


Figure 4 IC 50 value of all samples

Table 9 Observation of Desha, Kaala of Samples Collected Sites

Sample name	Changalpet	Chattisgarh	Mysore	Sri Lanka
Longitude	79.9888413	81.8661442	76.6393805	80.6326916
Latitude	12.6819372	21.2786567	12.2958104	8.1995638
Attitude/Elevation	Nonem (0ft)	279.15m	742.98m	136.52m
Annual high temperature	33.18ºC	33.23ºC	30.73ºC	32.18ºC
Annual low temperature	25.58ºC	22.72ºC	20.31ºC	23.64ºC
Average annual prcp.	86.78mm	67.04mm	140.02mm	132.42mm
Warmest month	May 39.13ºC	May 42.98ºC	April 36.12ºC	April 34.35ºC
Coldest Month	January 21.63ºC	January 14.65ºC	December 17.31ºC	January 20.71ºC
Wettest Month	November 277.14mm	August 260.61mm	October 321.09mm	October 302.2mm
Driest Month	March 8.36mm	April 5.76mm	January 7.99mm	June 32.19mm
Number of days with rainfall (≥ 1.0 mm)	119.27 days (32.68%)	87.91 days (24.08%)	179.64 days (49.22%)	165.45 days (45.33%)
Days with no rain	245.73 days (67.32%)	277.09 days (75.92%)	185.36 days (50.78%)	199.55 days (54.67%)
Humidity	65.36%	49.28%	66.56%	75.5%

OBSERVATION OF BHUMI (SOIL) of SAMPLES COLLECTED SITES

Showing table no 09

1. Chengalpettu, Tamilnadu

The soil in Chengalpettu is genarally considered to be of low quality.It is often described as highly stony or mixed with lime, gravel, soda, and laterite.This type of soil can pose challenges for agriculture and other land uses due to its poor fertility and drainage properties.

2. Tatamari,Chhattisgarh

ORIGINAL RESEARCH ARTICLE

The soil in Chhattisgarh, including the Tatamari region, varies significantly across different areas. Here are some key types of soil found in Chhattisgarh:

Bhata (Lateritic Soil): Found in the plains, this soil is rich in iron and aluminum.

Matasi (Sandy Loam): Also common in the plains, this soil is well-drained and suitable for various crops.

Dorsa (Clay Loam): This soil type retains moisture well, making it good for rice cultivation.

Kanhar (Clay Soil): Known for its high fertility, this soil is ideal for growing paddy and other water-intensive crops.Tatamari, being a hilly region, might have a mix of these soils, especially the lateritic and clay loam types, which are common in hilly and plateau areas.

3. Mysuru, Karnataka

Mysuru district features a variety of soil types, primarily categorized into three main types:

Red Sandy Soils: These soils cover most of the district and are known for their high permeability and neutral pH levels.

Red Loamy Soils: Found in certain areas, these soils are also quite permeable and support various agricultural activities.

Deep Black Soils: These are less common but are present in some parts of the district

4. North Central Province,Sri Lanka

Soil Types: The most common soil types in Sri Lanka include:

1. **Reddish Brown Earths (RBE)**: Found mainly in the Dry Zone.
2. **Red Yellow Podzolic (RYP)**: Predominantly in the Wet Zone.
3. **Low Humic Gley (LHG):** Typically found in poorly drained areas.

The relationship between soil (Bhumi) and phytoconstituents is quite significant. Phytoconstituents are the bioactive compounds found in plants, and their composition and concentration can be greatly influenced by the soil in which the plants grow. Here are some key points about this relationship:

NUTRIENT AVAILABILITY:

- The type and amount of nutrients available in the soil can affect the synthesis of phytoconstituents. For example, soils rich in nitrogen can enhance the production of alkaloids in certain plants[1].

Impact on Plant Phytoconstituents:

- Both iron and aluminum are abundant in soils and can form complexes with organic matter. These complexes can influence the soil's physical and chemical properties[2] .
- **Iron**: Adequate iron levels are crucial for the synthesis of chlorophyll and other phytoconstituents like flavonoids and phenolics. Iron deficiency can lead to chlorosis, reducing the plant's ability to produce these compounds[3].
- **Aluminum**: High levels of aluminum can inhibit root growth and function, affecting the plant's overall health and its ability to uptake nutrients necessary for phytoconstituent synthesis. Aluminum, while not essential for

ORIGINAL RESEARCH ARTICLE

plants, can become toxic at high concentrations, particularly in acidic soils[3] .

➢ As a feedback mechanism of the plants grow in such soil condition naturally prepared to fight with toxicity by enhancing anti toxicity and anti oxidant activity. Therefore more concentrated phytoconstituent such as phenolic compound could be found.

SOIL pH

According to the soil condition report of the four places, Chhattisgarh soil showing highest nutrition level of iron and aluminum.

According to the soil condition of all sites which samples were collected, Chatisgarh showing more sandy, clay combined with lateritic soil compared to the other sites of samples.

➢ The pH level of the soil can influence the availability of nutrients and minerals, which in turn affects the types and amounts of phytoconstituents produced. For instance, acidic soils might increase the availability of certain minerals that are crucial for the synthesis of specific phytochemicals[4].

➢ In previous study proven , that the availability of most micro nutrients decreases at higher soil pH levels. This data suggests that soil pH affect plant growth and phytochemicals content - Among phytochemical phenolics, tannins, flavonoids, saponins and alkaloids are major components.

➢ The quantity of these phytochemicals is mainly depending on available soil nutrients, minerals, environment water availability etc). directly. Although, field experiments are needed to clarify the effects of various physico-chemical factors like soil type and pH on nutrient availability and the threshold[5] .

SOIL TEXTURE AND STRUCTURE:

➢ The physical properties of the soil, such as its texture (sand, silt, clay) and structure, can affect water retention and root penetration, which are critical for the uptake of nutrients and minerals necessary for phytoconstituent synthesis.

Soil Texture

Soil texture refers to the proportion of sand, silt, and clay particles in the soil. It affects several key soil properties:

➢ **Water Retention**: Clay soils retain more water than sandy soils, which can influence the hydration status of plants and, consequently, the synthesis of certain phytoconstituents[6.]

➢ **Nutrient Availability**: Fine-textured soils (like clay) have a higher cation exchange capacity, meaning they can hold more nutrients that plants need for growth[7] .

➢ **Root Penetration:** Sandy soils are easier for roots to penetrate, which can affect the uptake of nutrients and water[8] .

Soil Structure

Soil structure refers to the arrangement of soil particles into aggregates. Good soil structure improves:

➢ **Aeration**: Well-structured soils have better air circulation, which is essential for root respiration and the synthesis of certain phytoconstituents[8] .

ORIGINAL RESEARCH ARTICLE

- **Microbial Activity:** A good structure supports a healthy microbial community, which can enhance the availability of nutrients through processes like nitrogen fixation[8].

Impact on Phytoconstituents

Phytoconstituents are the bioactive compounds in plants, such as alkaloids, flavonoids, and terpenoids. The soil's texture and structure can influence these compounds in several ways:

- **Nutrient Uptake**: The availability of nutrients in the soil directly affects the synthesis of phytoconstituents. For example, nitrogen availability can influence the production of alkaloids[9].
- **Stress Response:** Plants growing in soils with poor structure or texture may experience stress, leading to the production of secondary metabolites like phenoles, flavonoids, which help in stress mitigation[9].

DISCUSSION

DISCUSSION ON SELECTION OF TITLE AND DRUG .

Mere identification of the drug is not the only criteria for therapeutic efficacy,but knowledge of ***desha, kala, rutu, sangrahana vidhi*** etc. Plays a very important role in enhancing the potency of a drug.

Ancient *Acharyas* lived amidst the nature and collected drugs from its natural habitat, considering above factors. Now a days physicians depend upon the market for procurement of drugs. As demand and utility of the herbal drugs has increased considerably the aspects according to classical collection of drugs are not considered during the collection of a drug thus resulting in the substandard quality of the drug.

Hence *Kalamegha* which has a wide range of utility is collected from different geographical sites analyzed for qualitative and quantitative parameters and compared with the API standard.

DISCUSSION ON SELECTION OF SAMPLES COLLECTING PLACES

According to the authentic text there are three desha ***sadharana, jangala*** and ***anupa***. Based on thais for ***Sadharana desha*** : Mysuru, Karnatak sample was collected ***Anupa desha*** : Sri Lanka, North Central Province sample, for ***Jangala desha*** : Changalpet, Tamilnadu and Chatishgarh, Central Indian sample were selected as resources places.

DISCUSSION ON REVIEW OF LITERATURE:

Ancient texts identified the drugs by different synonyms. References of ***Kalamegha*** is found in ***Priya Nighantu*** and ***Nighantu Adarsha*** which describe ***Kalamegha*** as ***Bhunimba*** & ***Yavatikta*** . Morphologically, colour of the plant shape of the fruits and bitter taste suggests the identification of the drug. The synonyms and references in the classical literature suggest ***Andrographis paniculata*** as the source for ***Kalamegha***.

Kalamegha has ***tikta rasa,*** and ***katu vipaka*** its indicated in ***Kushta, Kandu, Yakritroga, Krimi, Jwara***.It is one of the ingredient of important formulations like ***Bhunimbadi churna***, ***Mahatiktaka ghritha, Chandraprabha vati,***

ORIGINAL RESEARCH ARTICLE

Dhanvanthara gutika and ***Manasamithra vati*** *etc.,*

Hence this study was taken up to evaluate the different Geographical samples of ***Kalamegha*** which is collected from different natural habitats and compared with the biomarker Andrographolide.

DISCUSSION ON ANALYTICAL STUDY:

ORGANOLEPTIC FEATURES:

All the samples collected from the natural habitat were extremely bitter and with characteristic odour suggesting the presence of Andrographolide .

MACROSCOPIC STUDY

Maximum plant height 61.30 cm,leaf length 7.12cm and width 1.88cm was recorded from Mysuru sample.Minimum height 53.40cm, leaf length 6.74cm recorded from Chatisgarh sample.

MICROSCOPIC STUDY

Powder microscopy of all samples of *Kalamegha* revealed the presence of Leaf epidermis, Trichome, Paranchyma Cells with cystolith.This confirms with the API standard and shows its genuinity.

PHYSICOCHEMICAL STUDY

- The drugs, Andrographis paniculata nees . all four samples were devoid of foreign matters, as it was collected personally indicating the absence of adulteration.
- Loss on drying of the sample is directly related to its moisture content. If the moisture content is very high then it may affect its preservation.Loss on drying was more in Mysuru sample compared to other three samples indicating high moisture content because of high humidity in ***Kalamegha*** sample of Mysuru .
- All the samples showed the values within the API standards suggesting that the samples were not adulterated and contaminated while collecting.Total ash of Mysuru sample was less when compared to others.Here the less ash content reveals that the drug is containing less amount of inorganic matter owing to the geographical and was within the standard limits as in API.
- All the samples showed the extractive values as per the standards.Water soluble extractive values were maximum in the sample collected from the Mysuru natural habitat.Minimal difference was seen in the extractive values of Sri Lankan sample when compared to the other samples.Alcohol soluble extractive value was more in Mysuru sample when compared to the other samples.
- Among the alcohol and aqueous extracts, the yield was more in aqueous extracts. showing aqueous soluble components are more in ***Kalamegha,*** thus suggesting that it can be better used in ***Kashaya*** form.
- Standard pH value of ***Kalamegha*** is 3 to 5. According to the all samples results **Chatisgarh** ***Kalamegha*** was acidic in nature . pH value of **Chatisgarh** ***Kalamegha*** was **6.36** which reveals presence of highest phenols more compared to other three samples.
- Mysuru and Sri Lanka samples were above 7 pH value indicating alkaline in nature .

ORIGINAL RESEARCH ARTICLE

PHYTOCHEMICAL STUDY

SECONDARY METABOLITES:

- The phytochemical study revealed the presence of **Phenol compounds, Flavonoid, Triterpenoids, Tannin, Saponins, Glycosides** and **Steroids** in all the samples. This proves that there is no significant difference in the active constituents.
- **Phenol,saponin, Steroids** and **alkaloid** were present in both aqueous and alcohol extracts of ***Kalamegha***.
- In addition **Flavanoids** and **Triterpenoids** were present only in alcohol extracts of ***kalamegha*** while **glycosides** only present in aqueous extracts of ***Kalamegha***.
- Presence of **Triterpenoides, Alkaloids** and **Glycosides** in all ***Kalamegha*** samples could be due to its ***Tikta rasa***.
- Presence of **Glycosides** are responsible for pharmacological activities like **Cardiotonic** and **Purgative**[10] **.**
- Presence of **Flavonoids**, which are **polyphenols**, have pharmacological actions like **Anti-oxidant**[11] may be correlated with ***rasayana karma.***
- **Triterpenoids** have pharmacological activities like **Anti-hyperlipidemic**[12] and **Immunomodulatory** activity .
- **Tannins** have **Anti-oxidant activity**[13] may be correlated with ***rasayana karma.***
- **Saponins,** have **Haemolytic activity**[14] and **Anti-inflammatory activity.**

PRIMARY METABOLITES:

- Carbohydrate and starch were present in all alcohol extracts of *Kalamegha.*
- Except in alcohol extracts of *Kalamegha* proteins were present in remaining all the four aqueous extracts of *Kalamegha.*

INORGANIC CONSTITUENTS:

- Presence of **Iron, sodium, calcium, potassium, sulphate, chloride, nitrate** and **phosphate** were screened in aqueous and alcohol extracts of ***Kalamegha.***
- The phytochemical evaluation of four different geographical samples of ***Kalamegha*** revealed the presence of certain common phytoconstituents indicating their similar pharmacodynamic activities like **Anti-oxidant, Anti-hyperlipidemic, Immunomodulatory activity, Anti-inflammatory activity.**

CHROMATOGRAPHIC ANALYSIS

ANALYSIS OF HPLC OF ANDROGRAPHOLIDE

- The photochemical Andrographolide was selected as standard, as it is the common phytoconstituent present in ***Kalamegha.***The HPLC profiles of four samples of ***Kalamegha*** showed resemblance in their peaks with similar retention time to the standard Andrographolide.
- Quantification of Andrographolide through HPLC showed highest result in both alcohol and aqueous extracts of sample of ***Kalamegha*** collected from **Chatisgarh**.
- Quantification of Andrographolide through HPLC showed lowest result in both alcohol and aqueous extracts of sample of ***Kalamegha*** collected from **Mysuru**.

ORIGINAL RESEARCH ARTICLE

TOTAL ANDROGRAPHOLIDE AS BITTERS

➢ The bitterness of ***Kalamegha*** is due to the presence of the Andrographolids. The amount of bitterness correspond to the amount of Andrographolid.

Total bitter was maximum in the sample collected from **Chattisgarh** .Among the all natural habitat samples least amount was seen in **Mysuru**.

➢ The difference in the above findings may be due to the impact of season, plant maturity, geographical variation, storage period etc.

ANALYSIS OF HPLC OF GALLIC ACID

➢ The HPLC profiles of four samples of ***Kalamegha*** showed resemblance in their peaks with similar retention time to the standard Gallic acid

➢ Quantification of Gallic acid through HPLC showed highest result in aqueous extracts of sample of ***Kalamegha*** collected from **Chatisgarh**.

➢ Quantification of Gallic acid through HPLC showed lowest result in aqueous extracts of sample of ***Kalamegha*** collected from **Mysuru**.

➢ The difference in the above findings may be due to the impact of season, plant maturity, geographical variation, storage period etc. Showing in table no 10.

Table 10 Analysis of HPLC of Gallic Acid

S.n.	Sample name	Andrographolide		Gallic acid		IC50 Value
		RT	Con: in %	RT	Con: in %	(Antioxidant)
		min	mg/ml	min	mg/ml	µg/ml
1	Changalpet Aq	3.637	2.3595	3.630	0.3353	32.158
2	Changalpet Al	3.530	0.6228	-	-	228.195
3	Chhatisgarh Aq	3.640	2.7086	3.400	0.4386	14.975
4	Chhatisgarh Al	3.717	1.7921	-	-	112.401
5	Mysuru Aq	3.593	1.2248	3.507	0.0370	69.127
6	Mysuru Al	3.547	0.4576	-	-	127.450
7	Sri Lanka Aq	3.410	1.3825	3.700	0.1079	52.325
8	Sri Lanka Al	3.440	0.6967	-	-	348.863
9	**Standard**	**3.520**	**100**	**3.623**	**100**	**1.91**

DISCUSSION ON RELATION BETWEEN DESHA KAALA AND PHYTOCONSTITUENTS

➢ Acharya Charaka quotes the importance of Rutu in germination and growth of medicinal plants[15].

➢ Proper season has been qualified as the season during which the plant intended for collection should have Rasa in abundance . Charaka has discussed the effect of stars, planets, moon, sun, and air, fire on manifestation of Rasa, Guna, Veerya, Vipaka and Prabhava of drugs[15].

➢ He highlighted the role of 'Kaala' in the formation of drug properties and mentioned specific seasons for the collection of different parts[15].

ORIGINAL RESEARCH ARTICLE

- He categorically pointed out that the drugs growing seasonally are only to be collected[15].
- Acharya Chakrapani further cleared that medicinal plant with Ushna Veerya should be collected in Greeshma Rutu and Sheeta Veerya drugs should be collected in Shishir rutu[15].
- In this study all samples collected in Greeshma ritu extends from mid-April to mid-July .
- In that Chatisgarh sample of Kalamegha showing highest amount of andrographolide as well as it shows highest potential of anti oxidant activity.
- According to the weather of Chtisgarh it belongs to the highest temperate and lowest humidity atmosphere among all four samples .
- High temperatures and low humidity can significantly impact phytoconstituents, which are the bio-active compounds in plants. These conditions can affect the plant's metabolism, growth, and the concentration of these compounds[16] .
- High temperatures and low humidity, Increased Secondary Metabolites: Stress conditions, such as high temperatures and low humidity, often lead to an increase in secondary metabolites like Phenolic compound flavonoids, alkaloids, and terpenoids. These compounds help plants cope with stress but can also alter the plant's nutritional and medicinal properties[16].
- In the study named Total Soluble Phenolic, Antioxidatant Enzymatic, and Osmolyte Regulation under Drought Stress Conditions had proven that, there was a 100% increase in phenolic content under drought stress conditions[17] .

DISCUSSION ON RELATION BETWEEN BHUMI(SOIL) AND PHYTOCONSTITUENTS.

NUTRIENT AVAILABILITY:

- The relationship between soil (Bhumi) and phytoconstituents is quite significant. Phytoconstituents are the bioactive compounds found in plants, and their composition and concentration can be greatly influenced by the soil in which the plants grow.
- According to the soil condition report of the four places, chatisgarh soil showing highest nutrition level of iron and aluminium. And its show high potential of anti oxidant activity.

SOIL PH:

- Usually the soil pH level correlate with growing plants pH in it. Lowest pH level of samples was shows by Chatisgarh Kalamegha.And it was showing highest anti oxidant activity as well as andrgrapholide content.
- Highest pH level of samples was Mysuru Kalamegha and it was showing lowest anti oxidant activity as well as minimum amount of andrographolid content.

SOIL TEXTURE AND STRUCTURE:

According to the soil condition of all sites which samples were collected Chatisgarh showing quality of texture such as sandy , clay combined with lateritic soil with poor structure compared

ORIGINAL RESEARCH ARTICLE

to the other sites of samples lead to more concentration of phytoconstituents.

Mysuru and Sri lanka kalamegha samples collected sites possess well structured soil condition which reduce the stress response of the plants, leading to the less production secondary metabolites like phenoles, flavonoids, which help in stress mitigation.

GEOGRAPHICAL VARIATIONS:

Different geographical regions have distinct soil compositions, which can lead to variations in the phytoconstituents of plants grown in those areas. For example, the medicinal properties of Phyllanthus niruri (Bhoomi Amla) can vary based on the soil it is grown in[1].

- Latitude

Latitudes of the sites of samples collected are showing as Changalpet - 12.6819372, Chattisgarh - 21.2786567, Mysore - 12.2958104 and Sri Lanka - 8.1995638.

Chattisgarh localized more far to the equator, than other samples collected sites.More close to the equator mean those places having comparatively more humidity level Chattisgarh far from the equator which showing less humidity level.

Therefore plants which grow in that area having more concentration of phyto constituents due to the influence of highest temperature and lesser humidity (draught condition) .

DISCUSSION ON IN - VITRO STUDY

- An in-vitro study to compare the Anti oxidant activity of four different geographical samples of *Kalamegha* was performed by assessing the Anti oxidant activity by ABTS assay.
- ABTS assay is Colorimetric method to evaluate the decay of $ABTS^{\bullet+}$ in the presence of an antioxidant agent.

ASSESSMENT OF ANTI OXIDANT ACTIVITY

- IC50 Value through ABTS assay, showed lowest result in both alcohol and aqueous extracts of sample of *Kalamegha* collected from Chatisgarh.
- IC50 Value through ABTS assay showed highest result: in alcohol extracts of four sample of *Kalamegha* collected from Sri Lanka.And in aqueous extracts of four sample of *Kalamegha* collected from Mysuru.
- The highest potential of anti oxidant activity of *Kalamegha* correspond to the lowest IC 50 value of sample.
- The lowest potential of anti oxidant activity of *Kalamegha* correspond to the highest IC 50 value of sample.

CONCLUSION

- Pharmacognostic, Physiochemical and Phytochemical studies showed the results as per API standards.
- The HPLC study carried out for qantification of Andrographolide in Kalamegha confirmed the genuinity of the trial samples.
- The HPLC study carried out for qantification of Gallic acid in Kalamegha

ORIGINAL RESEARCH ARTICLE

confirmed the anti oxidant property of the trial samples.

- The Aqueous extract of *Kalamegha* exhibited more concentration of Andrographolide when compared to Alcoholic extract. Hence, *Kashaya* dosage form may be more beneficial.
- The Aqueous extract of *Kalamegha* collected from Chattisgarh exhibited more concentration of Andrographolide compound when compared to other three samples.
- In- invitro ABTS assay, All four samples showed significant antioxidant results.
- The Aqueous extract of *Kalamegha* exhibited better anti oxidant property when compared to Alcoholic extract.
- The Aqueous extract of *Kalamegha* collected from Chattisgarh exhibited better anti oxidant property when compared to other three samples.
- The *desha* and *sangrahana kala* plays an important role in quality of the phytoconstituents which further exhibit better pharmacological action
- Collection of herbs From Jangala and Sadharana desha are said to be virya sampanna
- Hence , as per the reference of classics the samples were collected and then analytical and invitro assay were carried out which showed potential results from Chattisgarh sample which is predominantly a Jangala desha .
- The climatic conditions and other environmental factors has a major influence on morphology and growth which results in saviryata of the dravya I.e, better phytoconstituents.

REFERENCES

1. B. R. Annapoorna & P. Madan Kumar Bioactive Compounds of Bhoomi AmlaTherapeutic Use of Medicinal Plants and their Extracts: Volume 2(Phyllanthus niruri): Nutritional and Pharmacological Aspects first chapter -food production diversity and safety under climatic change pp 113-119

2. Hu, N., Mao, Q., Zhong, X. et al. Factors driving the positive resorption of aluminum and iron from old leaves. Plant Soil 488, 443–450 (2023).

3. Soil Texture and Structure is shared under a CC BY 4.0 license and was authored, remixed, and/or curated by Colby Moorberg & David Crouse (Prairie Press)Published:14 March 2023 .

4. Ravindra Kn2, Supreetha Bh1, Nithyanandha Kr3,Nutan Sr3, Deepdarshan Urs1, Dharmappa Kk1, Giresha Prabhudev sh et al, AsEffect of soil pH on plants growth, phytochemical contents and their antioxidant activity, journal of advanced applied scientific research-isentient-2022 issn(o): 2454-3225 joaasr-vol-5-5-september-2023 15-39

5. Rota Wagai, Masako Kajiura, and Maki AsanoIron and aluminum association with microbially processed organic matter via meso-density aggregate formation across soils: organo-metallic glue hypothesis Articles Volume 6, issue 2 SOIL, 6, 597–627, 2020

6. Joko Warino S.P M.Si What is Soil Texture? Components, Influence, and Classification, June 25, 2024

ORIGINAL RESEARCH ARTICLE

7. E-unit: Understanding Soil Texture and Structure Page 1-7 AgEdLibrary.co

8. The important role of soil texture on water Written by CHRISTINE ANNE CLARK info@extension.wisc.edu© 2024 The Board of Regents of the University of Wisconsin System

9. Alamgir, A.N.M. (2018). Phytoconstituents—Active and Inert Constituents, Metabolic Pathways, Chemistry and Application of Phytoconstituents, Primary Metabolic Products, and Bioactive Compounds of Primary Metabolic Origin. In: Therapeutic Use of Medicinal Plants and their Extracts: Volume 2. Progress in Drug Research, vol 74. Springer, Cham.

10. Kokate C. K, Purohit A. R, Gokhale S.B, Pharmacognosy, 36th edition, Pune, Nirali Praskshan, Sep 2006, T.pg. 634.

11. Avtar Chand Rana, Bhawna Guliya, Chemistry and Pharmacoloy of Flavanoids- A Review, Indian Journal Pharmaceutical Education and Research, Vol 53, Jan-March 2019.

12. Prithviraj Karak, Biological Activities of Flavanoids: An Overview, International Journal of Pharmaceutical Sciences and Research, Vol 10 (4) PP. 1567-1574, 2019.

13. Rebamang A. Mosa, Thabo Ndwandwe, Nontando F. Cele, Andy R. Opoku, Anticoagulant and Anti-inflammatory activity of a triterpene from Protorthus longifolia stem bark, Journal of Medicinal Plants Research, Vol 9 (19), pp.613-617, May 2015.

14. Mota ML Thomas G, Barbosa Filho JM. Anti-inflammatory actions of tannins isolated from the bark of Anacardium occidentale L. J Ethanopharmacol. 1985 Jul;13(3): 289-300.

15. Vaidta Yadavaji Trikamji Acharya Agnivesha,'Charak Samhita,' revised by Charak and Dridhbala with 'Ayurveda Dipika' commented by Chakrapanidatta, Chaukhamba Surbharati Prakashan,Gopal Mandir Lane,Varanasi-221001,India,reprint 2005, Vimansthana 8/87), (Sutrasthana 9/7), (Vimansthana 3/4), (Sutrasthana 11/23), Vimanasthana ¾,(Kalpasthana 1/10), (Sutrasthana 27/316-317).

16. Behera, S.K., Behera, M.D., Tuli, R. et al. Atmospheric temperature and humidity demonstrated strong correlation with productivity in tropical moist deciduous forests. Environ Monit Assess 195, 69 (2023).

17. Plants' Physio-Biochemical and Phyto-Hormonal Responses to Alleviate the Adverse Effects of Drought Stress: A Comprehensive ReviewAbdul Wahab1,Gholamreza Abdi2,Muhammad Hamzah Saleem ,Baber Ali,Saqib Ullah ,Wadood Shah 6,Sahar Mumtaz Ghulam Yasin 8,Crina Carmen Muresan and Romina Alina Marc .