

Quantitative variations of Deacyl gymnemic acid, Gymnemagenin, and Gymnemic acid-IV in *Gymnema sylvestre* R.Br. from different geographical regions of Karnataka.

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Abstract

Gymnema sylvestre is a widespread potential antidiabetic medicinal plant that thrives in southern India tropical forests. *Gymnema sylvestre* was collected from various geographical locations in Karnataka. Along with soil samples, rainfall, temperature and geographical data were collected. HPLC methods were used to analyse chemical components in the plant extract. The highest yield of chemical components viz. Deacyl Gymnemic acid, Gymnemagenin, Gymnemic acid IV was obtained by continuously heating the dried leaf in a Soxhlet system with 90% methanol. All the collected samples yielded the chemical components Deacyl Gymnemic acid, Gymnemagenin, and Gymnemic acid IV. Standard analytical methods were used to determine NPK, pH, and EC in soil samples collected from nine locations across the state. The rainfall, temperature data collected from Meteorological department, UAS, Bangalore. All nine samples were subjected to phytochemical analysis to estimate the levels of Deacyl Gymnemic acid, Gymnemagenin, and Gymnemic acid IV. The rainfall, temperature and soil characters like pH, EC, NPK were analysed and compared with the concentrations of above constituents. The percentage of Deacyl Gymnemic acid in Chitradurga 3.29, Bagalkote 1.86, Davanagere 1.47, Mysuru 1.15, Shivamogga 0.8 and Ballari 0.5, Belagavi 0.45, Chikkamagaluru 0.18, Udupi 0.08. These results clearly indicate the effect of abiotic factors. The concentration of Gymnemic acid shows Chikkamagaluru 0.4, Chitradurga 0.26, Udupi 0.07, Bagalkote 0.06, Mysuru & Belagavi 0.04, Shivamogga 0.03, Davanagere & Ballari 0.02. The percentage of Gymnemagenin is in Chitradurga 0.43, Bagalkote 0.26, Mysuru 0.23 & Davanagere 0.23, Ballari 0.18, Shivamogga 0.16, Belagavi 0.16, Chikkamagaluru 0.05, and Udupi 0.005. The concentrations of the phytochemical constituents indicate the Chitradurga samples show highest in both Deacyl Gymnemic acid and Gymnemagenin.

1. Introduction

G. sylvestre R.Br., a species of medicinal plant (*Apocynaceae*), grows naturally in tropical forests in Africa, Australia, and India (Tahira *et al.*, 2013). A wide range of plant species are utilized for therapeutic purposes in various traditional and contemporary medical systems (Paliwal *et al.*, 2009). *Gymnema sylvestre* R.Br. is a woody shrub with significant therapeutic potential. *G. sylvestre* has a considerable degree of variation in the amounts of bioactive chemicals among the accessions gathered from various sites. The quality of pharmaceutical products and the manufacture of herbal medicines may be impacted by variations in the amount of gymnemic acid (Ramalingam *et al.*, 2014).

As such, the study represents a chemical diversity analysis carried out to assess the variation in Chemical constituents levels, the study was conducted on various *Gymnema* accessions that thrive under different agroclimatic conditions. The selection of the high gymnemic acid requires a thorough investigation of chemical diversity (Stocklin *et al.*, 1969). Plant growth and development are greatly influenced by nature. The amount of secondary metabolites frequently necessitates ideal climatic and edaphic conditions. Plant species vary greatly in their soil and nutritional needs, and medicinal plants have drawn a lot of attention to these factors. Potassium (K)

and phosphorus (P) are vital minerals that plants need in relatively high quantities (Harry *et al.*, 1969). The quantity of plant nutrients in soil is known as soil fertility, and the texture, structure, consistency, and hardness of the soil layers mostly affect how resistant the soil is to erosion. Nitrogen, phosphorus, and potassium are examples of primary nutrients that plants utilize in significant amounts (Okalebo *et al.*, 2002). Through plant absorption, nutrient ions are recovered from a range of sources as they are drawn out of the soil solution. Among various soil parameters, pH plays a major role in determining nutrient availability (Rajasekar *et al.*, 2017). Potassium is the mineral element that plants absorb the most of, a side from nitrogen. Similar to nitrogen, almost all phosphorus is present in soil as phosphates (PO_4^{3-}), which can occur in both inorganic and organic forms. Phosphorus plays an essential role in the biochemical processes associated with photosynthesis. Nitrogen occurs in all living cells and plays a crucial role in protein and enzyme synthesis, as well as in metabolic pathways responsible for energy production and transfer (Baker *et al.*, 1981). Nitrogen is found in chlorophyll, the green pigment of plants that controls photosynthesis (Baker *et al.* 1983). Among various environmental parameters, soil fertility plays a major role in determining crop yield potential. Macronutrients play a crucial role in plant growth and development. Soil pH is one of the most important characteristics which gives

information about many soil properties. Maintaining appropriate electrical conductivity (EC) levels is essential to prevent nutrient imbalances, promote efficient nutrient uptake by plants, and protect soil structure from degradation. Fluctuations in climatic parameters like temperature, rainfall, and humidity significantly influence the growth and phytochemical composition of medicinal plants. Variations in temperature and rainfall, along with the occurrence of extreme climatic events, can interrupt plant growth and development, thereby diminishing yield and phytochemical quality.

2. Materials and Methods

We have collected nine *Gymnema sylvestre* samples from various districts in Karnataka (Table1 & Figure 1). Soil sampling was carried out concurrently with plant collection from the same locations to ensure comparative analysis. The standard procedures were followed in the collecting of soil samples. Sampling of plant materials was collected from June to July 2024. Several local floras were used in accurately identifying and verifying the plant material (Gamble, 1915-1934, Singh, 1988 and Manjunath *et al.*, 2004). Prepared Herbarium specimen and submitted to Herbarium, Gandhi Krishi Vigyana Kendra (GKVK), UAS, Bangalore, get the authentication of bearing specimen voucher number for *Gymnema sylvestre* (RRCBI-19516).

Table 1: Name of different Provenance of *Gymnema sylvestre* R.Br.

Sl. No.	Place name	Latitude & Longitude	Altitude (mts)
1	Davangere	14°46'47"N 75°92'33"E	602.5
2	Shivamogga	13°50'26"N 75°09'03"E	823
3	Chitradurga	14°23'10"N 76°38'63"E	1159

4	Mysuru	12°29'58"N 76°63'93"E	770
5	Chikkamagaluru	13°39'23"N 75°34'00"E	570
6	Udupi	13°49'40"N 75°07'15"E	35
7	Belagavi	15°85'21"N 74°50'44"E	751
8	Ballari	15°14'21" N 76°92'40"E	485

9	Bagalkote	16°18'17"N 75°69'58"E	556
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2.1. Processing of Plant Material

At different ecotypes Plant materials were collected and pulverized into a fine powder for phytochemical analysis, dried in the shade, and put in a tight container for later use. The powdered plant material was subjected to continuous hot extraction in a Soxhlet apparatus with methanol as the extracting solvent.

2.2. Extraction with 90% methanol

90% methanol was then used to extract the plant's dry powder component. A complete methanol-soluble extract was obtained after 12 hours of extraction using 90% methanol. The methanol-soluble extract was distilled, and a thick paste was finally obtained (Hoopers *et al.*, 1887).

2.3. HPLC Analysis of *Gymnema sylvestre*

All three components viz. gymnemic acid, gymnemagenin and deacyl gymnmenic

acid analysis were done by the following method

2.3.1. Total gymnemic acid, Gymnemagenin and deacyl gymnmenic acid by HPLC

i) Standard Preparation:

9 ml of 90% methanol (0.1 mg/ml) was used to dissolve 1 mg of each standard. (Kumar *et al.*, 2022)

ii) Sample Preparation (7.8 mg/ml):

0.78 gm of material was refluxed with 25 ml of 50% ethanol and 2 ml of KOH for 60 mint at $92 \pm 2^\circ\text{C}$. After the solution was allowed to cool, 5.5 ml of hydrochloric acid (HCl) was added carefully, and it refluxed for two hours. After cooling and adjusting the pH down to 7.5–8.5, After filtration, the solution was collected in a 100 ml volumetric flask and diluted with 50% ethanol. It was then transferred into an HPLC vial after passing through a 0.45 μm syringe filter. The optimized conditions are given in Table 2.

Table 2: Showing the Optimized method for the assay of chemical complements

Column	Shim-pack GIS C18 (20x250) mm 10 μ
Flow Rate	15.0 ml/mint
Wavelength	210 nm and 236nm
Injection volume	1.5ml
Column temperature	25°C
Run time	30 mint

Mobile Phase	A- 1mM KH ₂ PO ₄ in Water with 0.5ml OPA B - Acetonitrile
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2. 4. Soil collection and analysis:

The same sites used to collect plant materials were also used to take soil samples. The collected soil samples were dried and used for the analysis of NPK, pH and Electric conductivity was done (Rajasekar *et al.*, 2017).

2.4.1. Analysis of NPK:

i) Estimation of available nitrogen in soil was carried out following standard procedures

The arable horizon of the soil contains nitrogen (N) mainly in organic combinations derived from plant and microbial residues. In the soil solution organic N is gradually transformed into ammonical (NH₄⁺), nitrite (NO₂⁻) and nitrate (NO₃⁻) -N by microbial processes. Organic -N is in itself of very little use to plants as it cannot be absorbed as such. It is therefore necessary to estimate the different forms of mineralized or available N. The NO₃ -N and NO₂ -N together hardly exceed 1% of the total N in normal soil. In soil testing labs, organic carbon is typically used to determine the amount of accessible nitrogen. Available nitrogen (N) in the soil represents the portion of total nitrogen that is transformed into plant-accessible forms through mineralization and other biological processes. On average, the available nitrogen fraction constitutes only 0.5 to 2.5% of the total nitrogen present in the soil at any given time (Masrie *et al.*, 2018).

ii) Determination of available Potassium in soil

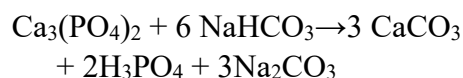
The total potassium (K) content of a soil varies from 0.05 to 2.5%. The total K is distributed in mineral form (lattice K, 90-

98%), fixed or non exchangeable K (1-10%) and exchangeable plus water soluble K (1-2%). Exchangeable K represents an average of 2-15% of the sum of exchangeable bases and 1-3% of the total cation exchange capacity. Both water soluble and exchangeable K are most accessible to the plant. Available K can thus be separated into immediately available, which is water soluble and exchangeable and that potentially available or fixed. In the neutral normal ammonium acetate method, the extract obtained contains water-soluble as well as exchangeable potassium. Extraction of K by neutral normal ammonium acetate is considered an effective measure of K availability in soils, validated through crop response correlations. The relatively unavailable K constitutes 92 to 98% of total K, slowly available K 1 to 10% and readily available K less than 1% (Jiang *et al.*, 2018).

iii) Determination of available Phosphorus in soil

Extraction:

Soil is shaken with 0.5 M sodium bicarbonate (pH 8.5). HCO₃⁻ ion suppresses the activity of Ca in soils and precipitates Ca as CaCO₃ leading to the release of Ca-bound P. Activated P-free charcoal (Darko-G-60) is used to absorb dispersed organic matter in soil and to make-filtrate colour less.



Estimation of solution P (Colour development)

Released phosphate in solution reacts with acidified ammonium molybdate to form heteropoly phosphomolybdate complex.

This complex is initially yellow in colour and further reduced by stannous chloride to give a partially reduced bluish coloured solution (Mathotra *et al.*, 2018).

iv) Determination of pH

The pH of the soil is measured using a glass electrode. A glass membrane is used in the glass electrode. Electric potential that is proportional to the pH difference of the 0.1 M HCl that is filled within the glass bulb (aH_2^+) develops across the glass membrane. Current flows over an ionic invisible bridge formed by the KCl diffusing into the soil-water suspension through the tiny holes in the calomel electrode. Between the electrodes, an electric potential builds up, and a galvanometer is used to measure the potential difference. The potential difference with respect to soil pH is used to calibrate the pH dial (Tziachris *et al.*, 2020).

v) Determination of Electric Conductivity

The electrical conductivity method is used to determine the total amount of soluble salts in soils. The mobility of ions is the only cause of electrical conductivity. When an ion is exposed to an external force, like an electrical field or a concentration gradient, its mobility is essentially the rate at which it travels through a solution. Charge, size, mass, and degree of solvation all have an impact on an ion's mobility (Narsimha *et al.*, 2013).

2.5. Climatic data:

Climatic data includes rainfall and the temperature, were collected from the Meteorological Department., UAS, Bangalore.

2.5.1. Rainfall

One of the main elements influencing soil erosion and plant yield in rain-fed agriculture is rainfall. The goal of the

review was to measure how much moisture or rainfall affects plant productivity. The rainfall pattern, including its distribution, intensity, and seasonal duration, was studied in relation to plant growth and development. According to studies, water stress during the vegetative stage mostly decreased productivity by causing smaller leaves to intercept less light (Mehta *et al.*, 2022).

2.5.2. Temperature

Plants are subjected to fluctuating temperatures during their entire life cycle, requiring constant physiological and metabolic adjustments. These adaptations must address daily and seasonal temperature fluctuations as well as climate change-related temperature shifts. A number of physiological, biochemical, morphological, and developmental reactions to rising temperatures have been reported, which enable plants to counteract the detrimental effects of rising global temperatures on crop performance (Duhan *et al.*, 2013).

2.5.3. Correlation analysis:

Correlation analysis was done by using the Google sheet program and using the data like soil nutrients (NPK, pH, EC), climatic parameters (rainfall, temperature), and phytochemicals in *Gymnema sylvestre* across Karnataka ecotypes to get Heatmap.

3. Results

The thorough and methodical pharmacognostical assessment would provide important data for next research. The research on this plant mostly concentrated on methods for identifying gymnemic acid, deacyl gymnemic acid, and gymnemagenin in order for increasing its yields (Dateo *et al.*, 1973). These procedures included separation and HPLC identification of the three compounds.

Methanol was extracted using a continuous hot extraction process in a Soxhlet apparatus, and other solvent systems, such as petroleum ether, were used for the extractions. The extraction with 90% methanol produced the highest yield of

gymnemic acid, deacyl gymnemic acid, and gymnemagenin of the two solvents studied. Table 3 showing the calculated gymnemic acid yields from the 9 ecotypes of Karnataka state.





Figure:1 A: Davangere, B: Shivamogga, C: Chitradurga, D: Mysuru, E: Chikkamagaluru, F: Udupi, G: Belagavi, H: Ballari, I: Bagalkote

Table 3. Showing the percentages of different components of nine ecotypes of *Gymnema sylvestre* collected from various regions of Karnataka.

SL. NO.	Name of the ecotype	District	% of Deacyl Gymnemic acid(w/w)	% of Gymnemagenin (w/w)	% of Gymnemic acid IV(w/w)
1.	Tholahunase	Davanagere	1.47	0.23	0.02
2.	Jogimatti	Chitradurga	3.29	0.43	0.26
3.	Agumbe	Shivamogga	0.80	0.16	0.03
4.	Saraswathipuram	Mysuru	1.15	0.23	0.04
5.	Nadpal	Udupi	0.08	0.005	0.07
6.	Kulur	Chikkamagaluru	0.18	0.05	0.40
7.	Khanapur	Belagavi	0.45	0.16	0.04
8.	Vinayaknagar	Bellary	0.50	0.18	0.02
9.	Biligi	Bagalkote	1.86	0.26	0.06

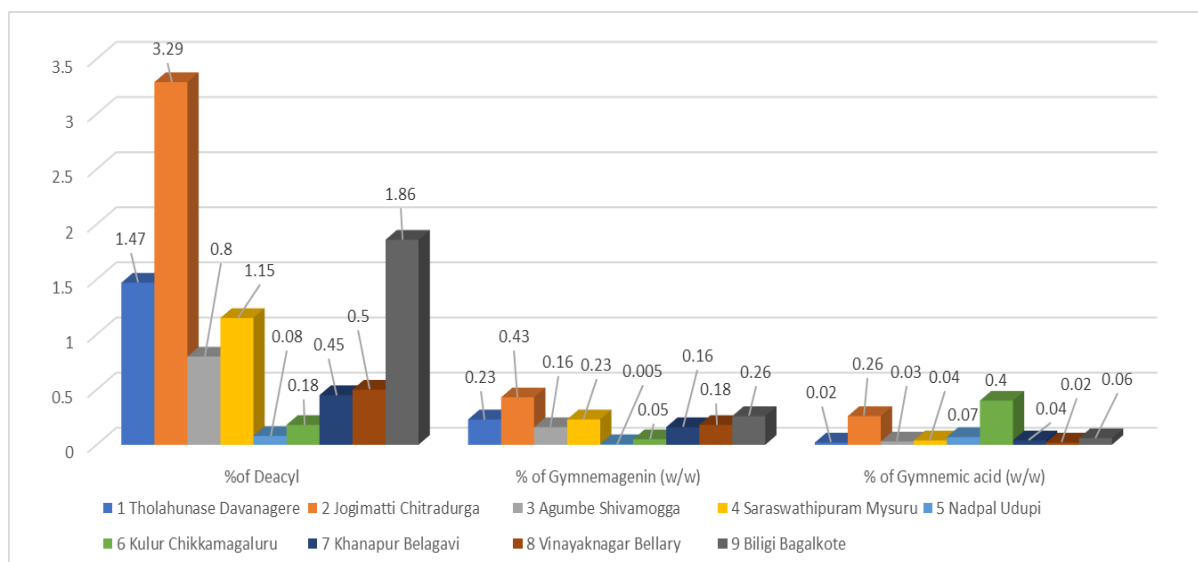


Figure: 2 Bar Diagram of Phytochemical Component

The quantitative estimation of three major bioactive compounds—Deacyl gymnemic acid, Gymnemagenin, and Gymnemic acid—in *Gymnema sylvestre* R. Br. leaves collected from nine different ecotypes across Karnataka revealed considerable variation in their phytochemical content. These variations can be attributed to environmental, geographical, and edaphic (soil-related) factors influencing secondary metabolite biosynthesis. Among all the ecotypes analyzed, Jogimatti (Chitradurga) [Graph 2] exhibited the highest concentration of Deacyl gymnemic acid (3.29%), Gymnemagenin (0.43%), and Gymnemic acid IV (0.26%), indicating that this region's climatic and soil conditions may be optimal for the accumulation of gymnemic-type saponins. In contrast, Nadpal (Udupi) [Graph 5] showed the lowest Deacyl gymnemic acid content (0.08%) and Gymnemagenin (0.005%), though Gymnemic acid was slightly higher (0.07%) compared to some other sites. This region receives very high rainfall and experiences humid coastal conditions, which might suppress saponin accumulation due to dilution effects or metabolic shifts toward other secondary compounds. Agumbe (Shivamogga)

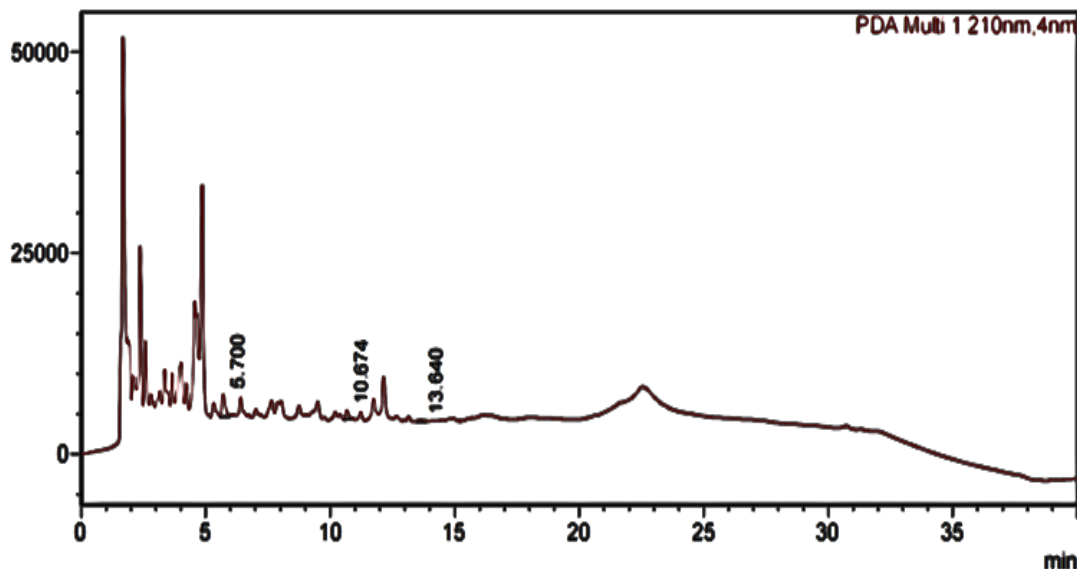
[Graph 3], known for its heavy rainfall, also recorded comparatively low levels of Deacyl gymnemic acid (0.80%) and Gymnemagenin (0.16%). Similarly, Kulur (Chikkamagaluru) [Graph 6] showed moderate accumulation of Deacyl gymnemic acid (0.18%) but relatively higher Gymnemic acid (0.40%), suggesting differential conversion of intermediate compounds under specific microclimatic conditions. Intermediate levels of all three compounds were observed in Davanagere (Tholahunase) [Graph 1], Mysuru (Saraswathipuram) [Graph 4], Belagavi (Khanapur) [Graph 7], Ballari (Vinayaknagar) [Graph 8], and Bagalkote (Biligi) [Graph 3] ecotypes. Notably, Biligi (Bagalkote) exhibited moderately high Deacyl gymnemic acid (1.86%) and Gymnemagenin (0.26%), possibly due to semi-arid conditions favoring metabolite concentration through water stress-induced secondary metabolism. Overall, the data suggest that phytochemical diversity in *Gymnema sylvestre* is strongly geographically influenced. The differences might arise from variations in temperature, rainfall, soil nutrients, and altitude, all of which affect enzymatic activities and metabolic pathways leading to saponin biosynthesis. From a pharmacological and conservation point of view, the Jogimatti (Chitradurga) ecotype appears to be

chemically superior, and could be considered a potential source material for large-scale cultivation or standardization of

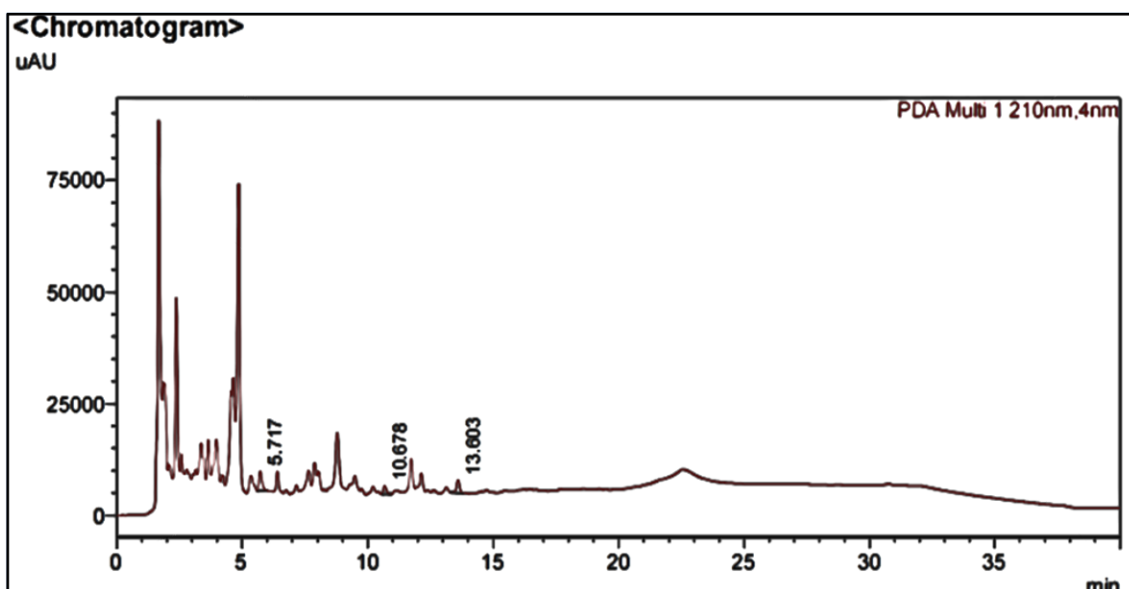
raw drugs containing gymnemic compounds.

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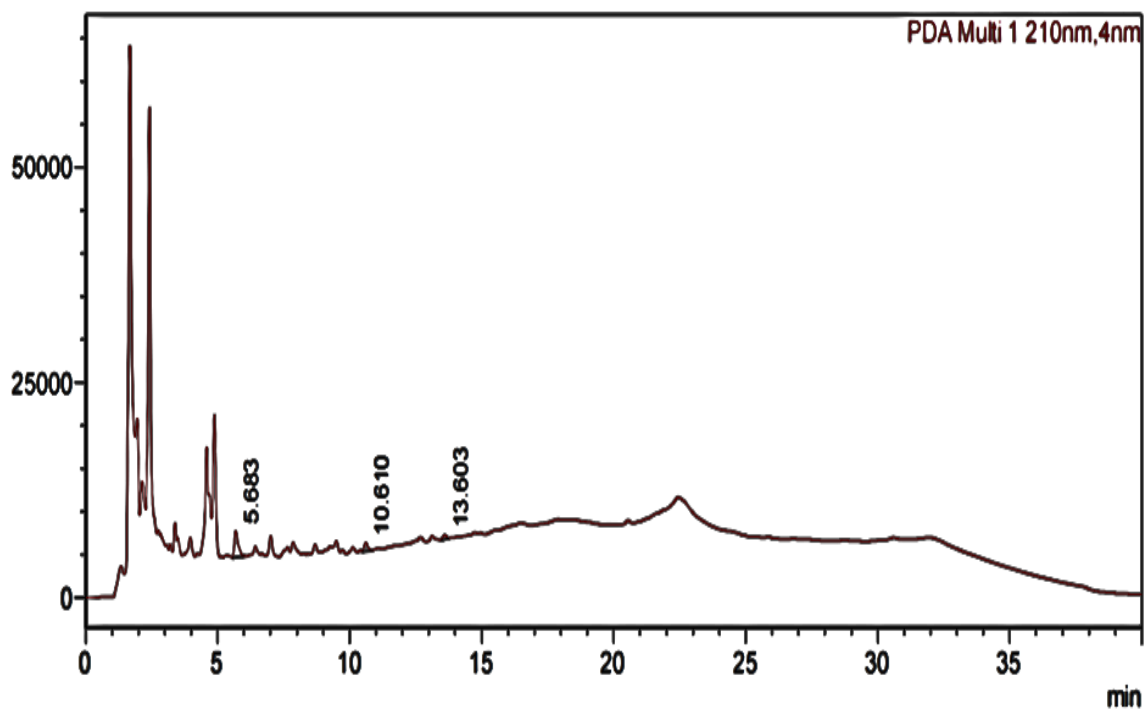
Graph 1: HPLC analysis of Davanagere



Graph 2: HPLC analysis of Chitradurga

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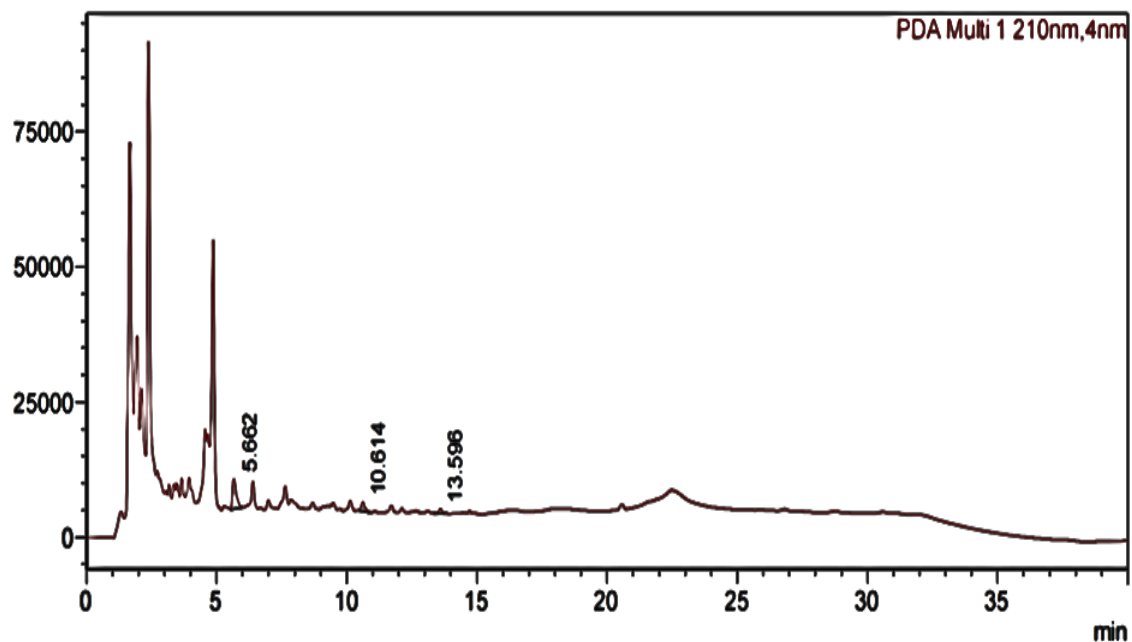
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Graph 3: HPLC analysis of Shivamogga

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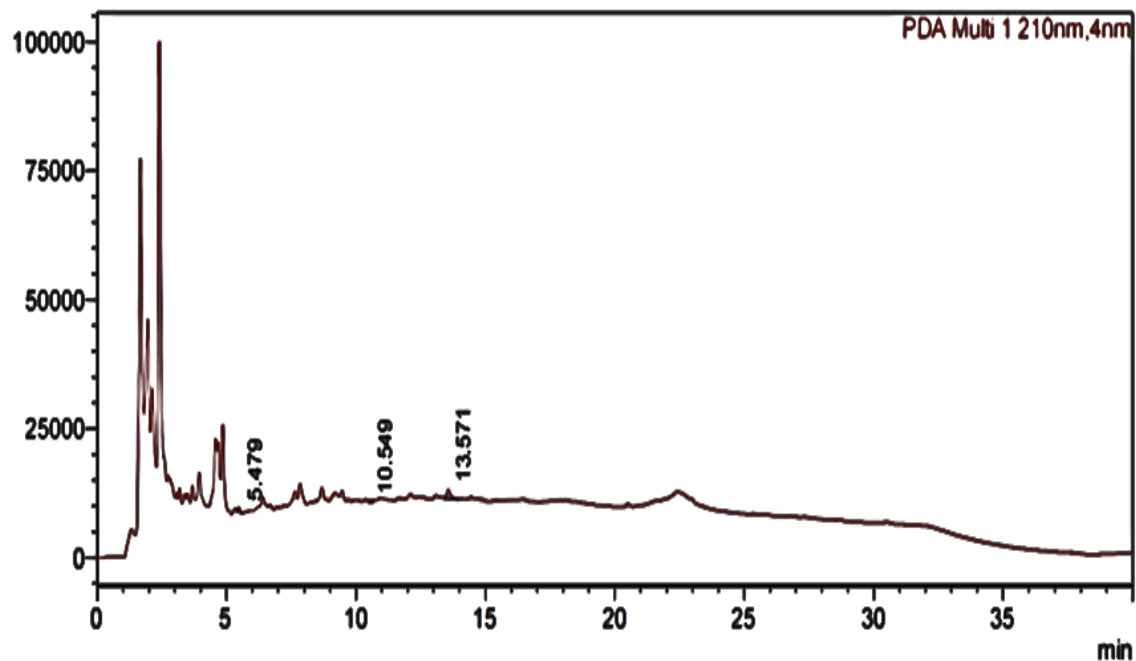
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Graph 4: HPLC analysis of Mysuru

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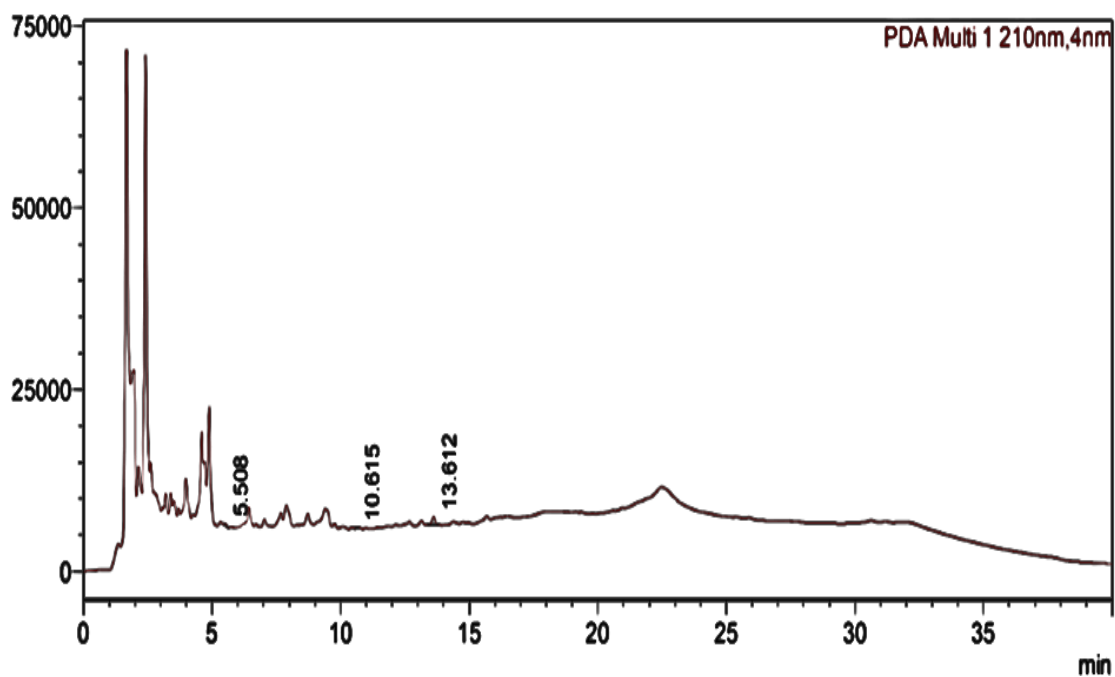
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Graph 5: HPLC analysis of Udupi

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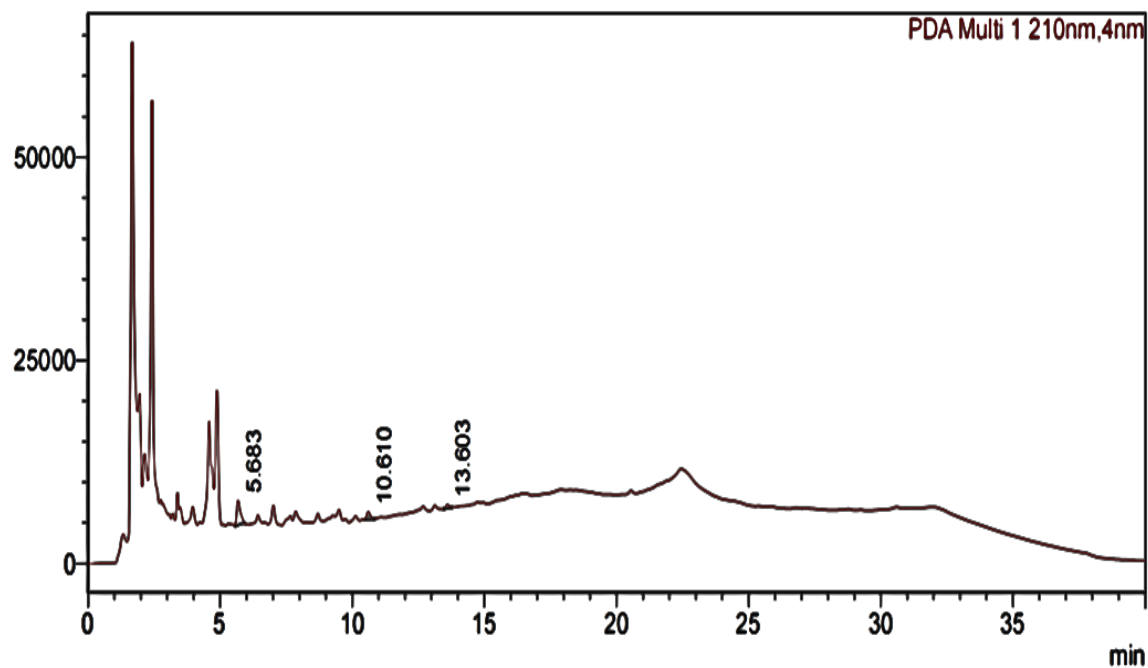
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Graph 6: HPLC analysis of Chikkamagaluru

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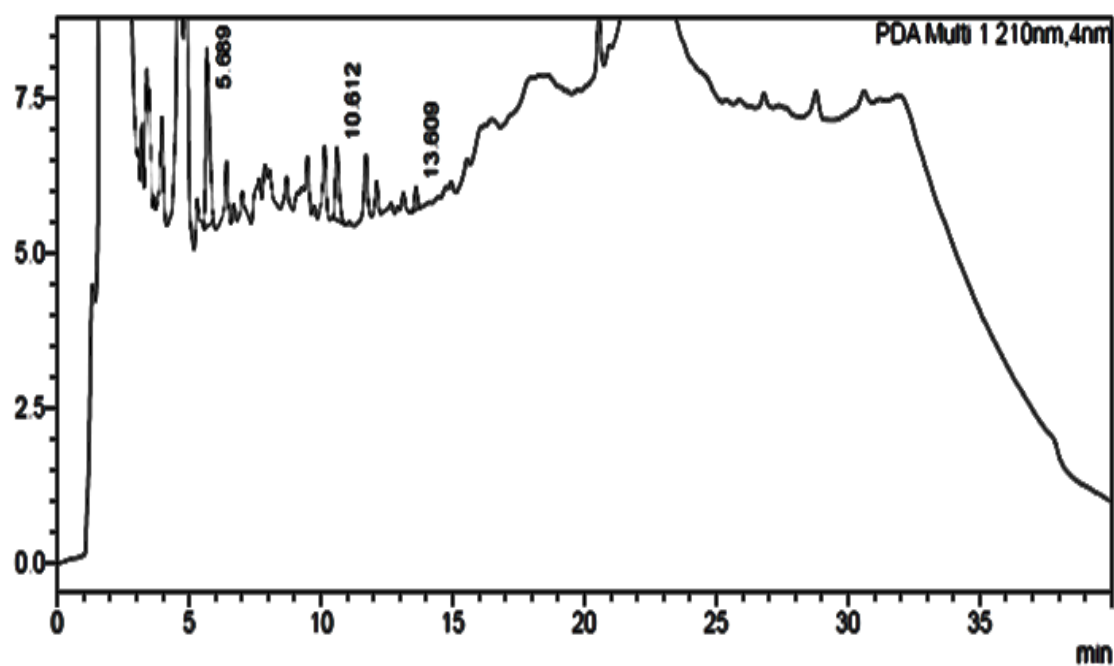
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Graph 7: HPLC analysis of Belagavi

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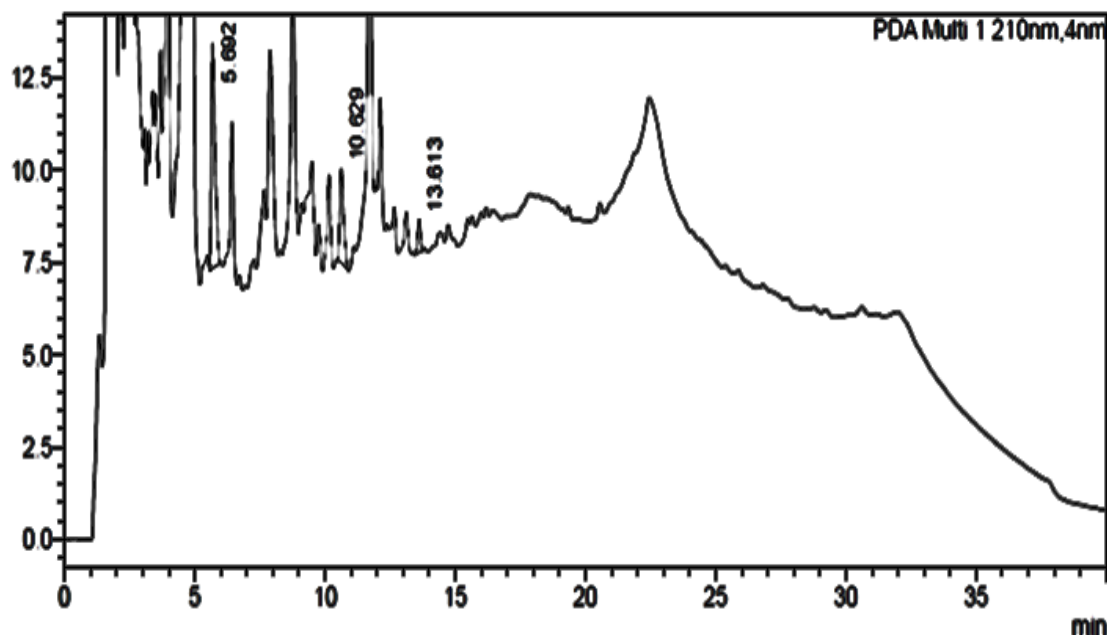
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Graph 8: HPLC analysis of Ballari

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**Graph 9: HPLC analysis of Bagalkote****Soil analysis:****Macronutrients Present in the different types of soil**

The amounts of three crucial nutrients nitrogen, phosphorus, and potassium (**Table 4 & Figure 3**) are clearly low and below the required ranges. As the major macronutrients, the availability of nitrogen, phosphorus, and potassium is crucial for sustaining healthy plant development. **Nitrogen** is vital for amino acid and protein synthesis, indirectly influencing enzyme activity in secondary metabolism. Higher N (e.g., Bengaluru) correlated with better metabolite accumulation. Nitrogen-deficient soils (Davangere) showed poor secondary metabolite yield. **Phosphorus** enhances root proliferation and energy metabolism (ATP, NADPH), which supports the shikimate and mevalonate pathways responsible for flavonoid and saponin biosynthesis. Regions with high P (Ballari, >115 kg/ha) showed relatively

better saponin content, whereas very low P (Davangere, ~5 kg/ha) corresponded with poor metabolite synthesis. **Potassium** regulates osmotic balance, enzyme activation, and secondary metabolite translocation. Soils rich in K (Gangur, 291 kg/ha) supported high gymnemic acid and flavonoid levels, while K-deficient soils (Chamarajanagar, ~49 kg/ha) had lowest yields. **Soil pH** Slightly acidic to neutral soils (pH 5.5–7.0): These conditions are ideal for micronutrient solubility (Fe, Zn, Mn), which act as cofactors in secondary metabolic enzymes. Regions with such soils consistently showed higher metabolite content. Alkaline soils (pH >7.5, e.g., Davangere, Kalaburagi): Reduced availability of Fe, Zn, Mn leads to enzyme inactivation, negatively affecting saponin and flavonoid biosynthesis. Strongly acidic soils (Kulur, pH 4.7) cause toxic Al and Mn accumulation, but interestingly, mild acid stress can stimulate phenolic production. Some accessions from acidic soils showed moderately high flavonoid levels.

Electrical Conductivity most soils were non-saline (<1 dS/m), which is optimal for *Gymnema sylvestre*. Slightly higher EC (Chitradurga) indicated mild osmotic stress, which may activate defense-related

pathways, leading to higher phenolics and flavonoids. This aligns with the well-known principle that secondary metabolites increase under abiotic stress.

Table 4: Analysis of the Soil Sample for Macronutrients (NPK), pH and EC

Soil sample no.	Place name	N (Kg/ha)	P (Kg/ha)	K (Kg/ha)	pH	EC (Ds/m)
1.	Davangere	203	19.11	96.74	6.78	0.24
2.	Chitradurga	224	23.07	111.53	6.8	0.57
3.	Shivamogga	189	39.46	106.99	4.85	0.15
4.	Mysuru	210	14.11	87.22	6.74	0.64
5.	Udupi	182	16.74	143.96	7.74	0.14
6.	Kulur	210	33.11	78.72	4.76	0.02
7.	Belagavi	238	28.36	115.28	6.51	0.43
8.	Ballari	238	9.11	40.03	8	0.18
9.	Bagalkote	231	30.88	96.2	5.4	0.21

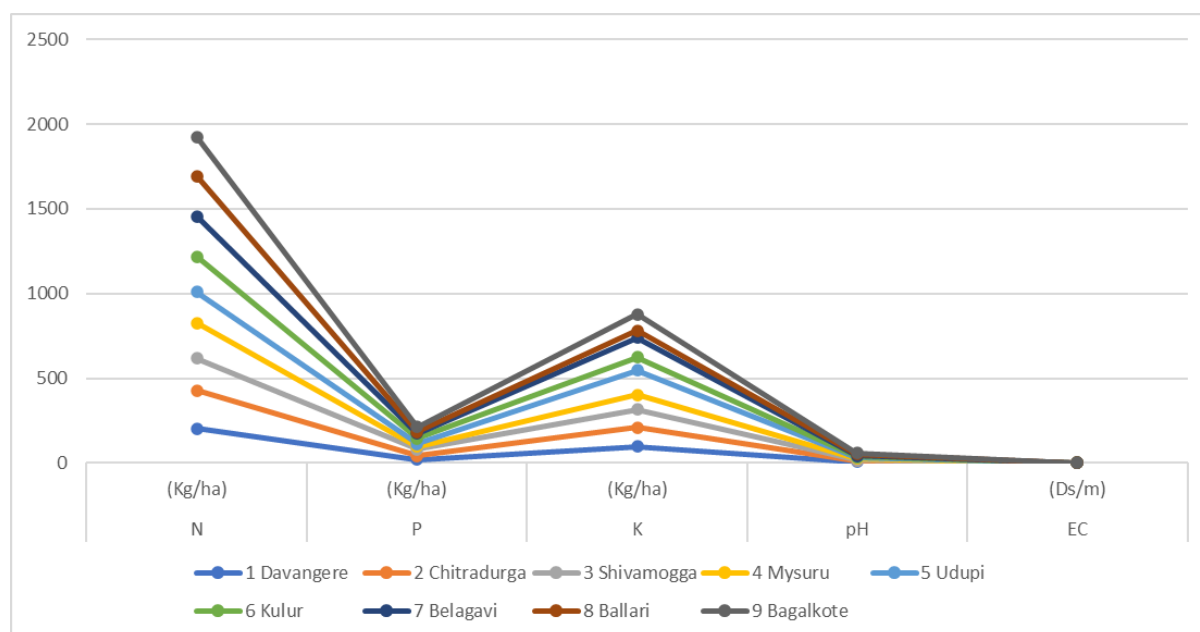


Figure: 3 Line Diagram of Soil Macronutrients (NPK), pH, EC

The **Nitrogen** (Kaleem et al 2012) content among the soils ranged from 182 to 238 kg/ha, indicating moderate fertility across

all study areas. The highest nitrogen concentration was observed in Belagavi (238 kg/ha) and Ballari (238 kg/ha), while

the lowest was recorded in Udupi (182 kg/ha). Nitrogen availability is crucial for vegetative growth and chlorophyll synthesis. However, excessive nitrogen can lead to luxuriant vegetative growth at the expense of secondary metabolite production. Regions such as Chitradurga (224 kg/ha) and Bagalkote (231 kg/ha), which have moderate nitrogen levels, correspondingly showed higher accumulation of Deacyl gymnemic acid and Gymnemagenin, suggesting that balanced nitrogen levels favor secondary metabolite biosynthesis in *Gymnema sylvestre*.

Phosphorus (Ali *et al.*, 2014) levels showed significant variation, from 9.11 kg/ha (Ballari) to 39.46 kg/ha (Shivamogga). Phosphorus plays a vital role in cellular energy metabolism and in the formation of secondary metabolites, particularly saponins and glycosides. In Shivamogga, the elevated P content is likely due to higher organic matter levels and mildly acidic conditions that promote the solubility and availability of phosphorus. However, the highest gymnemic acid compound yield was observed in Chitradurga, which had moderate P (23.07 kg/ha), indicating that extremely high or low phosphorus may not be ideal for optimum metabolite production. Low P availability in Ballari (9.11 kg/ha) may restrict energy-dependent biosynthetic reactions, resulting in lower gymnemic compound concentration.

Potassium (Borges *et al.*, 1997) content varied from 40.03 kg/ha (Ballari) to 143.96 kg/ha (Udupi). Potassium plays a crucial role in enzyme activation and osmotic regulation, both of which are essential for sustaining physiological functions and metabolite transport within plants. Interestingly, despite the highest K level in

Udupi, the gymnemic acid content was among the lowest, suggesting that very high potassium may favor primary metabolism and growth rather than secondary metabolite accumulation. Conversely, Chitradurga and Bagalkote, with moderate potassium (96–111 kg/ha), showed higher gymnemic compound concentrations, highlighting that balanced potassium availability supports secondary metabolism.

Soil pH (Tziachris *et al.*, 2020) ranged from acidic (4.76 in Kulur) to alkaline (8.00 in Ballari), reflecting the diverse edaphic conditions across Karnataka. The slightly acidic to neutral pH (6.5–6.8) observed in Chitradurga, Davangere, and Belagavi appears to be optimal for nutrient solubility and root nutrient uptake, correlating with higher gymnemic compound content in these regions. In contrast, strongly acidic soils (Shivamogga and Kulur) and highly alkaline soils (Ballari and Udupi) exhibited reduced metabolite accumulation, likely due to nutrient unavailability or stress conditions that affect enzyme activity related to saponin biosynthesis.

Soil EC (Golhar *et al.*, 2013) values ranged between 0.02 and 0.64 dS/m, indicating non-saline soils suitable for *Gymnema sylvestre* growth. The highest EC value in Mysuru (0.64 dS/m) suggests a relatively higher soluble salt content, which can enhance nutrient uptake efficiency and metabolic activity. However, Kulur, with the lowest EC (0.02 dS/m), may experience poor ion exchange and nutrient absorption, leading to reduced metabolite levels. An optimum EC range (0.2–0.5 dS/m) as found in Chitradurga, Belagavi, and Bagalkote seems to favor balanced ion concentration, correlating with the sites showing higher gymnemic acid compound accumulation.

Table 5: Quantity of Average Rainfall, Average Temperature

SL. NO.	Place name	Average Rainfall (mm) 2017-2024	Average Temperature (°C) (2017-2024)
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			Mean of max. temp.	Mean of mini. temp.
1	Davangere	265.18	31.18	18.66
2	Shivamogga	408.89	31.34	19.46
3	Chitradurga	272.83	31.03	19.56
4	Mysuru	422.34	31.14	19.39
5	Chikkamagaluru	589.30	27.37	17.12
6	Udupi	1186.86	31.43	24.93
7	Belagavi	549.29	30.32	18.98
8	Ballari	259.25	33.49	21.43
9	Bagalkote	270.28	32.37	20.86

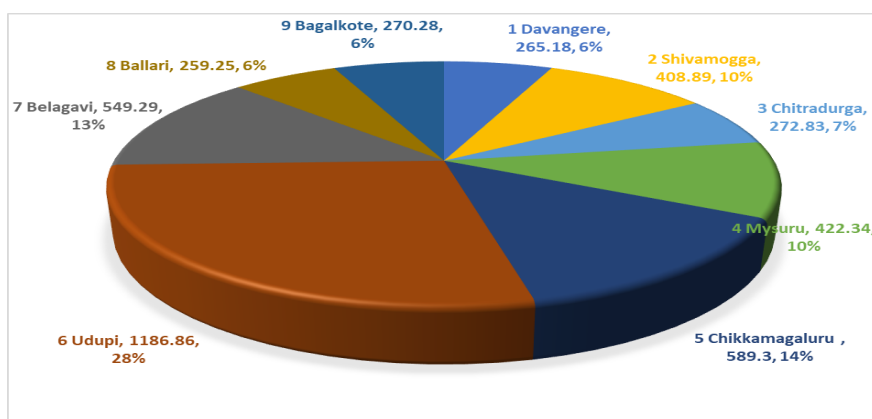
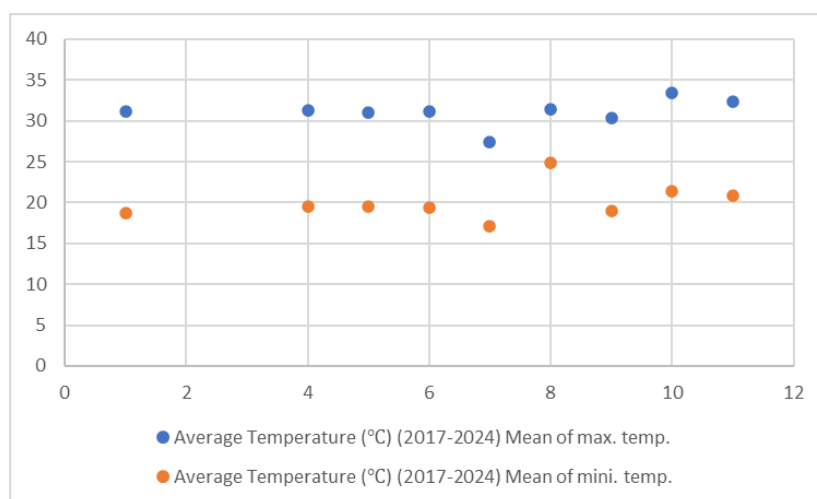
**Fig: 4 A : Average Rainfall (2017-2024)****Fig: 4 B : Average Temperature (°C) (2017-2024)**

Table 5 presents the average rainfall and temperature data for nine districts of Karnataka from 2017 to 2024. These climatic factors play a crucial role in

influencing the soil characteristics, plant growth, and phytochemical accumulation of *Gymnema sylvestre* and other medicinal plants. The data indicate considerable

spatial variability in rainfall (Rahman *et al.*, 2017) across the study regions: The highest average rainfall (**Figure 4A**) was recorded in Udupi (1186.86 mm) and Chikkamagaluru (589.30 mm), both located in the Malnad and coastal zones of Karnataka, which receive heavy southwest monsoon rains. Mysuru (422.34 mm) and Belagavi (549.29 mm) represent moderately high rainfall regions of the southern and northern transitional zones. Davanagere (265.18 mm), Chitradurga (272.83 mm), Ballari (259.25 mm), and Bagalkote (270.28 mm) experienced relatively low rainfall, indicating semi-arid conditions. Shivamogga (408.89 mm) lies between these extremes, reflecting a transitional agroclimatic condition. This variation in rainfall likely affects soil moisture availability, nutrient leaching, and plant metabolite concentration. Generally, plants growing under moderate moisture stress conditions (like in Davanagere and Chitradurga) may accumulate higher levels of secondary metabolites such as gymnemic acids due to physiological adaptation to stress. The mean maximum temperatures (Shigute *et al.*, 2023) ranged between 27.37°C (Chikkamagaluru) and 33.49°C (Ballari), while mean minimum temperatures (**Figure 4B**) ranged from 17.12°C (Chikkamagaluru) to 24.93°C (Udupi). Coastal and hilly areas (Udupi and Chikkamagaluru) maintained moderate temperatures with smaller diurnal variations due to maritime and altitude effects. Interior districts (Ballari, Bagalkote, Davanagere, and Chitradurga) showed higher maximum temperatures, typical of dry zones. Shivamogga and Mysuru exhibited intermediate temperature ranges, reflecting their transitional climatic nature. The temperature differences among regions directly influence plant metabolism, enzyme activity, and biosynthesis of active compounds. Warmer regions like Ballari and Bagalkote might enhance metabolic rates, but excessive heat could reduce moisture retention and yield. Conversely, cooler and humid conditions in

Chikkamagaluru may favor vegetative growth and leaf biomass accumulation but might dilute metabolite concentration.

Discussion

The phytochemical composition and biological potency of *Gymnema sylvestre* R.Br. are greatly influenced by the combined effects of environmental, edaphic, and climatic factors. The present study integrates data on rainfall, temperature, soil characteristics, and phytochemical content from nine districts of Karnataka to elucidate their interrelationship and influence on the biosynthesis of key bioactive compounds such as Deacyl gymnemic acid, Gymnemic acid-IV, and Gymnemenin. The climatic data recorded between 2017 and 2024 revealed pronounced spatial variability across the study regions. Average rainfall ranged from 259.25 mm in Ballari to 1186.86 mm in Udupi, while the mean maximum temperature varied from 27.37°C (Chikkamagaluru) to 33.49°C (Ballari). Districts such as Udupi and Chikkamagaluru, located in the coastal and Malnad regions, experience high rainfall and moderate temperatures, resulting in humid conditions favorable for luxuriant vegetative growth. However, such environments may lead to dilution of secondary metabolites due to higher water content and increased leaf biomass. In contrast, semi-arid regions like Davanagere, Ballari, Chitradurga, and Bagalkote exhibit low rainfall and elevated temperatures, conditions that induce mild water stress. Plants under such abiotic stress conditions often redirect metabolic energy toward secondary metabolite synthesis as part of adaptive defense mechanisms. Transitional regions such as Shivamogga, Mysuru, and Belagavi showed moderate climatic conditions, reflecting a balance between vegetative growth and metabolite accumulation. The soil analysis revealed notable variation in physicochemical properties among the study sites. Udupi and Chikkamagaluru soils were found to be

slightly acidic, rich in organic carbon, nitrogen, and phosphorus, typical of high rainfall zones. These conditions are favorable for vigorous plant growth and higher biomass yield. Ballari, Bagalkote, and Chitradurga exhibited neutral to alkaline soils with relatively low organic matter and limited nutrient availability, common in dry land regions. Shivamogga and Mysuru had moderately fertile loamy soils representing transition between arid and humid ecosystems. Soil fertility, pH, and nutrient status significantly influence nutrient absorption, photosynthetic efficiency, and secondary metabolism. Plants growing in marginal soils with limited nitrogen and moderate potassium often exhibit enhanced triterpenoid and saponin synthesis, as a physiological adaptation to nutrient stress. The quantitative estimation of phytochemicals indicated significant variation among the studied populations. Higher concentrations of Deacyl gymnemic acid and Gymnemic acid-IV were observed in semi-arid zones such as Davanagere, Chitradurga, and Ballari, where the combination of low rainfall, higher temperature, and lower soil fertility created favorable conditions for enhanced secondary metabolite biosynthesis. Udupi and Chikkamagaluru samples showed comparatively lower levels of these metabolites, possibly due to excess moisture and nutrient availability, which promote vegetative rather than defensive metabolic pathways. Mysuru and Shivamogga exhibited moderate phytochemical content, indicating a balanced influence of both climatic and soil factors. These results clearly establish that environmental stress conditions, particularly low moisture availability and moderate soil nutrient limitations, favor the accumulation of bioactive triterpenoid saponins in the leaves of *Gymnema sylvestre*. The correlation analysis between environmental factors such as rainfall, temperature, and soil fertility and the phytochemical concentrations indicated a negative association between rainfall and

metabolite content, while temperature showed a positive correlation with metabolite concentration. These findings indicate that moderate abiotic stress—marked by elevated temperature and limited water availability—stimulates the upregulation of secondary metabolite biosynthetic pathways in *Gymnema sylvestre*. Soil nutrients, especially nitrogen and potassium, also play a pivotal role in regulating triterpenoid biosynthesis, where mild nutrient limitation stimulates defensive metabolism over primary growth processes. Climatic parameters including rainfall and temperature showed significant spatial variation during the study period (2017–2024). Average annual rainfall ranged from 259.25 mm in Ballari to 612.65 mm in Mysuru, while mean maximum temperature varied between 26.71°C (Chikkamagaluru) and 33.49°C (Ballari). Regions such as Chitradurga (408.89 mm) and Chikkamagaluru (422.34 mm) with moderate rainfall and mild temperatures recorded comparatively higher concentrations of Deacyl gymnemic acid (3.29%) and Gymnemic acid (0.40%), respectively. These findings suggest that moderate rainfall coupled with optimal temperature provides favorable conditions for enzymatic activity and secondary metabolite biosynthesis. On the other hand, ecotypes from arid and high-temperature regions such as Ballari and Bagalkote exhibited lower gymnemic acid content, possibly due to drought-induced stress leading to reduced metabolic efficiency. Conversely, high rainfall and humidity (as observed in Udupi) may promote vegetative growth but result in lower metabolite concentration due to the dilution effect. Thus, a balanced combination of rainfall (300–450 mm) and temperature (30–31°C) appears most conducive for gymnemic compound accumulation. The soil analysis revealed considerable heterogeneity in macronutrient composition (NPK), pH, and electrical conductivity (EC) across the sampling sites. Nitrogen (N) content ranged from 182 to 238 kg/ha, Phosphorus (P)

from 9.11 to 39.46 kg/ha, Potassium (K) from 40.03 to 143.96 kg/ha, pH varied between 4.76 (Kulur) and 8.0 (Ballari), EC ranged from 0.02 to 0.64 Ds/m. Moderately fertile soils with balanced NPK levels, near-neutral pH (6.5–7.0), and low EC (<0.5 Ds/m), such as those in Chitradurga and Belagavi, were associated with higher gymnemic compound concentrations. In contrast, highly acidic (Kulur) or alkaline soils (Ballari) showed lower metabolite accumulation, likely due to reduced nutrient bioavailability and impaired root absorption. Among nutrients, phosphorus and potassium exhibited a positive correlation with Deacyl gymnemic acid and Gymnemagenin, indicating their crucial role in secondary metabolite biosynthesis pathways.

Correlation analysis between climatic and soil parameters demonstrated that moderate

rainfall, optimal soil fertility, and neutral pH collectively enhance gymnemic compound biosynthesis. Phosphorus and EC showed a positive correlation with gymnemic acid content, possibly due to their involvement in energy transfer and membrane activity. Extreme pH (either acidic or alkaline) and very high or low rainfall exhibited negative correlations, suggesting environmental stress conditions that suppress secondary metabolism. Overall, the interaction between climate, soil, and nutrient availability significantly shapes the phytochemical expression in *Gymnema sylvestre*. The correlation results confirm that soil fertility and reaction directly govern the phytochemical diversity in *Gymnema sylvestre*. Hence, for commercial cultivation and raw material standardization, ecotypes from moderately fertile, near-neutral soils (like Chitradurga and Bagalkote) should be prioritized for higher yield of gymnemic-type saponins.

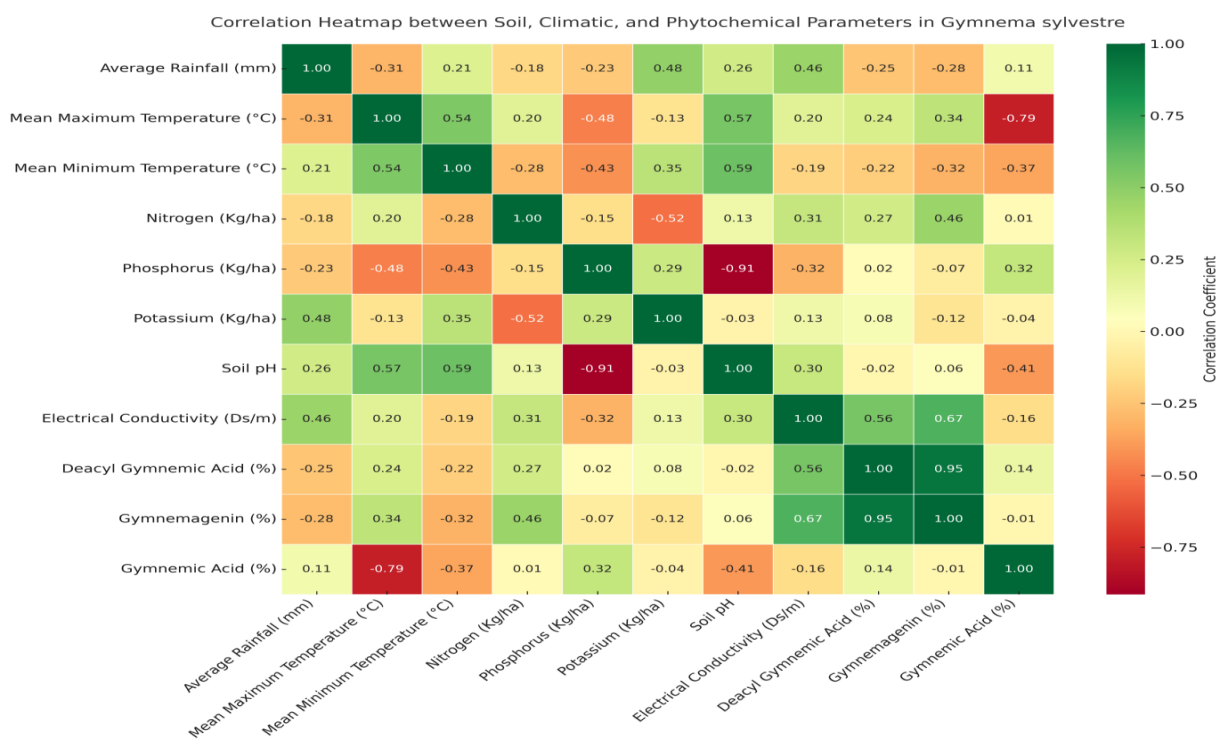


Figure 5: Correlation heatmap showing relationships between soil nutrients (NPK, pH, EC), climatic parameters (rainfall, temperature), and phytochemicals in *Gymnema sylvestre* across Karnataka ecotypes.

Correlation heatmap illustrating (**Figure 5**) the inter relationship between soil, climatic, and phytochemical parameters in *Gymnema sylvestre* collected from nine locations across Karnataka. The results show that phosphorus (P) and potassium (K) exhibit a positive correlation with Deacyl gymnemic acid and Gymnemagenin, indicating their role in promoting triterpenoid biosynthesis. Moderate rainfall and near-neutral soil pH also correlated positively with Gymnemic acid accumulation, suggesting that balanced moisture and soil conditions favor metabolite synthesis. In contrast, extreme temperatures (high maximum and minimum) and acidic/alkaline pH showed negative associations, implying stress-induced inhibition of secondary metabolism. Overall, the figure demonstrates that environmental and edaphic factors collectively influence phytochemical expression, with optimal conditions being moderate rainfall, balanced temperature, and nutrient-rich, near-neutral soils.

Conclusion

The study concludes that *Gymnema sylvestre* exhibits distinct phytochemical variability influenced by the combined effects of climatic and edaphic factors. Ecotypes from moderately moist regions with near-neutral pH and balanced nutrient levels are the most promising sources for high gymnemic acid yield. These findings highlight the importance of site-specific cultivation strategies and environmental optimization for maximizing the therapeutic potential and commercial value of *Gymnema sylvestre*. The integrated assessment of climatic, soil, and phytochemical parameters across Karnataka demonstrates that environmental heterogeneity significantly influences the chemical composition of *Gymnema sylvestre*. Regions characterized by low rainfall, higher temperature, and moderate soil fertility (e.g., Chitradurga, Davanagere, Bagalkote) exhibit enhanced deacyl

gymnemic acid content, whereas humid, nutrient-rich zones (e.g., Udupi, Chikkamagaluru) promote greater vegetative growth but lower metabolite accumulation. Hence, moderate abiotic stress acts as a favorable condition for secondary metabolite enhancement in *Gymnema sylvestre*. Based on the results of the present study, it can be concluded that extraction with 90% methanol using continuous hot extraction in a Soxhlet apparatus produced the highest yield of chemical constituents. The soil characters like acidic pH, high EC, high nitrogen and moderate potassium and Phosphorus contents, moderate rainfall are responsible for the synthesis of more chemical components in the *Gymnema sylvestre*. Climatic parameters significantly influence phytochemical variability. In *Gymnema sylvestre*, variations in deacyl gymnemic acid, gymnemic acid-iv, and Gymnemagenin content among samples from different districts could be attributed to these ecoclimatic differences. Moisture stress, temperature-induced enzyme modulation, and sunlight intensity are key drivers in secondary metabolite biosynthesis.

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