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Phytochemical Constituents of *Gymnema Sylvestre* R. Br. Influenced by Edhapic and Other Environment Conditions

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ABSTRACT
Gymnema sylvestre is a widespread medicinal plant that thrives in southern India tropical forests. There are different types of phytochemicals are found in Gymnema sylvestre. In general, most plants grow by absorbing nutrients from the soil. The makeup of a soil and its pH,EC, determine the extent to which nutrients are available to plants. Temperature plays a vital role in the overall growth and development of plants. Rainwater that falls to the ground usually filters slowly into the soil, where it will absorb ionized forms of essential plant nutrients. Gymnema sylvestre collected from different locations in Karnataka along with soil sample, rainfall, temperature and geographical data are 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 were obtained by continuously heating the dried leaf in a Soxhlet system with 90% methanol. In general, the majority of plants grow through taking nutrients from the soil. Plants take up these minerals with water that is pulled up through roots, and these essential nutrients are distributed throughout the stems and leaves of plants through vascular tissue. The highest yield of chemical components viz. Deacyl Gymnemic acid, Gymnemagenin, Gymnemic acid IV were obtained. Nitrogen, phosphorus, and potassium are examples of primary nutrients that plants utilize in significant amounts. Depending on the type of plant, temperature has a different impact on growth and development. There are minimum, maximum, and suitable temperatures required for each plant growth. All life depends on water, which is also necessary for the proper growth of plants. Concentrations of all soil nutrients and climate elements that are beneficial for the required level of plant growth and chemical components.
Keywords: *Gymnema sylvestre, Ecotypes, HPLC, DeacylGymnemic acid, Gymnemagenin, Gymnemic acid, Edaphic factors, Climatic factors.*

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INTRODUCTION

A species of medicinal plant (Apocynaceae), *G Sylvestre* R.Br. thrives wild in tropical forests in India, Australia and Africa [35]. Many different traditional and contemporary medical systems use a wide variety of plant species for therapeutic purposes (Paliwal et al.2009). One of the sectors with the quickest rates of growth for the manufacturing of herbal-based medications is the pharmaceutical and herbal drug businesses. Now a day, people are becoming more aware of the health risks connected to the careless use of contemporary medications. Found in the tropical and subtropical areas of the Indian subcontinent, *Gymnema sylvestre* R.Br. is a woody shrub with significant therapeutic potential [28]. *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 [29]. As such, it is one of the chemical diversity studies used to analyze the levels of gymnemic acid in the various Gymnema accessions that thrive under varied agroclimatic conditions. The selection of the high gymnemic acid requires a thorough investigation of chemical diversity [32]. Plant growth and development are greatly influenced by nature. The number 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. One of the most economical methods for managing nutrients is soil testing [16]. 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 [25]. Having the right amount of nutrients is essential for normal plant development and reproduction. Through plant absorption, nutrient ions are recovered from a range of sources as they are drawn out of the soil solution. The pH of the soil was also most important factors affecting the availability of nutrients [30]. In general, macronutrient availability is often lower in low pH soils where as micronutrient availability is generally lower in high pH soils. 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 is necessary for the processes involved in photosynthesis [4]. It is important because plants utilize nitrogen, which is the inorganic form of (NO^{3-}). Nitrogen is found in all living cells and is necessary for all proteins, enzymes, and metabolic processes that generate and transfer energy. Nitrogen is found in chlorophyll, the green pigment of plants that controls photosynthesis [5].

MATERIAL AND METHODS

Collection of plant material and identification

The plant samples of *Gymnema sylvestre* were collected from various parts of the Karnataka based on agroclimatic zones viz. Chikkamagaluru, Gulbarga and Chamarajnagara [Figure 1: 1a,1b,1c]. The plant material was properly identified and confirmed with help of various regional floras [14, 20].

Figure 1: *Gymnema sylvestre* plant collect from different District in the Karnataka

Processing of plant Material

About 25 gm cleaned leaves from each ecotype dried under shade, powdered and stored in closed vessel for further uses. The dried powder material was subjected to Soxhelt extraction with methanol for continuous hot extraction [11].

Extraction with 90% methanol

The plant dry powder material was then extracted with 90% methanol. 90% methanol was added and the extraction was carried out for 8 hours till the total methanol soluble extract was obtained [1, 33].

HPLC Analysis

A 0.45µm syringe membrane filter was used to filter all of the samples ready for HPLC analysis, and separation was done at 25°C. After filtering the solvents using a 0.45µm membrane filter with a 50 mm diameter, they were sonicated for 15 minut in a microclean 109 bath [36]. Throughout the whole analysis, chromatography was performed using a mobile phase consisting of phosphoric acid and water, flowing at a rate of 1 ml per minute [37]. Acetonitrile was utilized as part of a binary mobile phase made up of solvents. For a gradient elution with increasing polarity, the acetonitrile was 5–15 v/v at 0–10 min, 75 v/v at 35 min, 85 v/v at 50 min, and 95 v/v at 55 min for the best separation [39].

Soil Sampling

The soil samples were collected, where plant samples were collected. The soil samples collection following the standard methods [21].

Sampling procedure

Dead furrows, old manure, and places beneath or close to trees were avoided when collecting soil sample, a V-shaped trench that was 20–25 cm deep dug, from top to bottom. Three soil samples were collected near by the plant sample are collection [6].

Reduction of sample bulk

This was accomplished by dividing the 500 gm soil sample in half. The combined soil was quartered by splitting it into four equal portions and then throwing away two of them. The process was repeated until the necessary little amount was obtained after the two quarters were mingled and once more separated into four parts, discarding two. The dry material was run through a 2 mm filter to remove stones, big roots, and other coarse particles. The earth was gently crushed between polythene sheets [40].

Drying

Drying was done under moderate temperatures under shade where air circulation was possible [7].

Sample Storage and Transportation

The soil samples were put in paper bags with the labels. A slip-out with the following information was attached: the name of the place where the sample was taken; the depth of sampling; the number of samples mixed to create a representative sample; the type of soil (coarse or heavy); and plant growth (good, poor, or average) [17].

Determination of Nitrates

Reagents:

To determine the nitrates used some reagents viz. 0.32% $KMnO_4$ solution, 2.5% NaOH, 0.05 (N) H_2SO_4, 0.07 gm of methyl red, 0.1 g of Bromocresol green, 95% ethanol, Boric acid solution.

Method

Fill a dry Kjeldahl flask with 800 ml of soil (20 gm). Swirl the soil with 20 ml of distilled water. Add a few glass beads and 1 ml of liquid paraffin to stop the frothing and bumping, respectively. Fill a Kjeldahl flask with 100 ml of each of the 0.32% $KMnO_4$ and 2.5% NaOH solutions, then quickly attach it to a distillation apparatus. Fill a 250 ml conical flask with 20 ml of boric acid mixed indicator solution, then pipette the end of the delivery tube into the flask. The contents should be steadily distilled, and the released NH_3 should be collected in a conical flask with mixed indicator and H_3BO_3. When NH_3 is absorbed, the pinkish color changes to bluish green. Distill until approximately 100 ml of distillate are obtained. To restore the original pink color, titrate the distillate against a standard acid (0.05 N H_2SO_4) [22].

Determination of available potassium

Reagents:

Neutral Ammonium acetate solution, K-standard were used to analyse the Potassium.

Procedure:

To a 250 ml conical flask, add 10 gm of soil 50 cc of neutral normal acetate should be added. Use an electric shaker to shake the contents for 5 minute, then strain through a Whatman filter paper leach the soil with an extra 50 ml of ammonium acetate, then use NH_4OAC to get the amount up to 100 ml. Set the flame photometer to 100 readings and blank [NH_4OAC] or adjust it to 10 ppm K. Then, insert the K-filter and feed the flame photometer with K standards. Solution set to zero Plot the flame photometer to create the calibration curve. Using NH_4OAC solution, feed NH_4OAC soil extracts at the proper dilution to the flame photometer [2].

Determination of available Phosphorus

Reagents:

The following reagents were used to analyse viz. 0.5 M $NaHCO_3$, Darko-G-60,Chloromolybdic acid, stannous chloride, standard P solution.

Procedure:

Extraction**:** A 250 ml clean conical flask should contain 2.5 gm of soil. A pinch of P-free Darko-G-60 activated charcoal should be added. Pour 50 ml of 0.5M $NaHCO_3$ solution, for 30 minute, shake on a mechanical shaker. After passing the filter through Whatman No. 1 filter paper, gather the filtrate [8].

Colour development: 5 ml of the soil extract and 5 ml of ammonium molybdate should be pipetted into a 50 ml volumetric flask. After gently shaking the flasks and letting them stand for 5 min, dilute to 40 ml. Add 0.5 or 1 ml of the $SnCl_2$ working solution, then use distilled water to fill the remaining space (Sun et al 2019).

Determination of pH:

Reagent:

Distilled water, $CaCl_2$. $2H_2O$, KCl (1.0 M)

Procedure:

Fill a 100 ml beaker with 20 gm of soil. Pour in 40 ml of purified water. For 30 minute, stir the suspension sporadically [11, 12].

Determination of EC:

Procedure*:* Fill a 100 ml beaker with 20 gm of soil. Pour in 50 ml of distilled water and stir periodically for 30 minut. Give it an hour to resolve the suspension. Employing an EC bridge, determine the EC in the supernatant solution [13, 14].

Rainfall:

Rainfall is one of the main elements influencing plant growth and the accumulation of bioactive constituents. Among the many distinct climatic factors that affect the growth traits of plants is rainfall. Water is necessary to sustain the plant's chemical and physiological functions [23, 21]. The selected areas rainfall data was collected from Meteorological department, UAS, Bangalore for comparative studies.

Temperature:

Throughout their lives, plants are subjected to a broad range of temperatures, thus they must constantly adjust. 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 [40].

RESULTS AND DISCUSSION

Other solvent systems, like methanol, were utilized for the Soxhlet extractions, the extraction with 90% methanol yielded the largest amounts of gymnemic acid, deacyl gymnemic acid, and gymnemagenin. (Table 1 & Figure 2A,2B,2C,3). The calculated chemical components yield from *Gymnema sylvestre* at three ecotypes of Karnataka [27].

Table 1. The percentage of gymnemic acid in 3 ecotypes of *Gymnema sylvestre* collected from various regions of South India.

Sl.no	Name of the ecotype	Place of collection	% of Deacyl Gymnemic acid (w/w)	% of Gymnemagenin (w/w)	% of Gymnemic acid (w/w)
1.	GANGUR	CHIKKAMAGALURU	2.21	0.49	0.06
2.	KALABURAGI	GULBARGA	2.19	0.227	0.02
3.	GALIPUR	CHAMARAJANAGAR	0.55	0.18	0.02

The HPLC profile of methanolic extract showed that all the accession possesses the secondary metabolites of mid-polarity with considerable variability. The content of gymnemic acid varied from 0.02 to 0.06% as observed for accession 1,2 and 3 respectively (Table 1). The chemical content of gymnemagenin varied from place to place 0.49,0.227,0.18.**%** and Deacyl Gymnemic acid 2.21,2.19,0.55.

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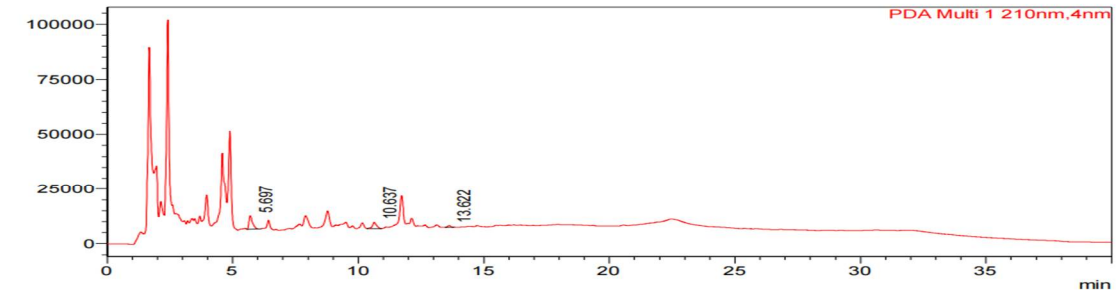

<Peak Table>

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	5.697	60665	6173	0.000			
2	10.637	31724	2797	0.000			
3	13.622	6780	851	0.000			
Total		99169	9822				

Figure 2:A- HPLC Chromatogram of *Gymnema sylvestre* leaf extracts (Chikkamagaluru)

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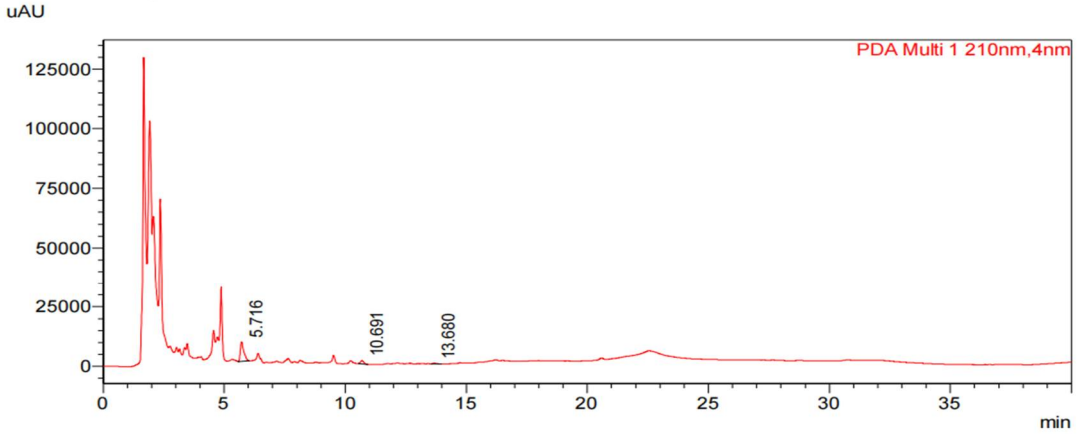

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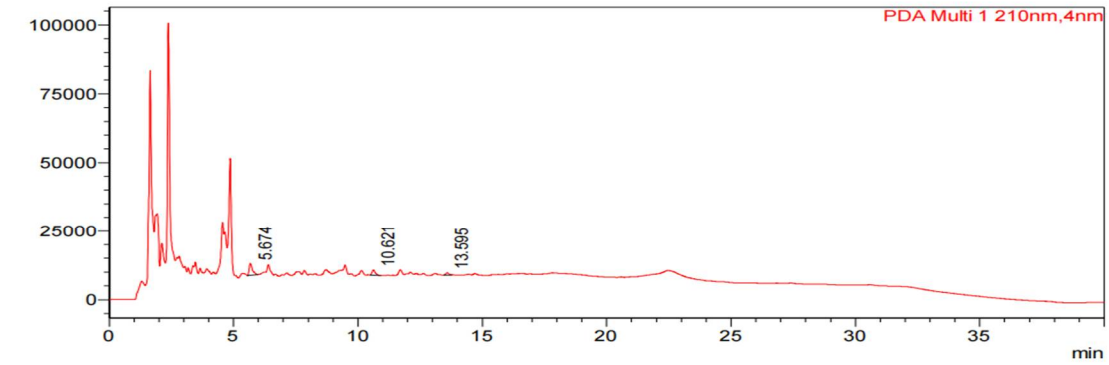
PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	5.716	82542	8265	0.000			
2	10.691	12140	1414	0.000			
3	13.680	2983	328	0.000		V	
Total		97665	10007				

Figure 2:B- HPLC Chromatogram of *Gymnema sylvestre* leaf extracts (Gulbarga)

<Chromatogram>



<Peak Table>

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	5.674	39368	4302	0.000			
2	10.621	18221	1943	0.000			
3	13.595	6577	835	0.000			
Total		64166	7079				

Figure 2:C- HPLC Chromatogram of *Gymnema sylvestre* leaf extracts (Chamrajnagar)

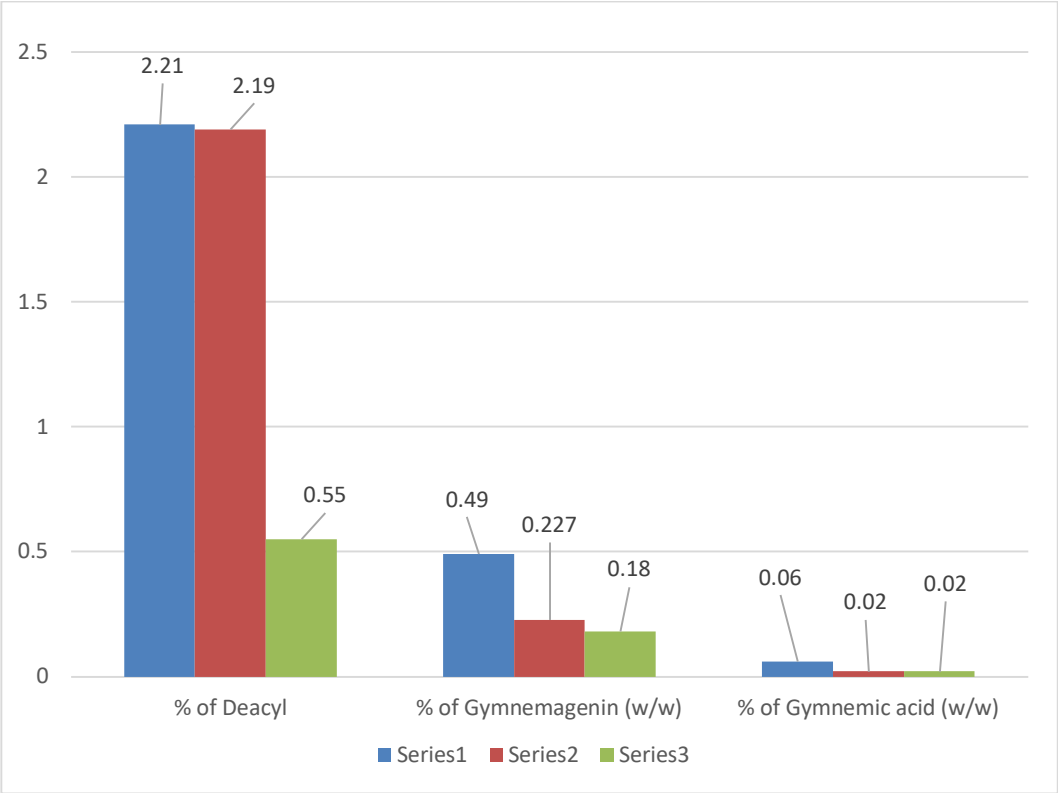


Figure 3: Graph of Chemical constituents of *Gymnema sylvestre*

HPLC Analysis

In this study, the active principal samples extracted from dried leaves were subjected to HPLC analysis [32]. HPLC chromatograms of *G. sylvestre* extract samples are displayed. The primary three chemical components of the ecotypes of *Gymnema sylvestre* found in the state of Karnataka make it abundantly evident that the climatic and edaphic conditions have a significant impact on these components. HPLC was used in this work to elute the total active ingredients from the *G. sylvestre* leaf sample. It was discovered that the leaf extract sample had more methanol-soluble chemicals than the other samples [24, 35].

Macronutrients Present in the different types of soil

The amounts of three crucial nutrients nitrogen, phosphorus, and potassium (Table 2, Figure 4) are clearly low and below the required ranges. The availability of these three nutrients is critical for healthy plant development because they are the main macronutrients [16].

Determine the pH and EC present in the different type of soil

The pH range of 5.68 to 8 (Table 2) is ideal for healthy plant growth. Every soil sample fell inside the permissible range and was acidic. Sample No. 3 had the highest pH of 8, while Sample No. 1 had the lowest pH of 5.68. There are more phytochemicals in acidic soil. An excessive amount of EC in the soil solution causes plasmolysis and exosmosis, which prevents plant roots from absorbing water and nutrients. When present in the right concentration, these soluble salts help plants grow, but when present in excess, they hinder plant growth [3].

Table 2: Analysis of the Soil Sample for Macronutrients (NPK) and pH and EC

Sl. No.	Place Name	Nitrogen (kg/ht)	Phosphorus (Kg/ht)	Potassium (Kg/ht)	pH	EC
1.	CHIKKAMAGALURU	196	27.5	291.16	5.68	0.08
2.	GULBARGA	210	15.32	138.1	7.52	0.17
3.	CHAMARAJANAGAR	210	29.15	49.06	8	0.17

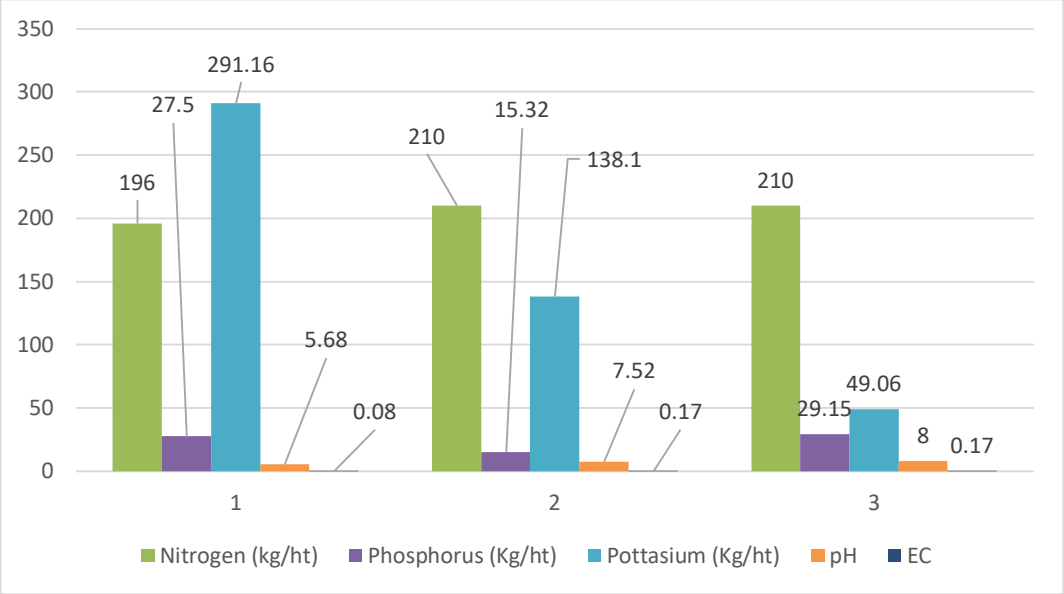


Figure 4: Graph of Analysis of the Soil Sample for Macronutrients (NPK) and pH and EC

Rainfall

The state receives 1270 mm of rainfall annually, of which roughly 10% comes from the pre-monsoon season, 74% from the south-west monsoon season, and 16% from the north-east monsoon season. In terms of time and geography, rainfall varies significantly from west to east across the state (Table 3), while the state north Karnataka, Gulbarga District receives 893.5 mm of rainfall, and Chamarajanagar receives 358.15 mm of rainfall, Chikkamagaluru receives moderate rainfall 612.65 mm (Figure 6) [15].

Temperature

The majority of plant functions, such as photosynthesis, transpiration, respiration, germination, and blooming, are influenced by temperature. Transpiration, respiration, and photosynthesis all rise with temperature (up to a certain point) (Figure 5). Temperature influences the transition from vegetative (leafy) to reproductive (flowering) growth in conjunction with day length. Temperature has the potential to accelerate or slow down this transformation, depending on the circumstances and the particular plant [9].

Table 3: Determination of Latitude & Longitude, Altitude(mts), Average Rainfall, Average Temperature, in the different District in Karnataka

SL. NO.	Place name	Latitude & Longitude	Altitude (mts)	Average Rainfall (mm) 2017-2024	Average Temperature (℃) (2017-2024)	
					Mean of max. temp.	Mean of mini. temp.
1	CHIKKAMAGALURU	13º54'75"N 75º82'41"E	680	612.65	27.37	17.12
2	GULBARGA	17º31'32" N 76º87'49"E	508	893.5	34.50	22.40
3	CHAMARAJANAGAR	11º92'65" N 76º59'15"E	910	358.15	31.6	19.88

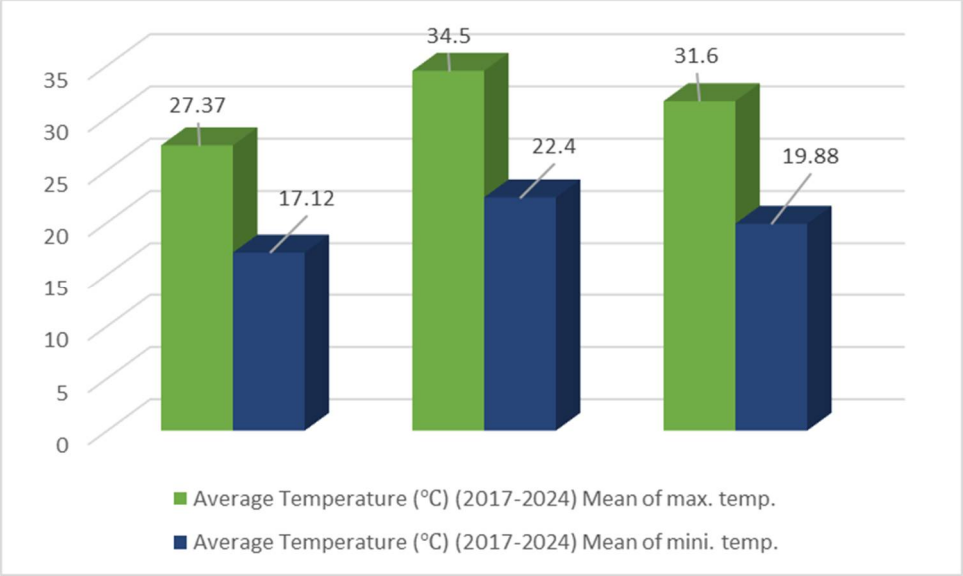


Figure 5: Graph of Average Temperature in the different District of Karnataka (2017-2024)

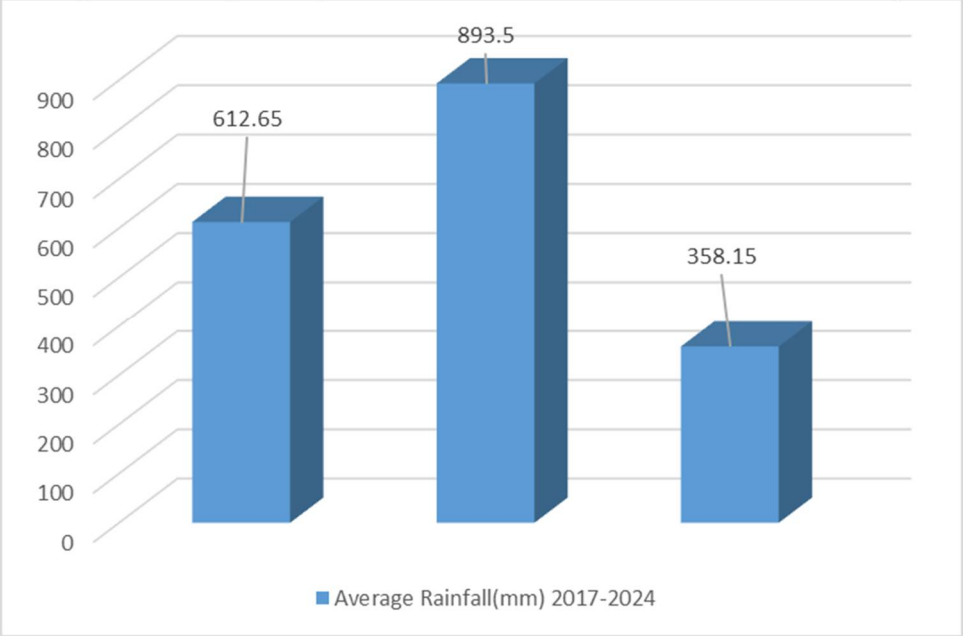


Figure 6: Graph of Average Rainfall in the different District of Karnataka (2017-2024)

Chikkamagaluru (Gangur) Sample showing the concentrations of Gymnemic acids IV: 0.06% , Gymnemagenin 0.49% Deacyl Gymnemic acid is 2.21% (Figure 2A). The Gangur area receiving moderate rainfall (612.65 mm), low temperature, pH is acidic (5.68) and nitrogen is low, phosphorus is moderate and potassium is high, might be the potassium content is responsible for the accumulation of Deacyl Gymnemic acid.

Gulbarga (Kalaburagi) Sample showing the concentrations of Gymnemic acid IV, 0.02% of Gymnemagenin, 0.227% Deacyl Gymnemic acid is 2.19% (Figure 2B). Kalaburagi area receiving high rainfall (893.5 mm), high temperature, nitrogen is high and phosphorus, potassium moderate then the other two district, might be the moderate climate indicates the good percentage of these active constituents especially Deacyl Gymnemic acid (2.19%).

Chamarajanagar (Galipur) Sample showing the concentrations of Gymnemic acids IV 0.02%, Gymnemagenin 0.18% Deacyl Gymnemic acid is 0.55% (Figure 2C). Galipur district receiving low rainfall (358.15 mm), moderate temperature, pH is slightly alkaline (8), High nitrogen (210), low phosphorus (29.15) and potassium (49.06) concentrations enhance the percentage of deacyl gymnemic acid (0.55%) among the samples.

The life cycle, distribution, and phytochemical makeup of the world flora, including fragrant and medicinal plants, are all being significantly impacted by climate change. Plant architecture, flowering, fruiting, phytochemical composition, and in situ competition with other species are all being impacted by

precipitation, which is being influenced by the shifting temperatures and wind patterns linked to climate change. The yearly monsoon significantly regulates India climate, which seems to be seeing more intense and unpredictable precipitation. In order to grow them in such climates and as certain how variations in temperature, moisture, and edaphic factors might impact the plants phenology, nutrient, and secondary metabolite levels, it is necessary to comprehend the effects of higher temperatures, different precipitation levels, and different soil moisture and fertility. Commercially, phytochemical analysis of plants is significant since pharmaceutical companies are very interested in creating novel medications to treat a number of disorders. Our research demonstrated that a number of medicinally significant phytoconstituents from the various *G. sylvestre* accessions were present in sufficient amounts, which supports the use of this plant species as a herbal remedy. India is known for its extremes in temperature and precipitation, as well as other seasonal variations in the environment and climate. Plant phytochemical composition is significantly impacted by various agroclimatic situations. According to earlier research, environmental temperature has a major impact on the assessment of phytochemicals, and this effect is particularly observed in colder regions. Thus, from January to June of 2024, we collected the plant material over the winter. We also discovered in this study that the highest concentration of phytochemical ingredients is found in the extracts from highland and semi-arid zones. In all tests, tropical zone accessions exhibited the lowest levels of phytochemical activity. Although *Gymnema sylvestre* can thrive in nearly any kind of environment, a number of variables can impact the amount and quality of a certain component. According to earlier research, a number of environmental factors, such as location, climate, soil type, sun exposure, grazing stress, seasonal variations, etc., affect the phytochemical composition of plants. Plants that are under stress create more phytochemicals to help them survive in a stressful situation. Plants during stress generated more flavonoids, anthocyanins, and mucilaginous compounds, according to studies. Phenolics are produced at higher temperatures and vice versa. From a nutritional and dietary perspective, plants from hilly regions are considered to be more nutritious. The amount of net photosynthesis per unit leaf area is reduced when there is water stress. The depth of water received during a shower (measured in millimeters) is known as the rainfall intensity. Large drops that fall harder on the soil surface are typically seen in high intensity rains. The raindrops are finer when it's not as intense. This portion of the total amount of rainfall that is required to meet plant growth needs is referred to as effective rainfall. Since *G. sylvestre* grows best in hot, humid climates with lots of rainfall, semi-arid climates with little rainfall are also unsuitable for the plant. While the majority of plant crops often thrive in semi-arid regions due to their soil structure and climate. Accessions from colder climates had strong antioxidant activity in the current investigation, suggesting the concept that plants under stress create more phytochemicals. Consequently, contracting environmental conditions can affect the phytochemical composition. The current study suggests that the phytoconstituents of *G. sylvestre* plants are significantly impacted by temperature, rainfall, and agroclimatic sites [12].

CONCLUSION

The results of the current investigation show that utilizing 90% methanol in a continuous hot extraction process in a Soxhlet apparatus produced the best output of gymnemic acid.

The resultant gymnemic acid can be further identified, purified, and characterized using HPLC techniques. Gymnemic acid analysis in traditional laboratory settings and the standardization of herbal remedies can both benefit from the accuracy, precision, and time-efficiency of HPLC procedures. Making natural medicines may benefit from these criteria.

In general, most plants grow by absorbing nutrients from the soil. Their ability to accomplish this is determined by the properties of the soil.The pH and content of the soil determine how much nutrition plants can absorb. Fertility and stability are two aspects of soil that are critical to plant growth. While soil stability is primarily influenced by the texture, structure, consistency, and hardness of layers, soil fertility is the quantity of plant nutrients that are available in the soil. Chikkamagaluru district exhibits the highest concentration of Deacyl Gymnemic acid (2.21) among the three samples. The synthesis of Deacyl Gymnemic acid in the plant is caused by soil characteristics such as acidic pH, high EC, moderate nitrogen and phosphorus, high potassium contents, and moderate rainfall. Of all the samples, the Chikkamagaluru sample is regarded as the best sample because the levels of both compounds, Deacyl Gymnemic acid and Gymnemic acids, are the highest. Variations in the concentration of active elements in the plant samples are reflected in variations in climatic and edaphic parameters.

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