Online at: https://researchtrendsjournal.com ISSN No: 2584-282X

INTERNATIONAL JOURNAL OF TRENDS IN EMERGING RESEARCH AND DEVELOPMENT

Volume 1; Issue 1; 2023; Page No. 175-180

Received: 16-10-2023
Accepted: 24-11-2023

Gather various plant components and measure stress-related physiological indicators such relative water content (RWC)

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DOI: https://doi.org/10.5281/zenodo.12663397

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Abstract

The physiochemical tests were conducted using *Vitex negundo* and *Eclipta prostrata*. To fully grasp the physiological state and therapeutic qualities of plants, seasonal fluctuation is the most crucial factor to consider. The various environmental factors have been observed to cause fluctuations in the concentration of inorganic elements, photosynthetic pigments (such as chlorophyll a, b, and carotenoids), photosynthetic enzymes (such as RuBPCase), oxidative enzymes (such as IAA oxidase), nitrogen metabolism enzymes (such as Nitrate reductase and Nitrite reductase), organic constituents (such as nitrogen, polyphenol, and proline), and inorganic elemental concentration in V. negundo and E. prostrata. Carbohydrates, proteins, glycosides, saponins, flavonoids, steroids, tannins, and phenolic substances were identified in the screening of early phytochemical studies and characterisation of several extracts of V. negundo and E. prostrata. In addition to lending credence to the traditional usage of E. prostrata, the results of the current study may aid in the fight against inflammation and allergies.

Keywords: Plant, components, stress, physiological, relative and water content (RWC)

Introduction

Humans have relied on medicinal plants for centuries for a variety of therapeutic purposes, including treatment of illness and prevention of disease, and the extraction of important bioactive substances. Traditional medicine and its associated products are said to be relied upon by around 80% of the global population, particularly in developing nations. Conventional and traditional medicine are often used together by ill individuals in underdeveloped nations. In rural areas of poor nations, people frequently turn to traditional medicines because they are both more economical and safer than contemporary alternatives. Rural dwellers tend to choose traditional medicines because they are more accessible to traditional healers, who are also more acquainted with the local environment, culture, and patients. There is a problem with people in rural parts of India being able to get healthcare in the West. In India, 7500 of the 17,000 species of higher plants are thought to have therapeutic uses. With the largest percentage of plants acknowledged for their therapeutic uses in India, this percentage of medicinal plants is the highest in the world for the present flora.

Several medicinal plants have been found in the region due to the increasing quantity of plant types. As a preventative measure, over 70% of Indians turn to homemade treatments, and over 90% of those individuals employ plant-based cures at least once. Traditional phytotherapy practises have been handed down through many generations of India's indigenous population. The lack of comprehensive documentation of medicinal plants and the inherent dangers of oral transmission mean that the traditional knowledge of many Indian ethnic groupings goes mostly unexplored.

Phytochemicals refer to the compounds that are naturally occurring in plants. The plant's main and secondary metabolism generates these. Insects, other plants and animals, and even microbes can't survive or even harm plants without these phytochemicals. Additionally, they aid plants and shield them from harm caused by pollution, ultraviolet light, stress, and drought. They have a long history of usage as both traditional medicine and poisons.

There is currently insufficient evidence to support the claim that phytochemicals provide any discernible health benefits for humans, so they should not be considered essential nutrients. It is well-established that they play a part in safeguarding human health. Phytochemicals, which number over four thousand, are categorised according to their chemical composition, physical properties, and protective roles.

Carotenoids and polyphenols, which include phenolic acids, stilbenes, and lignans, are the two main categories into which phytochemicals fall. For example, flavonoids are further subdivided into isoflavones, flavanols, anthocyanins, and flavones. Natural medicinal plants are a boon to human health because of all the ways they may improve our well-being. These medicinal herbs have a long history of usage in India, dating back hundreds of years or more. Traditional Indian medicine encompasses several different schools of thought, including Ayurveda, Siddha, Unani, and an extensive body of ethnomedicine. In the past, western culture had a significant impact on our medical knowledge, but now that their relevance is being recognised, along with the fact that these traditional healing practices have few side effects, they are making a comeback. Over eighty percent of people in poor nations rely on traditional medicines made from plants for their main health requirements, according to a World Health Organisation (WHO) research. "Phytomedicines" serve as a bridge between traditional and modern medicine because of the essential role that these traditional medicines play in the formulation of contemporary medicinal treatments.

Literature Review

Dixit, Himisha *et al*. (2019) [1]. Research has examined the antimicrobial properties of various plant extracts against various harmful bacteria, including Staphylococcus aureus, Escherichia coli, *Pseudomonas aureuginosa*, *Klebsiella pneumoiniae*, *Paenibacillus*, *Bacilus subtilis*, and *Datura metel*. The ethanol, methanol, and chloroform extracts showed a significant antibacterial action. Aside from P. aureuginosa, all of the bacteria examined had the highest levels of antibacterial activity against the ethanol extract of D. metel. No detectable antibacterial activity was seen in the aqueous extract. It was believed that inhibition was a phenomenon that depended on concentration. It was found to include alkaloids, flavonoids, glycosides, steroids, tannins, phenol, and saponin by phytochemical screening.

Saio, Valrielyn *et al*. (2015) [8]. Five medicinal plants from the northeastern area of India were analysed and compared for their phytochemical ingredient distribution. The main active principles that were evaluated were found in the following plants: Potentilla fulgens and Osbeckia chinensis roots; *Flemingia macrophylla* and *Hypochaeris radicata* leaves; *Solanum kurzii* fruits; and these plants' alkaloids, tannins, saponins, steroids, terpenoids, flavonoids, phlobatannin anthraquinones, and cardiac glycosides. Glycosides, alkaloids, tannins, steroids, terpenoids, and flavonoids were detected in every plant. In contrast to H. *radicata*, S. *kurzii* did not contain any tannins or saponins, and neither did H. terpenoids. Only P. fulgens was found to have plobatannins, but none of the plants tested had any anthraquinones. Additionally, we ran a quantitative study to determine the flavonoid content. Research like this is a step in the right direction towards our goal of fully understanding these plants and their bioactive constituents.

Bansal, Aanchal *et al*. (2021) [11]. As a defensive strategy against harmful organisms, plants create phytochemicals. As a component of traditional medicine, they are used to treat a range of metabolic, immunological, and neurological diseases in people throughout different regions of the globe. The commercial usage of medicinal plants derived from indigenous plants is on the rise in tandem with the global population. Demand rose as a result of plant extracts' antibacterial capabilities. However, plant tissue culture has shown to be a dependable substitute for extracting bioactive chemicals from plants. Medicinal plants' phytochemical output may be enhanced by artificial plant culture. The in-vitro synthesis of phytochemicals and their therapeutic qualities are the main topics of this review.

Valsan, Aswathy *et al*. (2022) [12]. Some research suggests that Ocimum species may have a variety of beneficial effects, including those against infertility, cancer, diabetes, fungal infections, microbes, the liver, the heart, nausea, vomiting, spasms, pain, and excessive sweating. Three species of the genus *Ocimum*-O. *tenuiflorum*, O. *basilicum*, and O. *gratissimum*-are the subject of this study's early phytochemical screening. Selected for the solvent extraction were six different substances: distilled water, petroleum ether, acetone, chloroform, ethanol, and methanol. A search for phytochemicals was conducted on the extracts. Bioactive chemicals including alkaloids, flavonoids, phenols, tannins, steroids, and terpenoids were detected in the examined samples' aqueous extracts. O. *tenuiflorum* had the highest concentration of phytochemicals among the samples that were analysed. Our research showed that several pharmacologically significant secondary metabolites are present in Ocimum species.

Devanesan, Arul Ananth. (2018) [13]. A perennial plant that bears fruits, *Pedalium murex* grows naturally in tropical Africa, Mexico, and southern India. The herb has anti-inflammatory properties and is used to treat a variety of illnesses, including stomach ulcers, asthma, heart conditions, and urinary tract infections in particular. Through the application of reasonable principles, traditional medicine has evolved into a competent method for treating a wide range of illnesses and creating a cheap phytotherapy. Rumour has it that P. *murex* is a pricey place to find rare bioactive chemicals that might be used to make natural remedies for a wide range of illnesses. Finally, this review delves into the traditional uses, phytochemical composition, biological activity, and ethno pharmacological significance of P. *murex*. Additionally, it offers a foundation for future research into topics like the plant extract's mode of action and the separation of its bioactive components.

Research Methodology

Medicinal plants were the subject of our University Grant Commission research. Here, fifteen medicinal herbs with antiallergenic properties were examined. Two antiallergenic medicinal herbs, *Vitex negundo* L. and *Eclipta prostrata* L., were chosen for the present investigation based on the findings made in that previous study. 1 gramme of freshly harvested plant leaf discs were collected. The discs of leaves were placed on petri dishes that contained water. Once the leaf discs had reached their maximum turgid level, which took about three hours, we weighed them and placed them in an oven set at 600C. After the leaf discs dried completely, their dry weight was recorded. The technique developed by Bates *et al*. (1973) [16] was used to estimate the proline content. The mixture was filtered through Whatman No. 1 filter paper after being homogenised in 10 ml of 3% aqueous sulfosalicylic acid with 0.5 g of fresh plant material. The reaction mixture of 2 millilitres of filtrate with 2 millilitres of acid ninhydrin and 2 millilitres of glacial acetic acid was incubated in a test tube for 1 hour at 100 ºC in a water bath. Subsequently, the tubes were promptly moved to an ice bath.

Data Analysis

Meteorological Data

The kinds and amounts of bioactive components found in medicinal plants may be affected by environmental conditions and the stress they cause. In the end, this impacts the medicinal plants' pharmacological qualities. Were we to examine the climatic changes over the last several years, we would see vast seasonal variations in every environmental variable (temperature, humidity, rainfall, etc.). Rain, heat, cold, and high humidity are constants throughout the year. Plants are severely impacted by these variables. Increased pollution, global warming, and other man-made factors may be to blame for the adverse circumstances. The present state of the environment is largely the result of human actions.

Table 1: Seasonal variation of maximum and minimum temperature in ºC.

Season	Max. Temp. ºC	Min. Temp. ºC	Average ºC
Monsoon 2015 -16	29.7	21.3	25.7
Winter 2015 – 16	31.5	15.5	23.3
Summer 2015 – 16	36.5	19.3	27.5
Monsoon 2016 – 17	29.3	22.3	27.5
Winter 2016 – 17	30	13.5	25.8
Summer 2016 – 17	35.7	16.3	26.3

The weather reports came from the India Meteorological Department in Mumbai, which is part of the Indian government's Ministry of Earth Sciences.

To analyse the impact of meteorological factors on the physiology and biochemistry of antiallergenic indigenous plants, a record of meteorological data is maintained continuously for about two years, spanning the Monsoon 2015–16, Winter 2015–16, Summer 2015–16, Monsoon 2016–17, Winter 2016–17, and Summer 2016–17. Temperatures ranged from quite high in the summer to very low in the winter, demonstrating seasonal fluctuation between the two years. On average, however, there was not much of a temperature difference. Tables 1 and 2 show that the 2016–17 monsoon had the most rainfall and relative humidity of any season.

Table 2: Seasonal variation of Rainfall (mm) and Relative humidity (%)

Season	Monsoon 2015 - 16	Winter 2015 - 16	Summer 2015 - 16	Monsoon 2016 - 17	Winter 2016 - 17	Summer 2016 - 17
Rainfall mm	82.7	34	5	123.3	29.7	5.08
Humidity %	80.3	48.8	39.3	82.75	50.5	42

Soil Parameters

Table 3: Seasonal variation of soil moisture (%)

Sr. No	Plant name	Soil Moisture (%)		
		Summer	Monsoon	Winter
1	*Vitex negundo* L.	4.236±2.3340	5.65 ±0.7610	5.503±0.6353
2	*Eclipta prostrata* L.	2.774±0.4170	7.07±0.9367	5.46±1.3976

Table 4: Seasonal variation of soil pH.

Sr. No	Plant name	Soil pH		
		Summer	Monsoon	Winter
1	*Vitex negundo* L.	8.76	8.99	8.58
2	*Eclipta prostrata* L.	8.63	8.90	8.91

From 2015 to 2017, the current research thoroughly examined environmental elements. The average temperature from 2015 to 2017 ranged from 23 to 28 degrees Celsius. However, there has been a notable disparity in the values obtained for relative humidity and rainfall. These variables cause stress on the plants because of how drastically different they are from one another.

As a result of the summer's increased temperatures and the monsoon and winter's consistent precipitation, soil moisture decreased significantly. Plants may only endure or thrive during dry spells if they are able to prevent soil moisture depletion caused by low precipitation. During the course of the research, the soil's pH was rather constant at about alkaline. Table No. 4 shows that in the alkaline soil pH range (pH 7), *Vitex negundo* L. and *Eclipta prostrata* L. both grew to a mature stage.

Plant water relation

The use of water as a solvent facilitates the dissolution of compounds. Water is constantly lost by plants via transpiration, even though they take in a lot of water every day. The amount of water needed by various plant species varies. There is a certain quantity of kinetic energy that molecules of water possess. Kinetic energy, also known as water potential, increases as the concentration of water in a system increases. Pure water has an atomic potential of zero at room temperature. The solute potential is the magnitude of the drop in water potential. The value of the solute potential decreases with increasing concentrations of dissolved solutes; this is always negative.

When it comes to the medium in which biological interactions occur, water remains paramount. Protoplasm does not exhibit any evidence of life until it comes into contact with water. Consequently, water plays a key role in the environment that plants rely on. Environmental variables impact the physiology, water relations, and metabolic activity of plants. The capacity of a plant to withstand water stress may be assessed by measuring its root system development, osmotic potential (OP), relative water content (RWC), and plant water potential (PWP).

Relative water content

The purpose of include these physiological data is to research plant stress physiology. Before morphological alterations become apparent, stressed plants go through a plethora of physiological and biochemical changes. The examination of the quality and quantity of the medicinal plants' bioactive components is most impacted by the stress circumstances. In response to stress, plants store most of their bioactive components in their tissues. There is a relationship between stress physiology and the amount and quality of the bioactive components found in medicinal plants. The relationship between plant water and stress physiology is the most crucial variable.

One way to characterise a leaf's relative water content is as the ratio of its actual water content to its maximum water capacity, as measured at the time of sampling. For example, it enables the calculation to be made using the following inputs, making it a crucial parameter in research of plant water relations. Cell membrane properties including permeability and sustainability are altered in a water-deficit environment (Blokhina *et al*., 2003) [17]. Damages, such as membrane cleavage and sedimentation of cytoplasm content, were discovered upon microscopic examination of dehydrated cells.

Since cellular water deficiency has physiological consequences, relative water content (RWC) is likely the best indicator of a plant's water condition. The monsoon season has the highest relative water content percentage of *Vitex negundo* L., followed by winter and summer. According to Table 5, *Eclipta prostrata* L. had a larger relative water content % during the monsoon season compared to summer and winter. Similarly, research by Jyothi and Jaya (2013) [18] indicated that the monsoon season had the highest relative water content, while the winter and summer seasons trailed closely behind. According to research by Jyothi and Jaya (2013) [18], plants retain protoplasmic permeability by increasing the water content of their leaves under stressful situations.

Table 5: Seasonal variation of relative water content (%).

Sr. No	Plant Name	RWC (%)		
		Monsoon Season	Winter Season	Summer Season
1	*Vitex negundo* L.	73.67	65.78	61.2
2	*Eclipta prostrata* L.	84.01	70.33	77.8

Photosynthetic pigments

Chlorophyll concentration provides insight into a plant's photosynthetic efficiency since it is associated with photosynthesis. The concentration of chlorophyll in leaves is one way to measure the physiological state of plants, including their nitrogen status and photosynthetic ability (Chang and Robison, 2003; Gitelson *et al*. 2003) [19, 20]. A plant's ability to grow, develop, and produce is proportional to how well its photosynthesis processes light. One essential characteristic for understanding the adaptation capability of various plants is their photosynthetic pigments, which change in response to changes in temperature, humidity, and rainfall.

Table 6: Seasonal variation of Chl.a, Chl.b, Total Chlorophyll Content & Chl a/b. ratio

S.N.	Name of the Plants	Season	Chl.a (mg/ 100 g)	Chl.b (mg/ 100g)	Total chl. content (mg100/g)	Chl. a/b ratio
1.	*Vitex negundo* L.	Monsoon	133± 0.018	32.5± 0.018	166± 0.009	4.18±0.299
		Winter	139± 0.019	55± 0.002	195.1±0.023	2.51±0.022
		Summer	166±0.0198	40.8±0.0072	206.9±0.022	4.07±0.076
2.	*Eclipta prostrata* L.	Monsoon	154± 0.065	62.1± 0.062	216± 0.06	2.68± 0.43
		Winter	145± 0.014	68± 0.026	213.3± 0.05	2.14±0.022
		Summer	139± 0.017	57.2±0.0138	196.3±0.0126	2.44±0.081

Various medicinal plants have distinct patterns in their seasonal fluctuations when it comes to photosynthetic pigment production. In order to engage in photosynthesis, the two photosynthetic pigments chlorophyll a and chlorophyll b create light-harvesting complexes. Aside from chlorophyll b, which provides additional light energy, the only other pigment that plays a significant role in the creation of the reaction centre is chlorophyll a. Ensuring a constant ratio of 3:1 between chlorophyll a and chlorophyll b is critical for life. The amount of chlorophyll an is consistently higher than that of chlorophyll b. Since chlorophyll b is a derivative of chlorophyll a, the former is present in greater concentrations than the latter.

Chart no. (4.9) shows the seasonal changes in the photosynthetic pigments chlorophyll a and chlorophyll b of the plants *Vitex negundo* L. and *Eclipta prostrata* L. The amount of chlorophyll an in the leaves of *Vitex negundo* L. was found to be higher in the monsoon season (154±0.065 mg/100gm) than in the summer (139±0.017 mg/100gm) or winter (145±0.014 mg/100gm), but it was lower in the summer (133±0.0183 mg/100gm) and winter (139±0.019 mg/100gm) seasons.

Compared to the summer (40.8±0.0072 mg/100gm) and monsoon (32.5±0.018 mg/100gm) seasons, the winter season had a larger amount of chlorophyll b concentration in *Vitex negundo* L. (55.0±0.002). Compared to the monsoon season (62.1±0.062 mg/100gm) and summer season (57.2±0.0138 mg/100gm), the winter season had a larger amount of chlorophyll b content in *Eclipta prostrata* L. (68±0.026 mg/100gm). During summer, winter, and the monsoon season, the total chlorophyll content of the *Vitex negundo* L. leaves was highest at 206.9±0.022 mg/100gm, 195.1±0.023 mg/100gm, and 166±0.009 mg/100gm, respectively.

Organic Constituents

Proline

Table 7 shows that there is a large amount of variance in the quantity of proline content among the chosen antiallergenic plants when looking at their seasonal change.

Table 7: Seasonal variation of Proline (mg/100gm)

Sr. No	Season	Proline (mg/100gm)	
		Vitex negundo L.	*Eclipta prostrata* L.
1	Summer	0.012 ± 0.0001	0.222 ± 0.0004
2	Monsoon	0.693 ± 0.0008	0.623 ± 0.0007
3	Winter	0.301 ± 0.0004	0.301 ± 0.0004

During the monsoon season, the plants' proline content accumulated the most, followed by the winter and summer seasons. Compared to *Eclipta prostrata* L. leaves, which had a maximum proline accumulation of 0.623 ± 0.0007 mg/100 g during the monsoon season, *Vitex negundo* L. leaves had a maximum of 0.693 ± 0.0008 mg/100 g. Both the *Vitex negundo* L. leaves (0.301 ± 0.0004mg/100gm) and the *Eclipta prostrata* L. leaves (0.301± 0.0004mg/100gm) accumulated proline concentrations in the winter season. During the summer, *Eclipta prostrata* L. leaves had a higher proline concentration accumulation (0.222± 0.0004mg/100gm) compared to *Vitex negundo* L. leaves (0.012± 0.0001).

Proline is an osmolyte that plants store in plenty as a defence mechanism against a wide range of environmental challenges, including but not limited to: cold, lack of nutrients, excessive salinity, drought, heavy metals, excessive acidity, and pathogen infections. The calibration curve was produced using pure proline as the standard. Current findings emphasise the seasonal stress and their adaptability, even though proline has long been thought of as a suitable osmolyte. Proline buildup under stress serves many critical functions, including stabilising proteins and protein complexes in the chloroplast and cytosol, protecting the photosynthetic machinery, and enhancing enzymes involved in ROS detoxification (Szabados L., 2009) [21].

Perhaps because of the less-than-ideal weather circumstances during the monsoon season, proline builds up more at that time of year than throughout winter and summer. An important part of a plant's ability to recover from stress is proline's dual role as an osmolyte and a signalling molecule that may affect mitochondrial function, cell proliferation/cell death, and the activation of certain genes (Szabados L., 2009) [21].

Polyphenols: An essential part of a plant's defence mechanism against the negative effects of biotic and abiotic stresses is the presence of polyphenols, also known as phenolic compounds, which are powerful antioxidants (Sharma *et al*, 2015; Gills and Tuteja *et al*, 2010; Sroka *et al*, 2005) [8, 22, 23].

Table 8: Seasonal variation of Polyphenols (mg/100gm)

Sr. No.	Season	Polyphenols (mg/100gm)	
		Vitex negundo L.	*Eclipta prostrata* L.
1	Summer	95.18±0.04	69.83±0.01
2	Monsoon	141.18±0.02	211.64±0.08
3	Winter	249.69±0.09	182.65±0.04

In (Table No.8), we can see how the total polyphenol content of several antiallergenic plants changed with the seasons. The number of polyphenols in leaves varied with the seasons and other environmental factors. Compared to *Eclipta prostrata* L. leaves, which had a total polyphenol content of 69.83±0.01 mg/100 gm throughout the summer, *Vitex negundo* L. leaves had a higher concentration of 95.18±0.04 mg/100 gm. During the monsoon season, *Vitex negundo* L. leaves had a concentration of 141.18±0.02 mg/100 g while *Eclipta prostrata* L. leaves had a concentration of 211.64±0.08 mg/100 g. In contrast to *Eclipta prostrata* L. leaves, which had a total polyphenol content of 182.65±0.07 mg/100 g during the winter season, *Vitex negundo* L. leaves had a greater concentration of 249.69±0.09 mg/100 g. Total polyphenol concentrations in the leaves of *Vitex negundo* L. and *Eclipta prostrata* L., two of the chosen antiallergenic plants, were found to be highest in the winter and lowest in the monsoon, respectively.

However, both *Vitex negundo* L. and *Eclipta prostrata* L. leaves show a decrease in total polyphenol content over the summer. The calibration curve was produced using tannanic acid as the standard.

Conclusions

Vitex negundo L. and *Eclipta prostrata* L., two plants chosen for their photosynthetic pigments, vary greatly in content and change with the seasons, according to estimates. Plants may benefit from accumulating proline amid stressful environmental circumstances in order to mitigate the negative effects of stress and lessen the rate at which their growth and development are stunted. In response to natural environmental stress, the enzyme activities of IAA oxidase, nitrate reductase, and nitrite reductase in *Vitex negundo* L. and *Eclipta prostrata* L. *Eclipta prostrata* L. and *Vitex negundo* L. have a high chlorophyll stability index, which demonstrates their strong stress tolerance capability. *Eclipta prostrata* L. and *Vitex negundo* L. had greater concentrations of total proteins, carbs, and lipids throughout winter and monsoon season, according to the findings of the study of the primary and secondary metabolites.

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