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CHLOROPHYLL ESTIMATION AND QUALITATIVE PHYTOCHEMICAL SCREENING OF *VITEX NEGUNDO LINN*.

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ABSTRACT

Many historically used plants are important in modern medicine because they have been shown by science to have a variety of desirable activities. One such plant is *Vitex negundo Linn*., which is found all over India. This big, fragrant shrub is a member of the Verbenaceae family and grows near water courses or in damp environments. *Vitex negundo Linn*. qualitative phytochemical screening was investigated. Three different solvents—water, ethanol, and methanol—were utilized to extract the leaves that had been powdered. Using accepted practices, the extracts underwent qualitative phytochemical screening. Eight phytochemicals are found, according to the results. These include carbohydrates, steroid, alkaloids, flavonoids, glycosides, terpenoids, tannins, and phenols. Overall, extracts made with water include more phytochemicals. The variety of phytochemicals that have been discovered indicates that *Vitex negundo Linn*. could be a good source of therapeutics. All photosynthetic organisms include green pigments called chlorophylls, which are the basic building blocks of photosynthesis. They exhibited weak protein binding, but could be easily removed using organic solvents as acetone, ether, etc. Chemically each chlorophyll molecule has a porphyrin nucleus with a chelated magnesium atom at the core and a long chain hydrocarbon (phytal) side chain bonded through a carboxylic acid group. Plants contain at least five different forms of chlorophylls. Using an 80% acetone extract, chlorophyll is extracted, and a spectrophotometer is used to measure the absorbance at 663, 645, and 470 nm. The amount of chlorophyll is computed using the absorption coefficients.

KEYWORD: ***Vitex negundo***, phytochemical analysis, chlorophyll estimation, spectrophotometer.

INTRODUCTION

Ethnobotany is defined as the study of indigenous plant applications and the interactions between humans and plants. *Vitex negundo* has been utilized as a female medicine from ancient times, according to ethnobotanical research.[1] It was discovered to reduce libido, and it is documented that Roman wives whose husbands were away with the legions used *V. negundo* leaves to cover their beds.[2] It was subsequently given the name "chaste" berry tree, which denotes innocence or faithfulness! The chaste berry was known as "Monk's pepper" or "Cloister" (a covered arcade typically used by monks) paper in monasteries during the Middle Ages due to the belief that it had a sexually stimulating effect that led to its progressive use as a food spice. Additionally, it was recognized in European folklore as a significant treatment for managing and regulating the female reproductive system. An ancient remedy for amenorrhea (abnormal suppression of menstruation) and dysmenorrhea (painful menstruation) was its usage as a menstrual period regulator.

Additionally, it eased the process of giving birth and menopausal symptoms.[5]

Fig: -1 *V.negundo* In Campus of Ranchi University, Ranchi.

Study of Moorphotaxonomy of plants taken

Vitex negundo is a small tree or upright shrub that reaches a height of 2 to 8 metres.[3] In most cases, the bark is reddish-brown. Its leaves have three to five finger-like lanceolate leaflets that branch off of them.[4] Every leaflet range in length from 4 to 10 cm, with the longest leaflet being located in the centre and having a stalk. The underside of the leaf is hair-covered, and its edges are saw-toothed or notched like a saw.[5][6] The abundant purple-white

blooms are carried in panicles that range in length from 10 to 20 cm. The middle lower lobe has the longest petal, though they are all different lengths. Dense hairs cover both the corolla and calyx.[6] Insects pollinate the fragrant flowers, which are naturally hermaphrodite[5] (having both male and female organs).

Nirgundi literally translates to "which protects the body from ailments" in Sanskrit. This powerful herb is used to treat a wide range of illnesses. The source of this aromatic shrub is *Vitex negundo*, which has small brown fruits that are used medicinally and rose-coloured flowers with a pungent, astringent, and bitter taste.

It is a multifunctional and adaptable herb that can be taken orally as a powder, leaf juice extract, or water decoction, or applied externally as an oil or ointment. It has antibacterial, anti-inflammatory, and analgesic qualities. Fever, arthritis, headaches, swelling, digestive issues, and oral health issues can all be effectively treated with it. This plant has two varieties based on flower types: blue and white flowered. The white-flowered plant is known as siduwar (*Vitex trifolia*), while the blue-flowered plant is called nirgundi (*Vitex negundo*).

Nirgundi is primarily composed of the following chemical components: dulcitol, alkaloid-Vitridine, B-sitosterol, camphene, orientin, artemetin, monoterpenes, anguside, eurotoside, and aucubin. Additionally, it contains flavonoids such as vitexin, which itself contains chrysosplenol D, casticin, and chryso-splenol. It is well known for having muscle relaxant and anti-histamine qualities. It facilitates sound sleep induction and functions as a mild topical analgesic.

V. negundo is a non-native shrub or small tree that thrives in full sun during hot weather. Since it blooms on new growth, it can be trimmed to the ground in the early spring to stimulate growth, much like a perennial. It has intriguing foliage and is shaped like an open vase with loose branches. Full sun and loose, moist, well-drained soil are preferred. Mid- to late-summer panicles bearing 5–8-inch flowers emerge. Remove deadheads to promote more blooms.

Its natural habitat is the wastelands of the Himalayas and the mixed thickets that grow on mountain slopes at an elevation of between 600 and 6500 feet. It can reach a maximum height of 15 feet, and in regions with mild winters, it may grow even higher. When woven with other materials, the young stems can be used for construction as well as for making

baskets. Grows in zones 6 through 9, though zone 6 is not a good place to plant it for the winter. In harsher winters, it might die to the ground or suffer from dieback. In this zone, where it can grow as a 3–5-foot perennial, planting in a sheltered spot is crucial.

TAXONOMICAL CLASSIFICATION

Kingdom - Plantae **Division** - Magnoliophyte Class - Magnoliopsida Subclass - Asteriidae Order - Lamiales Family - Verbenaceae Genus - Vitex Species – *V. Negundo.*

Distribution

Vitex negundo is native to tropical Eastern and Southern Africa and Asia.[7] The following nations are among its native territories: Afghanistan, Bangladesh, Bhutan, Cambodia, China, India, Indonesia, Japan, Kenya, Madagascar, Malaysia, Mozambique, Myanmar, Nepal, Pakistan, the Philippines, Sri Lanka, Taiwan, Tanzania, Thailand, and Vietnam.[7] In India, it is typically found in warmer regions and can reach elevations of up to 1500 metres in the outer Western Himalayas.[5] It is best suited for sandy and loamy soils with neutral, basic, or acidic characteristics; well-drained soil is also necessary. Insufficiently fertile soil can also support its growth.[5] Near water bodies, grasslands, and mixed open forests are where the plant is typically found.[8]

Medicinal Importance

It's common knowledge that natural products have a place in contemporary medicine.[9] Inspection of medical indications by source of compounds has demonstrated that natural products and related drugs are used to treat about 87% of all categorised human ailments.[10] [11][12] According to a World Health Organisation report[13], traditional medicine serves the primary healthcare needs of over 80% of the global population. The widespread perception that herbal remedies are safer than expensive synthetic (primarily allopathic) medications, which typically have side effects, is the primary driver of the growing interest in plant-derived pharmaceuticals. However, the ongoing emergence of resistant strains necessitates the development of novel therapeutics in order to treat illnesses.[14] Typically, the goal of herbal remedies is to restore the body to its optimal state of health, as opposed to just treating a specific illness.[15] Medicinal plant phytochemical constituents frequently enhance health in one of three ways: singly, additively, or jointly.[16] After having analysed the various chemical components available in different parts of *Vitex negundo*, it is essential that focus shifts to the medicinal applications of the plant. *Vitex negundo* is a plant with many recognised medicinal uses, and it has been widely used to treat a wide range of illnesses.[17] Three categories—

traditional medicine, folk medicine, and pharmacological studies—have been used to group these characteristics.[18]

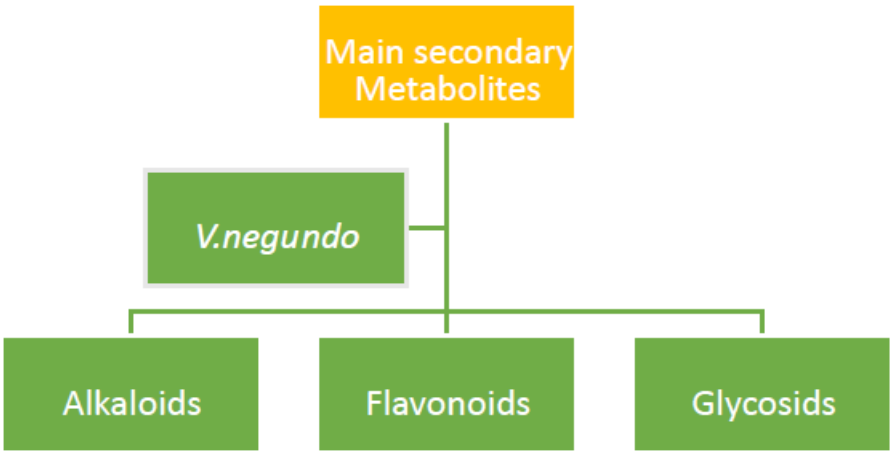
Ayurveda: The most ancient and authoritative text on Indian Ayurveda, the Charaka Samhita, contains references to *V. negundo* in its verses. The plant is described in the Charaka Samhita by Sharma.[19] as a vermifuge and has been classified as an anthelminthic (drugs that remove parasitic worms, or helminths, from the body). Other Ayurvedic uses of *Vitex negundo* are illustrated by Tirtha.[20] People use the leaves of *V. negundo* to smoke the leaves in order to relieve headaches and catarrh, which is the secretion of excess mucus through the nose and throat due to a cold. A crushed leaf poultice is used to treat sinusitis, tubercular neck swellings, headaches, and neck gland sores. Additionally effective in treating STIs and other syphilitic skin conditions is the essential oil derived from the leaves. When a person has catarrhal fever with dull hearing and a heaviness in the head, a leaf decoction containing Piper nigrum is used. Jadav and Bhutani[21] report on the Ayurvedic use of *V. negundo* for the treatment of dysmenorrhea. In reference to the preparations listed in Anubhoga Vaidya Bhaga, a compilation of cosmetic formulations, Patkar[22] described the application of their leaves in conjunction with those of *Azadirachta indica*, *Eclipta alba*, *Sphaeranthus indicus*, and *Carum copticum* in a well-known renovation remedy called Kayakalpa. Extracts of various *V. negundo* parts are found in *Vishagarva taila*, a significant Ayurvedic nervine sedative.[23]

Secondary Metabolites

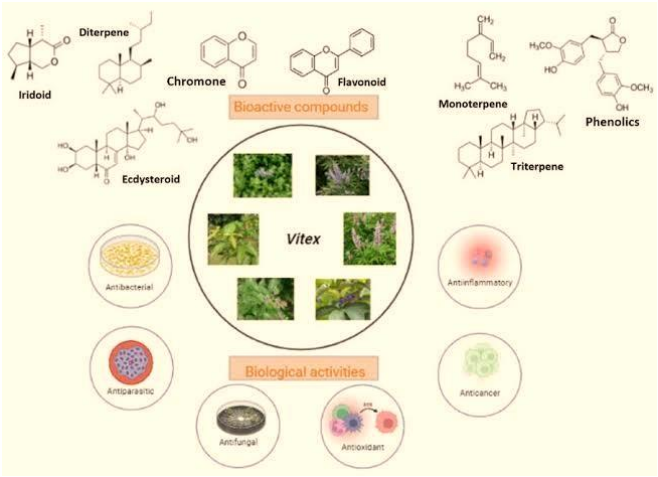
The substances that are produced by secondary metabolic pathways and are not necessary for cell survival are known as secondary metabolism products. These substances are not necessary for the survival of cells. The production of these compounds is not ongoing. Secondary metabolites, such as lignins, resins, phenols, steroids, alkaloids, and tannins, are frequently the byproducts of primary metabolites. Figure1.

Albrecht Kossel, who received the 1910 Nobel Prize in physiology or medicine, was the first to define the term "secondary metabolite."

The main secondary metabolites and leaf & flower contents of *Vitex negundo* were given in figure: -



(Figure 2: - Main Secondary Metabolites *of V. negundo)*



(Figure 3: -Bioactive secondary metabolites and biological activity *of Vitex sp.*)

MATERIALS AND METHODS

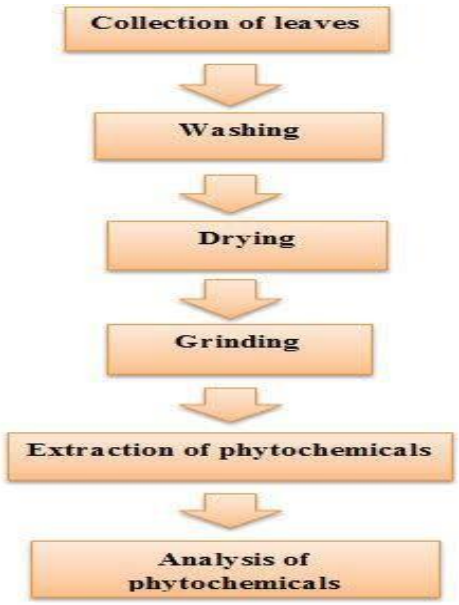
Collection of plant

In April 2023, fully grown fresh leaves of *Vitex negundo* were collected from the Ranchi University campus in the Ranchi district. The plant taxonomist Prof. Anita Mehta of the Ranchi University Department of Botany performed the taxonomic identification of these plantlets. Figure1.

Processing of plant material

Vitex negundo Linn. fresh leaves were repeatedly washed with water to get rid of dust, and after drying in the sun to get rid of any remaining moisture, the leaves were ground into a powder. In order to extract ten grammes of *Vitex negundo Linn* powder, ethanol, methanol,

and aqueous solution were combined with the powder using a Soxhlet extraction apparatus. The solvent was allowed to boil for 4872 hours, or until the extracted solvent turned clear. The extracts were then filtered using filter paper, and a rotary evaporator was used to evaporate the solvent, giving the extracts a syrupy consistency. For upcoming trials, the extract was then stored in a refrigerator at 4°C. (Figure3)



(Figure 4: - Methodology summary)

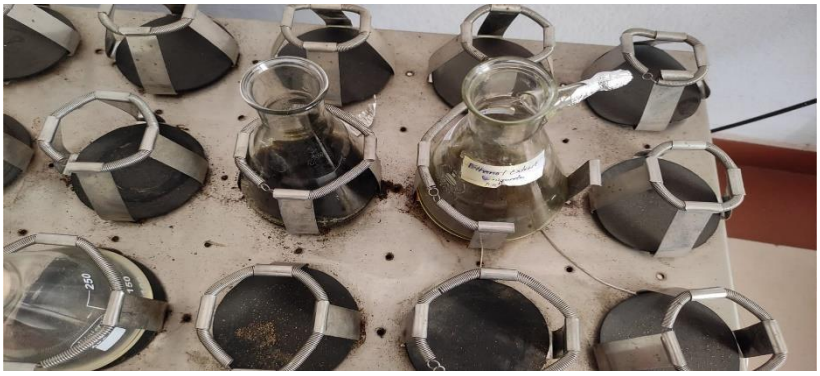
Extract preparation

The powdered plant leaves were soaked with (10g/100ml) in 70% different solvent of ethanol, methanol, and distilled water for overnight in rotator shaker. Figure2 a-f.

Figure 5a: -Fresh leaf

Figure 5b: -Dry leaf

Figure 5c: -Extract powder.



(Figure 5d -Rotatory Shaker)

(Figure 5e Extract filtration)

(Figure 5f Extract after filtrated)

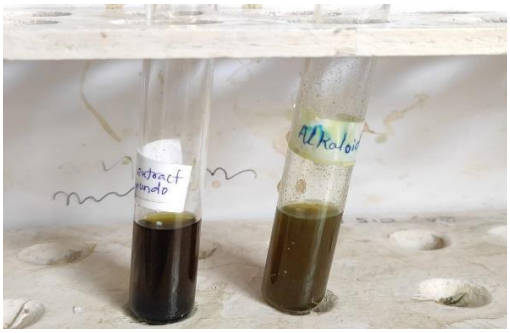
Figure [5a-f] Picture shows Extracted plants leaves powder Preparation.

Procedure of phytochemical screening from three type of extracts

The presence of different metabolites was quantitatively screened by using the standard procedure to identify the constituents *(Harborne J.B 1998; Sazada S et al. 2009; Wagner et al. 1996; solohokara et al. 2015; Solomon CU et al. 2013; yadav m et al. 2014*)

Test for Alkaloids (Mayer's test)

5ml of each plant extract were taken and 3-5 drops of Mayer's reagent were added and observed for the formation of white yellowish precipitate indicates the presence of Alkaloids.[3]



(Fig 6a: -Ethanol extract)

(Fig 6b:- Methanol extract)

(Fig 6c: -Aqueous extract)

Test for Phenol

5 ml of each Extracts were treated with 3-4 drops of 5% ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.[3]

(Fig 7a: -Ethanol extract)

(Fig 7b:-Methanol Extract)

(Fig 7c: -Aqueous extract)

Test for Tannins

To the extract of 5ml each 0.1% ferric chloride solution were added, formation of a dark blue or greenish black colour showed the presence of tannins.[3]

(Fig 8a: -Ethanol extract)

(Fig 8b:-Methanol extract)

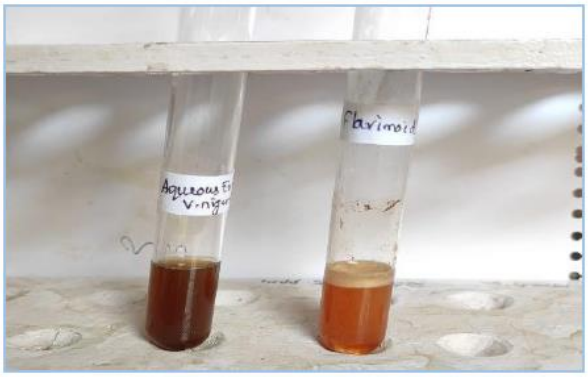
(Fig 8c Aqueous extract)

Test for Flavonoids

5ml of each extract were mixed with small amount of magnesium and HCl were added drop wise. Formation of yellow colour precipitate indicates the presence of flavonoids.[3]

(Fig 9a: -Ethanol extract)

(Fig 9b: -Methanol extract)



(Fig 9c: Aqueous Extract)

Test for Glycosides

5ml of each extract were treated with 1 ml water and 1 ml sodium hydroxide. Formation of yellow colour indicates the presence of glycosides.[3]

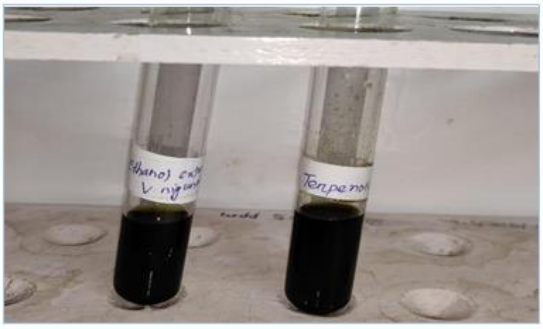
(Fig 10a: -Ethanol extract)

(Fig 10b:-Methanol extract)

(Fig: 10c Aqueous Extract)

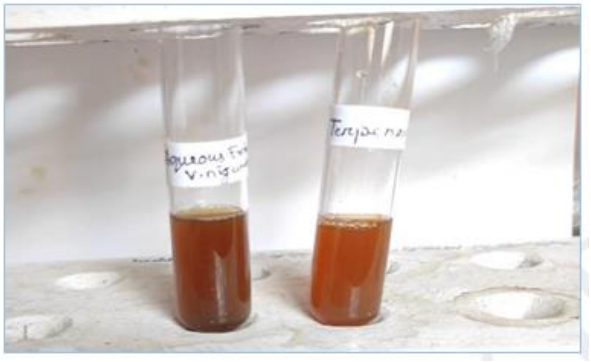
Test for Terpenoids

5 ml of each extract was added to 2 ml of chloroform and 3 ml of concentrated sulphuric acid to form a monolayer of reddish-brown coloration of the interface was showed to form positive result for the terpenoids.[3]



(Fig 11a:-Ethanol extract)

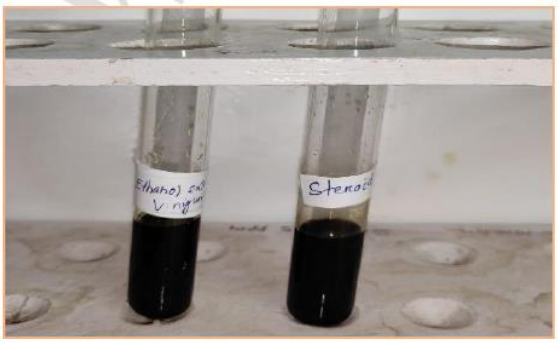
(Fig 11b:-Methanol extract)



(Fig 11c- Aqueous extract)

Test for Steroids (Salkowski Test)

Extracts were treated with few drops of chloroform and concentrated sulphuric acid. Formation of bluish red to cherry colour in chloroform layer green fluorescence acid layer indicates the presence of steroids.[3]



(Fig 12a: -Ethanol extract)

(Fig 12b: -Methanol extract)

(Fig 12c: Aqueous extract)

Test for Carbohydrates

Fehling's test: Equal amount of Fehling A and Fehling B reagents were mixed and 2ml of it was added to the plant extract and then gently heated the sample. Appearance of brick red precipitate indicated the presence of reducing sugars.[3]

(Fig13a: -Ethanol extract)

(Fig13b:-Methanol extract)

(Fig13c:-Aqueous extract)

RESULT

Result of phytochemical screening

Table 1: Phytochemical analysis of *Vitex negundo* was carried out by the following solvents Methanol, Ethanol and Aqueous.

		Extracts		
Sl.no	**Tests**	**Ethanol**	**Methanol**	**Aqueous (H_2O)**
1.	Alkaloid test	++	++	+++
2.	Phenol test	+++	+++	+++
3.	Tannin test	+++	+++	+++
4.	Flavonoid test	++	+++	+++
5.	Glycosides test	+++	++	++
6.	Terpenoid test	_	_	+++
7.	Steroid test	_	++	+++
8.	Carbohydrate test	+++	_	++

+++ =Highly present

++ =Moderate

-ve =Absent

With respect to extractive solution

Estimation of chlorophyll pigments

In this process, 80% acetone is used to extract chlorophyll, and a spectrophotometer is used to measure the absorption at 643, 645, and 663 nm. Green pigments called chlorophylls are found in chloroplasts in all photosynthetic plant tissues and are vital components of photosynthesis.

Living things require the plant pigments carotenoids and chlorophyll, both of which are very significant. Chlorophyll used as food additive. Fruits and veggies get their colour from carotenoids. Together with their anti-oxidant properties and potential to combat various forms of cancer, these two photosynthetic pigments are also recommended for conditions involving the skin and eyes. This study's goal is to identify the precise window of time during which the concentrations of these two pigments are at their highest, and then suggest a plant from which the food and pharmaceutical industries can extract these pigments for use in the production of herbal products intended for human consumption.

Extraction of Chlorophyll (Arnon, 1949).[24,25,26,27,29]

We took 100 mg of fresh *Vitex negundo* leaves, chopped them finely, and ground them with about 20 ml of 80% acetone. Next, it was centrifuged for eight minutes at 8000 rpm. After transferring the supernatant, the process was repeated until the residue lost its colour. Up to 50 millilitres of volume makeup have been applied. The solution's absorbance was measured at 470, 645, and 663 nm in relation to the solvent (80% acetone) as a reference.

(Fig 14:- *Vitex negundo* extract of 80% acetone)

Chlorophyll A.

Table 2: For the estimation of chlorophyll, a, the absorbance of the solution was taken at 663nm against the solvent (80% acetone) for three time, the mean value is taken for the calculation as reference.

	Absorbance value (3times)	Mean value
Chlorophyll a (A_{663})	0.613	0.616
	0.618	
	0.618	

Chlorophyll B.

Table 3: For the estimation of chlorophyll b, the absorbance of the solution was taken at 645nm against the solvent (80% acetone) for three time, the mean value is taken for the calculation as reference.

	Absorbance value (3times)	Mean value
Chlorophyll b (A_{645})	0.256	0.253
	0.250	
	0.255	

Carotenoids

Table 4: For the estimation of carotenoids, the absorbance of the solution was taken at 470nm against the solvent (80% acetone) for three time, the mean value is taken for the calculation as reference.

	Absorbance value (3times)	Mean value
Carotenoids (A_{470})	0.642	0.642
	0.643	
	0.641	

Estimation of chlorophyll content.[25, 26, 27, 28, 29]

The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equation (Arnon, 1949): Chlorophyll a (mg/gm tissue): [12.7(A_{663})-2.69 (A_{645})]*V/1000*W

Chlorophyll b (mg/gm tissue): [22.9(A_{645})-4.68 (A_{663})] *V/1000*W

Total Chlorophyll (a+b) (mg/gm tissue): [20.21(A_{645}) +8.02(A_{663})] *V/1000*W

A = Absorbance of specific wavelength;

V = Final volume of Chlorophyll extract in 80% Acetone;

W = Fresh weight of Tissue extract.

Estimation of Carotenoids (Lichtenthaler and Wellburn Method)[27, 28, 30, 31, 32, 33]

The concentration of Carotenoids was estimated by using well-known Lichtenthaler and Well burn method. The 80% acetone extract was measured at 470 nm in spectrophotometer to quantify the total carotenoid (xanthophylls + carotene) concentrations.

Total Carotenoids (mg/gm tissue):

$C\ x + c = (1000A_{470} – 1.82Ca – 85.02Cb) / 198$

Where, A = Absorbance at respective wave length,

Ca = Chlorophyll-a, Cb=Chlorophyll-b

Results of chlorophyll estimation

Table 5: Pigment concentration expressed in mg/gm tissue.

Month	Chlorophyll-a	Chlorophyll-b	Chla:Chlb	Total Chlorophyll	Total Carotenoids	Total Chlorophyll: Total Carotenoids
April 2023	2.8570	1.1643	2.453:1	4.0213	2.7162	1.480:1

DISCUSSION

The study analyzed the phytochemical properties of *Vitex negundo*, a plant known for its health benefits. The methanol extract of *Vitex negundo* contained alkaloids, flavonoids, tannin, glycosides, phenolic compounds, and steroids, with no terpenoids or carbohydrates. The extract was found to protect rats' brains from ethanol-induced oxidative stress, demonstrating its antioxidant potential. The extract also showed potential as a neuroprotective agent in drug development.

The study supports the use of medicinal plants as an alternative source of treatment for various diseases and infections. The powdered leaves of *Vitex negundo* contained carbohydrates, protein, alkaloids, saponins, and flavonoids. Plants contain two major bioactive molecules: carotenoids and chlorophylls, which can be beneficial in herbal medicine. The study found that the total chlorophyll content was 4.0213 and total carotenoid was 2.7162.

CONCLUSION

Vitex negundo ethanol and methanol leaf extracts show activity against various fungus strains, with the highest activity against *Penicillium sp*., *Aspergillus niger*, *Candida albicans*, and *Cryptococcus neoformans*. Understanding the inhibition mechanism of bioactive

compounds in plant extracts could help treat bacterial and fungal infections. Developing nations have the greatest diversity of medicinal plant species, offering new markets. Considering plant issues is crucial for obtaining positive drug effects. *Vitex negundo*, also known as "sarvaroganivarini," is considered a cure-all in traditional Indian communities. Optimizing the use of naturally occurring resources is essential for knowledge advancement.

Future Prospectus

1. Optimization of other culture conditions (light, temperature, aeration, elicitors biotic and abiotic) are to be used to increase alkaloid production in invitro grown plants.
2. comparative biochemical analysis of in vitro and in vivo plants.
3. Genetic modification can be very useful for the production of the desired level of secondary metabolites.
4. Salt tolerance varieties can be screened using physiological parameters.
5. Hairy roots culture can be developed by biotechnology for large-scale alkaloid production.

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