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Studies of Phytochemical screening of *Vitex peduncularis* in different solvents and estimation of Chlorophyll Pigment by Spectrophotometer

Dr. Shweta Nag[1], Riya[2]

Abstract

Vitex peduncularis Wall. ex-Schauer, commonly identified as Charaigorh (or Charaigorwa), is an evergreen tree having several ethnopharmacological usages and have its place to the family Lamiaceae. It's a go-to for tackling various health issues in both modern and traditional medicine. Packed with vitexin, flavonoids, iridoids, and ursolic acid – that's why Vitex peduncularis is a bioactivity powerhouse. Owing the high needs of Vitex peduncularis into the pharmaceutical manufacturing, this species is being oppressed indiscriminately, pushing into the direction of abyss of extinction. Urgent conservation efforts are needed to safeguard Vitex peduncularis for future generations. This research highlights Vitex peduncularis' pharmacological potential and therapeutic uses. Research gaps need to be addressed to ensure sustainable use and preserve Vitex peduncularis' genetic resources. Vitex peduncularis leaves contain flavones like 4'-acetoxy-5-hydroxy-6, 7-dimethoxyflavone, crisimartin, and genkwanin. A drug's effect comes from its active ingredient, and modifying its chemical structure can alter its properties. Herbal medicine is a big deal in Bangladesh for preventing and treating diseases, including infections. Leaves and barks of the Vitex peduncularis are used for diabetes in the hill area of khagrachari district, Bangladesh. Dried leaves yielded some key compounds: pachypodol, ursolic acid, vitexin, and peduncularisin. Kalazar, or visceral leishmaniasis, is caused by Leishmania parasites.

Keywords: *Vitex peduncularis, Vitexin, Lamiaceae, Iridoid, Pharmacological, Bangladesh*

Introduction

Vitex peduncularis, vernacularly known as "Charaigorwa" is a large evergreen tree having numerous ethnopharmacological uses and have its place to the family *Lamiaceae*. The species is globally acknowledged for its usage to curve multifarious human health issues in traditional as well as contemporary medicines. These plants have also been crucial in traditional medicine practices right from the start. Most medicines are derived from plants with anti-cancer, immune-boosting, antiviral, anti-inflammatory, and antibacterial properties.

This species is super bioactive thanks to its rich mix of secondary metabolites like flavonoids, vitexin, iridoid glycosides, pachypodol, ursolic acid, and 2-hydroxy-ursolic acid terpenoids. *Vitex peduncularis* is being over-exploited in the pharma industry, pushing it towards extinction. We need urgent conservation efforts and scientific interventions to save it. Flowers are yellow in colour, panicled cymes, in axillary many-flowered, 20-25 cm long-peduncled. Fruit drupe, bulbous, size of a black pepper, and black when ripe.

Leaf extracts of *Vitex peduncularis* and *Vitex pinnata* show promise against leishmania donovani. In simple terms, antioxidants are substances that prevent or slow down cell damage caused by oxidation. So, it's a broad spectrum of protection, covering lipids, flavonoids, terpenoids, and iridoids. Antioxidants are big players in supplements and research for preventing diseases like cancer, heart disease, Alzheimer's, and more. India's got a treasure trove of medicinal plants, and most of its population still swears by traditional remedies for healthcare

Description Of the Plant

A low medium-sized to a big deciduous tree, with 6-12 m high, leaves are 3-foliate entire, and rarely with 4, long acuminate, base cuneate, petiole up to 10 cm, the leaflets are narrow elliptic or lanceolate, 15-20 cm long entire, long acuminate, and the base cuneate. The flowers are yellow in colour, many flower, in axillary, cymes panicled, 20-25 cm big. Fruit is drupe, globular, size of s black pepper, black when ripe.

Pharmacological Activity

In pharmacology, biotic movement refers to a medication's effects (good or bad) on living tissue, driven by its active ingredient, which can be tweaked chemically.

In Bangladesh, herbal medicine is a big deal, especially *Vitex peduncularis*, used for diabetes in Khagrachari. Its leaves pack a punch with compounds like pachypodol and vitexin.

In Orissa, people drink a leaf decoction like tea to ward off cold. Leaf infusion boosts cell resistance and prevents breakdown in rabbits, whether given orally or via injection. Chakma people use the bark paste to treat jaundice. Mix root extract with hot water, take 2-3 spoons twice daily till it works. Apply the extract externally on affected areas for paralysis-like symptoms in eyes and face. Marma people use bark extract with water to treat urethritis.

Phytochemical Constituents

Vitex peduncularis is packed with flavones like 4'-acetoxy-5-hydroxy-6, 7-dimethoxyflavoe and known compounds like crisimartin and genkwanin. *Vitex peduncularis* stem bark yields peduncular side and agnuside. Previous studies found flavonoids like vitexin, pachypodol, and ursolic acid in *Vitex peduncularis*.

Estimation Of Chlorophyll Pigments

The process where chlorophyll is extracted in 80% acetone and the absorption is read at 643 nm ,645 nm, and 663 nm in spectrophotometer.

Chlorophyll's the green pigment in chloroplasts, crucial for photosynthesis. Totally, plant pigments like chlorophyll are a big deal for life on Earth. Chlorophylls used as a natural food colouring and additive. Carotenoids are behind those vibrant colours in fruits and veggies. Those pigments, like carotenoids and chlorophyll, are packed with antioxidant powers, fighting diseases and cancers, and helping with skin and eye issues.

Method And Materials

- **For bioactive compound screenings:**

Collection of plant were done, fresh matured leaves of *Vitex peduncularis* were collected from home garden in the month of May, 2024.

- **For chlorophyll pigments:**

Collection of plant were done, fresh fully natured matured leaves of *Vitex peduncularis* were collected from home garden in the month of May, 2024.

PROCESSING OF PLANT

Fresh leaf was washed trice with running tap water to remove the dust particles and then dried in the dark room to remove the residue moisture and grinded to form powder. 5 gms of leaf powder were used for the extraction with aqueous and ethanol solution by soaking it for 48-72 hours in conical flask covered it from aluminium foil or until the solution become thicker in consistency. Then the solution filtered with help of Whatman filter paper no 1 in another conical flask.

Extract Preparation for Phytochemical Screening

The powdered plant leaves were soaked with 1:10 ratio i.e., (5 g / 50 ml) in 70% different solvent of ethanol and aqueous for 48-72 hours.

- *Test for Alkaloid*

3 ml of each plant extract were taken and 4-5 drops of Mayer's reagent were added and observed for the formation of white yellowish precipitate indicates the presence of Alkaloids.

- *Test for Tannins*

3 ml of each plant extract were taken and 0.1% ferric chloride solution were added to it; formulation of a dark blue or greenish black colour shows the presence of tannins.

- *Test for Phenol*

3 ml of each plant extract were taken with 4-5 drops of ferric chloride solution. Formation of bluish colour indicates the presence of phenol.

- *Test for Flavonoids*

3 ml of each plant extract were mixed with a small amount of Magnesium and then HCL were added drop wise. Formation of yellow colour precipitate indicates the presence of flavonoids.

- *Test for Coumarins*

3 ml of each plant extract were taken and treated with 1 ml of distilled water and 1 ml of sodium hydroxide. Formation of yellow colour indicates the presence of Coumarins.

- *Test for Terpenoids*

3 ml of each plant extract were taken and 2 ml of chloroform with 3 ml of concentrated sulphuric acid were added to into it to form a monolayer of reddish-brown colouration of the interface was showed to form positive result for the terpenoids.

- *Test for Resins*

3 ml of each plant extract were taken and were treated with few drops of chloroform and concentrated sulphuric acid. Formation of light-yellow colour in chloroform layer, green fluorescence acid layer indicates the presence of resins.

- *Test for Saponins*

3 ml of each plant extract were taken and treated with Magnesium turning ribbon to form to form appearance on upper layer of the mixture. The foam layer appearance on upper layer indicates the presence saponins.

- *Test for Quinone*

3 ml of each plant extract were taken and treated with few drops of concentrated sodium hydroxide. Formation of yellowish colour appearance indicates presence of quinone.

Extraction Of Chlorophyll

100 mg of finely chopped fresh leaves were taken and grinded with approximately 20 ml of 80% Acetone. It was then centrifuged at 500 rpm for 8 minutes. The supernatant was transferred and the procedure was repeated till the residue becomes colour less. The absorbance of the solution was taken at 470 nm, 645 nm, and 663 nm against the solvent (80% acetone) as reference.

Result And Disscusion

- For phytochemical screenings:

Screening of Phytochemicals			
Sl. no	**Name of Test**	**Aqueous solution**	**Ethanol solution**
1	Alkaloids	++	--
2	Flavonoids	++	++
3	Phenols	++	++
4	Saponins	++	++
5	Tannins	++	++
6	Terpenoids	++	++
7	Quinones	++	++
8	Resins	--	++
9	Coumarins	++	++
(++) Shows Present **(--)** Shows Absent			

Table.1: *Above table shows presence and absence response of different tests done.*

- For Chlorophyll reading under spectrophotometer:

Chlorophyll-A

For the estimation of Chlorophyll A, the absorbance of the solution was taken in triplicate at 663 nm, against solvent (80% acetone), the mean value is taken for the calculation as reference.

	Absorbance (triplicates)	Mean value
Chlorophyll A	0.614	
	0.619	0.617
	0.619	

Chlorophyll-B

For the estimation of Chlorophyll b, the absorbance of the solution was taken in triplicate at 645 nm, against solvent (80% acetone), the mean value is taken for the calculation as reference.

	Absorbance (triplicates)	Mean value
Chlorophyll B	0.254	
	0.251	0.252
	0.253	

CAROTENOIDS

For the estimation of Carotenoids, the absorbance of the solution was taken in triplicate at 470 nm, against solvent (80% acetone), the mean value is taken for the calculation as reference.

	Absorbance (triplicates)	Mean value
Carotenoids	0.641	
	0.644	0.642
	0.642	

Estimation Of Chlorophyll Content

The estimation of Chlorophyll A & 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): $[20.2(A_{645}) + 8.02\ (A_{663})] * V/1000*W$

A = Absorbance of specific wavelength;

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

W = Fresh weight of tissue extracted

Estimation Of Chlorophyll

Month	Chlorophyll A (mg/gm)	Chlorophyll B (mg/gm)	Chl A: Chl B	Total Chlorophyll (mg/gm)	Total Carotenoids (mg/gm)	Total Chlorophyll: Total Carotenoids
May	0.71	2.89	0.24	6.4	1.995	3.22

Table.2: Pigment estimation of in-vitro leaf expressed in mg/gm.

Estimation Of Carotenoids (Lichtenthaler and Welburn Method)

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

Total Carotenoids (mg/gm tissues):

$C\ x + c = (1000\ A_{470} - 1.82\ C_A - 85.02\ C_B) / 198$

Where, A = absorbance at respective wavelength,

C_A = Chlorophyll A

C_B = Chlorophyll B

Conclusion

This article comprehensively outlines the significance of the species, focusing on its specialized pharmacological properties and diverse therapeutic applications, showcasing its potential benefits in various medical contexts. This article also underscores the need for further research to ensure the species' sustainable use and conservation of its dwindling genetic resources. Plants are like nature's gift, providing essentials for our survival and health.

Plants have been a cornerstone of traditional medicine for thousands of years, with many modern medicines still derived from plant-based compounds. They're a rich source of bioactive molecules, offering potential treatments for various diseases. Many life-saving drugs originated from plants, like aspirin (willow bark) and quinine (cinchona tree). Even today, scientists look to plants for new leads in medicine. People are turning to plants for health solutions due to their accessibility, potent bioactive components, and the rich traditional knowledge behind them. Medicinal and aromatic plants have been a game-changer in healthcare and pharma over the past few decades. India's got a treasure trove of medicinal plants, and a huge chunk of the population still swears by traditional remedies for healthcare. Many life-changing medicines have originated from plants, with a history dating back thousands of years.

The WHO survey highlights the significant role traditional medicine plays globally, with up to 70% of people relying on it for basic healthcare needs. This shows the trust people have in conventional healthcare systems, especially in developing countries where access to modern healthcare might be limited. Human activities and ecological changes are pushing many medicinal plants to the brink, making them rare or endangered. If we keep going at this rate, we're looking at a potential extinction crisis for these medicinal plants, which would disrupt the ecosystem's balance.

The Vitex genus is pretty diverse, with around 270 species spread across tropical and sub-tropical regions. Pretty much all Vitex species are used in traditional medicine, they've got a lot of potential. Vitex plants are a valuable resource, providing food and medicine. Their fruits are packed with nutrients, making them a great option for low-income populations. Plus, they've been used to treat various diseases, showcasing their versatility and importance in traditional healthcare. Over-reliance on Vitex plants can disrupt the ecosystem's balance, threatening their sustainability and availability.

Hence, both the socioeconomic and ecological significance of vitex cannot be denied. *Vitex peduncularis* Wall. ex Schauer belongs to the family Lamiaceae and it is traditionally consumed for health reasons throughout Southeast Asia and India. Not looking good for this species - it's already rare in India and threatened in Bangladesh.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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