

**PHYTOCHEMICAL ANALYSIS OF LEAVES
EXTRACT OF *Celastrus paniculatus* Wild. PREPARED
IN VARIOUS SOLVENTS**

Project Report

Submitted to the “**Jaypee University of Information Technology**” in the
fulfilment of Major Project for the award of the Degree of

B. Tech

In Discipline **Biotechnology**



Submitted By

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To

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Also, I am extremely grateful to my parents for their constant encouragement and for providing me with all the necessary resources required for the successful completion of the project.

Date: 14th May 2022

DECLARATION

I Mahak Rustagi hereby declare that the project report titled “Phytochemical analysis of leaves extract of *Celastrus paniculatus* Wild. prepared in various solvents” has solely been submitted by me to “Jaypee University of Information Technology” under the guidance and supervision of Dr. Gopal Singh Bisht and the work for the same has been carried out at Jaypee University of Information Technology, Solan H.P. (173234).

All the information & statement made by me are correct to the best of my knowledge. My candidature is liable to be cancelled and legal action may be initiated against me in case the information provided is found to be false or incorrect in any way.

Place: Waknaghat, Solan

Date: 14th May 2022

Name: Mahak Rustagi

SUPERVISOR'S CERTIFICATE

This is to certify that work contained in the report of “Phytochemical analysis of leaves extract of *Celastrus paniculatus* Wild prepared in various solvents” submitted to the Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, in the partial award of the degree of Bachelor of Technology in Biotechnology, is a record of bona fide work carried out by Mahak Rustagi, enrolment no. 181836, under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

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ABSTRACT

Celastrus paniculatus Wild is a plant that is underappreciated. In Uttar Pradesh and Uttarakhand, it has been designated as a rare and endangered species. It's most frequent between 1800 and 2000 metres above sea level. It is also known as Malkagni or Jyotishmati. It has a long history of being used to improve intelligence, memory, and learning ability. The presence of the most chemicals was found in the water extract, which was made from the leaves. DCM extract included sterols, methanol extract contained triterpenoids and glycosides. The greatest activity of DCM and methanol extract was found in an 8:2 solvent mixture of ethyl acetate: hexane and chloroform: DCM. Rheumatism, gout, arthritis, and a variety of gastrointestinal disorders have all been traditionally treated using seeds and their oil. Seeds, leaves and oil are widely used for their traditional and ethnological properties.

INTRODUCTION

Celastrus is a critically endangered plant in Uttar Pradesh and Uttarakhand, as well as a rare and endangered species in the Himalayan tropical and subtropical climates. The leaves are smooth and leathery in texture and are green oval or elliptic in shape. The fruits are globose, golden, and have 3-6 seeds. The seeds are reddish brown ellipsoid in shape and grow inside the fruit, encased in an orange, red fleshy aril. When seed oil comes into touch with something, it leaves a golden orange stain. Seeds, bark, and leaves are widely used due to their ethnobotanical and traditional significance. Celastrine and paniculatin are alkaloids found in the seed and its oil. Malkanguinol, Malkangunin, and Paniculatadiol are found in 80% of the methanolic extract of seed oil. Wifornine F, Paniculatine A and B, and other alkaloids are abundant in the stem. Alkaloids such as n-triacontanol and pristimerin, benzoic acid, uncharacterized quinine, and golden-yellow oil are found in a petroleum ether extract of *Celastrus paniculatus* root bark. Tannins are present in an ethanolic extract of *Celastrus paniculatus*. Steroids, alkaloids, and dulcitol are abundant in flowers. Seeds are used externally to treat filthy, indolent ulcers and scabies, and are also used internally to treat rheumatism, gout, paralysis, leprosy, and weakness, pruritis, skin illnesses, leukoderma, cephalalgia, arthralgia, asthma, cardiac debility, inflammation, strangury, neuropathy, amenorrhoea, dysmenorrhoea, epilepsy. Oil is used for intellect promoting, shows beneficial effect on the learning and memory process in mentally retarded children; thermogenic and rubefacient, stomachic, tonic, useful in abdominal disorders, enriches blood; powerful stimulant for neuromuscular system. It is used for treating beriberi (vitamin B1) deficiency disease; powerful against malaria; useful in treating ADD, anxiety, "brain fog", etc.; works against cough and asthma; cures headaches and leukoderma. The anti-oxidant capabilities of methanol extracts from seeds and flowers, as well as analgesic and anti-inflammatory characteristics, were dose dependant. Seed oil extract promotes seminiferous tubule vacuolization, germ cell loss, and exfoliation, resulting in anti-fertility properties. Various seed and seed oil extracts showed wound healing, anti-malaria, anti-bacterial, and anti-fungal properties. Triterpenoids were found only in DCM extract, while tannins and fixed oil were also present. In an 8:2 ratio of chloroform: DCM and ethyl acetate: hexane, the maximum number of peaks for DCM extract were achieved. In a 9:1 chloroform: DCM and ethyl

acetate: hexane solution, there were two peaks. All other solvents yielded negative results in chloroform extract, with only one peak in chloroform: DCM (8:2) solution.

REVIEW OF LITERATURE

Celastrus paniculatus is a woody climbing shrub belonging to family Celastraceae (Figure 1). Due to its cognitive boosting characteristics, it is known as Malkagni, Malkakni in Hindi, black oil tree, climbing staff tree in Gujarati, and jyotishmati in Sanskrit. It is native to the Indian subcontinent, but can also be found in Australia, China, Taiwan, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Nepal, Sri Lanka, Thailand, Vietnam, and many Pacific islands. It can be found between 1800 and 2000 meters above sea level in the Himalayan mountains, the western and eastern ghats, and other high altitudes. The Simlipal Biosphere Reserve, the Karlapat Sanctuary, and the Niyamgiri Hills are all rich in tropical forest. *Celastrus* is a critically endangered plant in Uttar Pradesh and Uttarakhand, as well as a rare and endangered species in the Himalayan tropical and subtropical climates. This deciduous vine can reach enormous proportions. The leaves are smooth and leathery in texture and are green oval or elliptic in shape. They alternate between having acute and acuminate apices. Their base is obtuse or rounded, and their edge is toothed. The primary stem can reach a diameter of 10 cm and a length of 6 cm. The bark is abrasive and cracked. The outside bark is pale to reddish brown in colour, while the inner bark is light yellow. Flowers are tiny, measuring 3.8 mm in diameter and ranging in colour from greenish white to yellowish green. The fruits are globose, golden, and have 3-6 seeds. They are pubescent and hermaphrodite. The seeds are reddish brown ellipsoid in shape and grow inside the fruit, encased in an orange, red fleshy aril. They have a strong odour and a bitter flavour. When seed oil comes into touch with something, it leaves a golden orange stain. Seeds, bark, and leaves are widely used due to their ethnobotanical and traditional significance [1,2].

Figure 1: Classification of *Celastrus paniculatus* Wild [1].

KINGDOM	PLANTAE
CLASS	ANGIOSPERMAE
ORDER	CELASTRALES
FAMILY	CELASTRACEAE

GENIUS

CELASTRUS

SPECIES

PANICULATUS

1.1. Phytochemical Constituents

Many minerals, vitamin C, proteins, carbohydrates, and lipids are abundant in the plant (mono and polyunsaturated fats). Celastrine and paniculatin are alkaloids found in the seed and its oil. 20.2 percent normal triglycerides, 44.4 percent triglycerides, 23.5 percent polar non glyceridic ester, and 11 percent non-polar non glyceridic ester make up fatty acid. Palmitic, stearic, oleic, linoleic, and linolenic acid make up the majority of these fatty acid components. Seeds strengthen the intellect and memory, and have a medicinal impact on gout, rheumatism, scabies ulcers, and other ailments. Celapanin, Celapagine, and Celapanigine are among the sesquiterpene alkaloids found in seeds. Malkanguinol, Malkangunin, and Paniculatadiol are found in 80% of the methanolic extract of seed oil. Wifornine F, Paniculatine A and B, and other alkaloids are abundant in the stem. Alkaloids such as n-triacontanol and pristimerin, benzoic acid, uncharacterized quinine, and golden-yellow oil are found in a petroleum ether extract of *Celastrus paniculatus* root bark. In the outer root bark, Zeylaseral, zeylasterone, and celastrol are discovered as quinine, methide, and phenolic triterpenoids. Tannins are present in an ethanolic extract of *Celastrus paniculatus*. Alkaloids, glycosides, and a colouring substance are also abundant in the leaves. Dulcitol is present. Steroids, alkaloids, and dulcitol are abundant in flowers [1,2].

1.2. Extraction methods for oil

Methods of the past: - The seeds are first soaked for several hours in a saucepan of boiling water or goat's milk. Essential oils float to the top of the water table. The seeds are squeezed out, and the rest of the mixture is let to settle. Finally, the oil is sucked into a new container [1].

Modern technique: - A screw press is used to press a large number of seeds. Under the pressure of the press, seed oil is squeezed out. After that, the oil is collected [1].

1.3. Properties of Oil

Protein, carbs, and lipids are all found in seed oil. Fats contain 0.022 percent saturated fat, 0.035 percent polyunsaturated fat, and 0.032 percent monounsaturated fat. It also contains a lot of vitamin C, sodium, potassium, ash, calcium, iron, sesquiterpenes, and polyesters. Even at the maximum dose, it does not induce brain damage or toxic effects. It has been discovered to be beneficial in stimulating memory and increasing cognition. When taken sublingually, 10-15 drops of oil have been shown to have the most effect. It has a maximum shelf life of two years in its raw form. It is best to keep it in a cool, dark place [1].

1.4. Traditional Uses

Many ayurvedic practitioners employ stem leaves and oil to treat a variety of ailments. Seeds have long been used as an anti-inflammatory, diuretic, and remedy for a variety of digestive problems. Men have always used seeds as a sexual stimulant. Many doctors in India advocate starting with 1 seed per day and gradually going to 100 seeds per day if you have dementia. It has been found to be effective both externally and internally in the treatment of rheumatism and gout. Even in retarded youngsters, oil is commonly used to improve memory and learning processes, sharpen memory, and boost intellect. It acts as a potent neuromuscular stimulant. It can also be used to treat vitamin B1 deficiency, asthma, and anxiety. As a daily supplement, a few drops of oil have been ingested as a part of healthy diet [4,5].

Seeds have appetizer, emetic, expectorant, sordific, aphrodisiac, thermogenic, emollient, nervine, stimulant, anti-inflammatory, diuretic, emmenagogue, diaphoretic, febrifuge, digestive, laxative, abdominal disorders, thermogenic, emollient, nervine, stimulant, anti-inflammatory, diuretic, emmenagogue, diaphoretic properties [1].

Seeds are used externally to treat filthy, indolent ulcers and scabies, and are also used internally to treat rheumatism, gout, paralysis, leprosy, and weakness, pruritis, skin illnesses, leukoderma, cephalalgia, arthralgia, asthma, cardiac debility, inflammation, strangury, neuropathy, amenorrhoea, dysmenorrhoea, epilepsy, neuropathy, amenorrhoea, dysmenorrhoea, amenorrhoea, dysmenorrhoea, epilepsy [1].

Oil is used for intellect promoting, shows beneficial effect on the learning and memory process in mentally retarded children; thermogenic and rubefacient, stomachic, tonic, useful in abdominal disorders, enriches blood; powerful stimulant for neuromuscular system. It is used for treating beriberi (vitamin B1) deficiency disease; powerful against malaria; useful in

treating ADD, anxiety, "brain fog", etc.; works against cough and asthma; cures headaches and leukoderma [1].

1.5. Ethnobotanical Study

The methanolic extract of seeds and oil has been found to boost learning and memory capacity as well as brain intelligence. They've been shown to help people recover from mental illness. Sedative and convulsant characteristics are also depicted. The anti-oxidant capabilities of methanol extracts from seeds and flowers, as well as analgesic and anti-inflammatory characteristics, were dose dependant. The use of a 50 percent seed ethanol extract appeared to lower blood cholesterol and LDL cholesterol levels. Petroleum ether and alcohol extract lower the pace of weight loss and paw edema in arthritis sufferers. Seed oil extract promotes seminiferous tubule vacuolization, germ cell loss, and exfoliation, resulting in anti-fertility properties. Various seed and seed oil extracts showed wound healing, anti-malaria, anti-bacterial, and anti-fungal properties [4,5].

OBJECTIVE

To prepare various leaves' extract and carry out the phytochemical analysis and thin layer chromatography.

MATERIALS AND METHODS

2.1. Collection of plant sample

The leaves of *Celastrus paniculatus* was taken from herbal garden of Jaypee University of Information Technology, Solan. The plant sample was collected in the month of April. Healthy leaves were selected. The leaves were washed in distilled water to remove dust particles and were air dried for 2 days. They were then grounded into powder form. For phytochemical analysis water extract was prepared as per standard protocols.

2.2. Preparation of water extract

4g of leaves' powder was dissolved in 200 ml of distilled water. It was then placed on hot plate at 60°C for 20 minutes and continuously stirred. After cooling down the mixture was filtered using Whitman filter paper. The filtrate was then centrifuged at 8000 rpm for 20 min and again filtered using Whitman filter paper. The final filtrate obtained was syringed filtered using 0.2 μ filter. The filtrate was stored at 4°C or used immediately [7].

2.3. Preparation of dichloromethane (DCM) extract

4g of leaves' powder was dissolved in 40 ml of DCM and incubated for 24 hr at room temperature. It was then filtered using Whitman filter paper. 40 ml of DCM was again added and incubated for 24 hr and filtered. This process was repeated until clear DCM solution was obtained. The filtrate was stored at 4°C or used immediately [6].

2.4. Preparation of methanol extract

Maceration of coarsely powdered sample was done in 80% methanol at room temperature for 3 days to obtain hydro-alcoholic crude extract. The marc was filtered and whole process was repeated twice [8].

2.5. Preparation of chloroform extract

4g of leaves' powder was dissolved in 40 ml of chloroform and incubated for 24 hr at room temperature. It was then filtered using Whitman filter paper. 40 ml of DCM was again added and incubated for 24 hr and filtered. This process was repeated until clear DCM solution was obtained. The filtrate was stored at 4°C or used immediately [9].

2.6. Phytochemical Analysis [3]

1. Alkaloids (Mayer's test)- In 1 ml of extract solution add a few drops of Mayer's reagent. A creamy white precipitate is formed.

2. Carbohydrates (Molisch's test)- In 1 ml of extract solution add Molisch's reagent and then slowly add 0.2 ml of concentrated sulfuric along the sides of test tube. Formation of violet to purple ring at the junction indicates the presence of carbohydrates.

3. Glycoside (Legal test)- To 1 ml of solution, add 1 ml of pyridine and few drops of sodium nitroprusside solution. It was made alkaline by adding a few drops of sodium hydroxide. Formation of red to pink colour indicates the presence of glycosides.

4. Sterol and triterpenoids (Salkowski's test)- Chloroform extract of each solution was prepared and then add a few drops of concentrated sulfuric acid, shake well and allow it to rest for some time. Formation of yellow colour in the lower layer indicates the presence of sterols and red colour in triterpenoids

5. Phenol (Ferric chloride test)- Add a few drops of ferric chloride to the extract solution. Formation of blue green colour indicates the presence of phenols.

6. Flavonoids (Alkaline reagent test)- Add a few drops of sodium hydroxide solution to the extract solution, formation of yellow colour and then turning colourless. Addition of few drops of dilute acetic acid indicates the presence of flavonoids.

7. Tannins (Ferric chloride test)- Add a few drops of ferric chloride solution to the extract solution. Formation of blue to green colour indicates the presence of tannins.

8. Fixed oil- A few drops of the extract solution were pressed in between the filter paper. Presence of stain on the filter paper confirms the presence of oil.

9. Gums and mucilage- The extract was added in a test tube containing alcohol with continuous stirring. Formation of precipitate confirms the presence of gums and mucilage.

2.7. Preparation of Thin Layer Chromatography (TLC)

Silica coated TLC plates were taken. Lines were drawn 0.5 cm above and below the edges. A drop of the various was placed on the bottom line with some distance apart. Sample was placed in a such a way that it takes minimum area and was placed in the centre of the line. Two solvents ethyl acetate: hexane and chloroform: DCM was prepared in two different

concentrations- 9:1 and 8:2 in 10 ml. TLC plates were prepared for solution in each concentration. The prepared plates were then placed in the solution and covered with aluminium foil and were kept in it till the solvent reached the upper line. Plates were taken out and left to air dry. Plates were then placed iodine chamber for 5-7 minutes and observed in UV chamber under visible lights. The Rf value of different peaks obtained were calculated [11].

RESULT AND DISCUSSION

3.1. EXTRACT PREPARATION

The extracts were prepared using maceration technique. The plant sample was coarsely powdered and solvent was added until the plant sample was completely submerged. The mixture was left to incubate at room temperature and stirred continuously. Filtration was carried out to separate marc from micelle. Two polar solvents, water and methanol, were selected for the extraction of polar compounds and two non- polar solvents, dichloromethane and chloroform, were chosen for the extraction of non- polar compounds. Chloroform is least polar compound with polarity of 0.259 and water being the highest with a polarity of 1. After the extract preparation, the extract was stored at 4C for further use [10].

3.2. PHYTOCHEMICAL ANALYSIS

Phytochemical screening is also known as chemical screening. It is used to detect the presence of primary and secondary metabolite in various sample. They are used to detect the presence of alkaloids, flavonoids, tannins, etc. in a sample [10]. Following table represents the phytochemical results.

Table 1: Phytochemical results

Compound	Water Extract	DCM extract	Chloroform Extract	Methanol Extract
Alkaloids	+	-	+	+
Carbohydrates	+	-	-	+
Sterol and triterpenoids	+	+	-	+
Phenol	+	-	-	+
Flavonoids	+	-	-	+
Tanin	+	-	+	+
Fixed Oil	+	+	+	+
Gum and mucilage	+	-	-	-
Glycoside	-	-	-	+

Water extract includes all of the components except glycoside, according to phytochemical research. Triterpenoids were found only in DCM extract, while tannins and fixed oil were also present. Alkaloids, tannins, and fixed oil were found in chloroform extract. Except for gums and mucilage, methanol extract was positive for all components, was specific for sterols, and was negative for triterpenoids.

3.3. THIN LAYER CHROMATOGRAPHY (TLC)

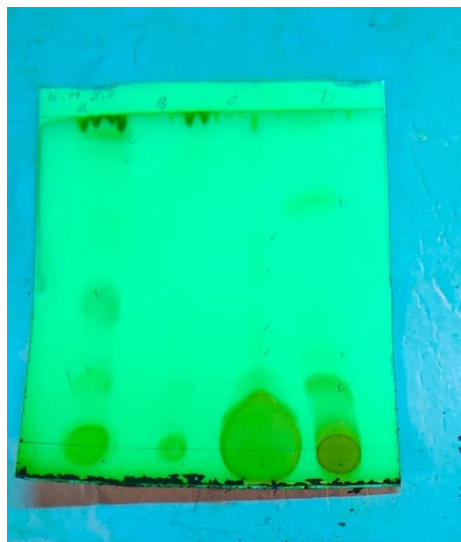
TLC was used to determine the number of components in the respective extract. TLC is primarily used to evaluate the existence of chemical constituents in plant extracts, both qualitatively and quantitatively. R_f value was calculated by dividing the distance carried out by solute to distance varied by solvent of the different peaks obtained [11]. Table 2 summarises the R_f value.

3.3.1. Ethyl Acetate: Hexane (9:1)



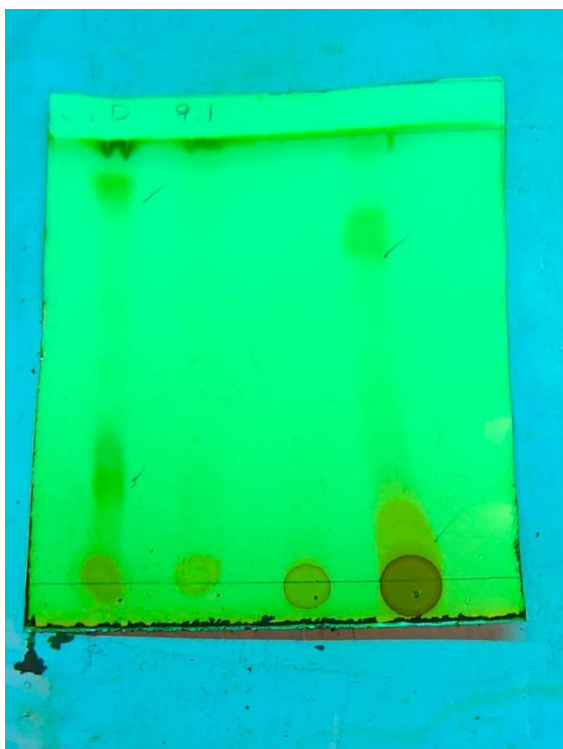
- A- Methanol Extract
- B- Water Extract
- C- DCM Extract
- D- Chloroform Extract

3.3.2. Ethyl Acetate: Hexane (8:2)



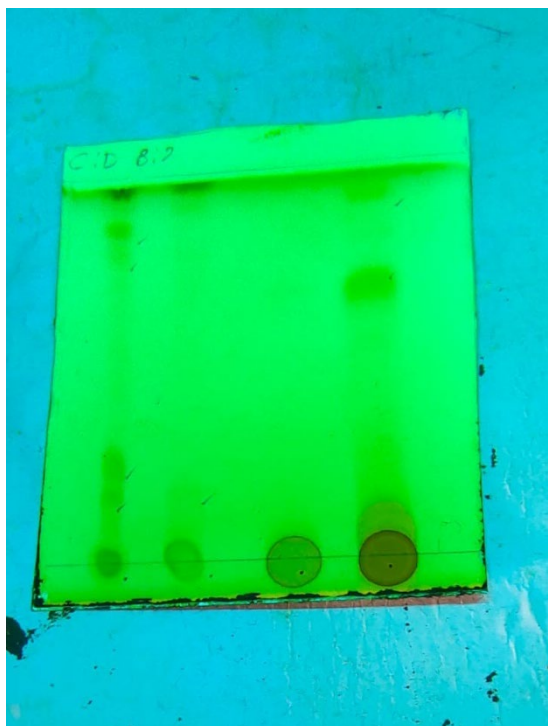
- A- DCM Extract
- B- Chloroform Extract
- C- Water Extract
- D- Methanol Extract

3.3.3. Chloroform: DCM (9:1)



- A- DCM Extract
- B- Chloroform Extract
- C- Water Extract
- D- Methanol Extract

3.3.4. Chloroform: DCM (8:2)



- A- DCM Extract
- B- Chloroform Extract
- C- Water Extract
- D- Methanol Extract

Table 2: Calculation of R_f value

S.No.	DCM Extract			Chloroform Extract			Water Extract			Methanol Extract		
	Solvent	Solute	R_f	Solvent	Solute	R_f	Solvent	Solute	R_f	Solvent	Solute	R_f
Ethyl Acetate: Hexane (9:1)												
1.	3.1	0.2	0.065	3.7	-	-	3.9	0.6	0.154	3.9	-	-
2.	3.1	0.5	0.161	-	-	-	-	-	-	-	-	-
Ethyl Acetate: Hexane (8:2)												
1.	5	1	0.2	5	-	-	5	1.5	0.3	5	0.5	0.1
2.	5	2.5	0.5	-	-	-	5	1.8	0.36	5	1	0.2
3.	5	4.3	0.86	-	-	-	5	2.3	0.46	5	3.8	0.76
4.	5	4.6	0.92	-	-	-	5	2.8	0.56	-	-	-
Chloroform: DCM (9:1)												
1.	5	1.2	0.24	5	-	-	5	-	-	5	0.5	0.1
2.	5	4.5	0.9	-	-	-	-	-	-	5	3.6	0.72

Chloroform: DCM (8:2)												
1.	5	0.8	0.16	5	0.8	-	5	-	-	5	0.7	0.14
2.	5	1.3	0.26	-	-	-	-	-	-	5	3.5	0.7
3.	5	3.9	0.78	-	-	-	-	-	-	5	4.5	0.9
4.	5	4.5	0.9	-	-	-	-	-	-	-	-	-

In an 8:2 ratio of chloroform: DCM and ethyl acetate: hexane, the maximum number of peaks for DCM extract were achieved. In a 9:1 chloroform: DCM and ethyl acetate: hexane solution, there were two peaks. All other solvents yielded negative results in chloroform extract, with only one peak in chloroform: DCM (8:2) solution. Water extract revealed four peaks in ethyl acetate: hexane (8:2) with four peaks in ethyl acetate: hexane (8:2). In other solvents, it demonstrated little to no activity. The activity of the methanol extract was comparable to that of the DCM extract, with the highest activity in the 8:2 chloroform: DCM and ethyl acetate: hexane ratios.

CONCLUSION

Celastrus paniculatus Wild. is a rare and endangered species with a great deal of untapped potential. It can be found in the world's sub-tropical and tropical regions. Jyotishmati is a popular moniker for it because of its cognitive and memory improving abilities. Alkaloids, lipids, carbohydrates, and a variety of vitamins and minerals are the most prevalent phytochemical constituents. Alkaloids, fixed oils, and carbohydrates were found in phytochemical study of diverse extracts. TLC activity was highest in DCM and methanol extracts.

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