# Isolation of Phytoconstituents from Ethanolic Bark Extract of *Terminalia Paniculata* (Combretaceae)

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## Abstract

The Terminalia genus is the second largest in the Combretaceae family. All around the world, species belonging to the genus Terminalia have been utilised in antiquated forms of popular medicine. A brief report on Terminalia paniculata L. (TP) was later made, nevertheless. Comprehensive information regarding Terminalia plants, with an emphasis on TP and its biological and separated components, is presented in this literature review. Tannins, flavonoids, phenolic acids, triterpenes, triterpenoid glycosides, lignan, and lignan derivatives are only a few of the phytochemicals that have been isolated from Terminalia plants through plant-based research. A wide range of actions were documented by extracts and isolated components of several Terminalia plants. Part of TP are polyphenols such flavellagic acid, ellagic acid, and 3,3'-di-O-methyyl ellagic acid. Researchers have uncovered a wide variety of active chemicals in the Terminalia genus. The use of Terminalia in the treatment of many diseases in plant-based medicine has been validated by numerous biochemical potentials. The

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pharmacological mechanisms responsible for bioactive components should be the focus of future research.processes involving pharmaceuticals

Keywords: Iaolation, phytochemistry, Terminalia paniculate, Ethanolic Extracr.

#### 1. Introduction

Medicinal plants have extensive usage in the treatment of illness on a global scale. Over eighty percent of the world's population is interested in traditional plant medicines for medical purposes, according to a World Health Organisation report [1]. To maintain health and to treat, diagnose, or halt illness, conventional medicine incorporates a wide range of practices, including but not limited to: spiritual healing, manual expertise, examination, and medicines derived from plants, animals, or minerals [2]. A campaign called "Saving Plants for Saving Lives" has been supported by the World Health Organisation (WHO). For the simple reason that herbal therapies for health epidemics rely heavily on medicinal herbs [3,4]. The World Health Organisation (WHO) has defined total health as a condition of complete mental, social, emotional, and spiritual wellness as well as the absence of sickness. Stress and unhealthy eating are major contributors to modern health problems including depression, cancer, and heart disease. Due to the increasing prevalence of mental and emotional aspects in these disorders, allopathy is not only unable to treat them but only provides short-term symptom relief. Alternative medicine must be available so that everyone can enjoy good health. If you want to get well, herbal treatment is your best bet [5].

Researchers have used follow-up studies to confirm the legitimacy of an estimated 75% of the biologically active chemicals produced from plants that are currently used globally.

Data from traditional and ethnomedicinal applications. Therefore, there is a lot of room for innovation in medicine stemming from conventional plant medicine [6].

Isolation and characterisation of bioactive substances may be initiated by the ethnobotanical knowledge gathered from traditional herbal practitioners.

Alkaloids, flavonoids, saponins, terpenoids, phenolics, tannins, etc. are considered major components in crude drugs; phytoconstituents are natural bioactive compounds that have the ability to heal and integrate with nutrients and fibres to create an integrated defensive system [6]. Plants and plant extracts are the primary means of medical treatment for more than three quarters of the global population. There was a time when almost 30% of all plant species were used medicinally. An estimated 2,00,000 crores of rupees is the potential global market for pharmaceuticals generated from plants. The current offering in India is below 2000 crores. The amount of crude medicines exported from India rose from 130 crores in 1991–92 to 165 crores in 1994–95, a 26% rise. There is a yearly production value of 200 crores of rupees from medicinal and aromatic plant crude material. With any luck, this will reach \$1,150,000,000 by the year 2000 and \$5 trillion,000,000 by the year 2050 [7]. The phytochemicals found in medicinal plants are diverse. There are various phytochemicals that might cause various ailments. These include alkaloids, flavonoids, tannins, saponins, steroids, phenols, and many more. Medicinal plants contain phytochemicals that have several diseasepreventing actions, including alkaloids, tannins, saponins, flavonoids, phenols, steroids, carotenoids, and so on [8]. The anti-inflammatory, anti-aging, and antidiabetic effects of these plant-based chemical compounds are significant.

Antibacterial, wound healing, antioxidant, antidepressant, and anticancer. The majority of therapeutic plants include flavonoids, which are bioactive compounds. Antimicrobial, antioxidant, anticancer, wound-healing, and anti-inflammatory are just a few of their many disease-prevention functions in humans [8]. The author of this dissertation has chosen a significant medicinal plant that is both widely available and still utilised by rural people today to cure a wide range of illnesses and disorders. A



scant amount of phytochemical data pertaining to the chosen plant could be found in published works. Chemical components will be isolated, purified, and their structures will be elucidated in order to assess their biological activity. One of the plants chosen is Terminalia paniculata Roth, which belongs to the Combretaceae family. One species of tropical tree is Terminalia paniculata. Originating in Africa and Australia as well as the West Indies and Bermuda. It inhabits the semi-evergreen and damp deciduous woodlands of India's western and eastern ghats. Its distribution includes peninsular India, West Bengal, Bihar, Odisha, and Andhra Pradesh [9–11]. A large deciduous tree that grows between 20 and 30 metres tall with a clear bole about 10 metres in diameter and flaking brown to dark brown bark. The tree's leaves are simple, opposite, upper and lower, oblong or elliptic, acute or acuminate, pale brown, and there are two glands near the base of the midrib below. The tree has 10-15 pairs of main nerves. The flowers are reddish brown, sessile, and in rusty pubescent spikes. The fruits are reddish brown-winged, with one wing broad and the other two narrow [9,10].



Fig. 1. Photographs of Terminalia paniculata Roth.

## Plant materials:

The fresh plant (bark) materials of *Terminalia paniculata* was collected during December 2023 from rural areas of Nainital forest area, Uttarakhand, India.

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**Drugs and chemicals used:** Standard manufacturers supplied all of the medications and chemicals employed in this dissertation, and they were all of analytical grade or laboratory grade. The following ingredients are needed: Mayer's reagent, Wagner's reagent, Dragendorff's reagent, Hager's reagent, hydrochloric acid, sodium hydroxide, chloroform, acetic anhydride, metallic tin, lead acetate, Millon's reagent, sodium bicarbonate, hydrogen cyanide, fresh-prepared  $\alpha$ -naphthol (5%) in ethanol, 0.2% anthrone solution in concentrated H2SO4.

Fehling's solution A: Bring 35 grammes of CuSO4.5H2O to a dissolved state in 500 millilitres of water. B. Fehling's solution: combine 120 g of sodium hydroxide and 173 g of sodium potassium tartrate (Rochelle salts) in 500 ml of water.

#### **Benedict's reagents:**

**Apparatus:** A number of items are needed: a test tube, a measuring cylinder, a conical flask, a beaker, a petridish, a pipette, a thin-layer chromatography plate, a unit for distillation, a UV chamber, a glass column, a hot plate, and a hot air oven.

First, dissolve 173 grammes of sodium citrate and 100 grammes of anhydrous Na2CO3 in 600 millilitres of boiling water; this is Reagent No. 1. Prepare a dilution by adding 800 cc of distilled water.

Separate 17.3 grammes of CuSO4.5H2O into 100 millilitres of boiling water; this is Reagent No. 2. Before diluting to 100 ml with distilled water, let it cool.

Reagents No. 3, combine the Reagents No. 1–2 in a 100 ml volume of dissolving water while stirring constantly.

#### **Preliminary Phytochemical Examination of Bark Extract:**

Plants provide humans with the primary macronutrients (carbs, proteins, and fats) in their diet. Alkaloids, glycosides, volatile oils, saponins, and many more compounds have physiological effects. Consequently, the plant serves as a veritable biosynthetic laboratory. For medicinal benefits, look no further than the molecules known as secondary metabolites. Primary and secondary metabolites can be synthesised by plants through metabolic processes. A preliminary phytochemical screening of the plant material can be performed to identify different plant components [25-27].

#### 1. Alkaloids tests:

- 1.36 grammes of mercuric chloride dissolved in 60 millilitres of water is Mayer's reagent. A solution of distilled water. Combine 5 grammes of potassium iodide with 60 millilitres of distilled water (b). Combine (a) and (b), then add 100 millilitres of distilled water to make the final volume. The precipitate that results from reacting with alkaloids is white or buff in colour.
- a) Wagner's reagent: In 5 millilitres of water dissolve 1.27 grammes of iodine and 2 grammes of potassium iodide. Add 200 millilitres of distilled water to produce the total volume. Using alkaloids with Wagner's reagent results in a precipitate that is reddish-brown in colour.
- c) Dragendorff's reagent: 14 grammes of sodium iodide, 5.2 grammes of bismuth carbonate, 50 millilitres of glacial acetic acid, and a brief boil. remained overnight and strained the sodium acetate crystal precipitate. A bottle of amber-colored stoke solution. When needed, dilute this stock solution to 100 ml with water by adding 20 ml of acetic acid to 10 ml. It creates a precipitate that is orange-brown when combined with alkaloids.

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d) Hager's reagent: To detect alkaloids, it is employed a saturated aqueous solution of picric acid. The crystalline precipitate it produces contains a high concentration of alkaloids.

Second, check for glycosides.

## 2. Find cardiac glycosides via testing:

a) Keller-Kiliani test: an aqueous drug extract in glacial acetic acid is mixed with a few drops of ferric chloride and concentrated sulfuric acid. The upper layer becomes bluish-green, and a reddish-brown hue is produced at the layer junction. Cardiac glycosides containing digitoxose as the glycone moiety are confirmed by the test.

Analyse for glycosides of anthraquinones:

a) In the Borntrager's test, the powdered medication is boiled with 5 millilitres of 10% sulfuric acid for 2 minutes.

Once filtered, allow the filtrate to cool. Then, add an equal volume of benzene and gently mix. Please allow the benzene layer fully separate from the bottom layer. Extract the benzene layer using a pipette and place it in a fresh test tube. Combine approximately 50% of its volume with a 10% aqueous ammonia solution. The solution was kept separate after gentle shaking. When free anthraquinones are present, the bottom ammoniacal layer takes on a pinkish-red hue.

b) A variant of Borntrager's analysis: Because anthraquinone C-glycoside hydrolysis requires more extreme conditions, a variant of the preceding test is employed. Hydrochloric acid and ferric chloride both influence oxidative hydrolysis. After boiling 0.1 g of the medication with 5 ml of diluted hydrochloric acid and 5 ml of a ferric chloride solution at 5% for five minutes, the mixture is cooled and filtered. Benzene is used to shake this filtrate. Cut the benzene layer in half and mix in the same amount of diluted ammonia. This layer of ammonia is coloured pinkish-red.

## 3. Carbohydrate testing:

- a) The Molisch test involves adding a 10% alcoholic solution of alpha-naphthol to a test tube containing an aqueous or alcoholic solution of the drug. After giving the mixture a good shake, add a few drops of concentrated sulfuric acid to the side of the test tube. Carbohydrates could be the reason why a violet ring forms when two liquids mix.
- b) Flehling's test: In a mixing tube, combine 2 millilitres of Fehling's solution A and 2 millilitres of Fehling's solution B. Then, boil 2 millilitres of liquid extract. A yellowish or brickred red precipitate forms when reducing sugar is present.
- c) Benedict's test: In a test tube, combine 3 millilitres of test solution with 5 millilitres of Benedict's reagent. Boil the mixture over a water bath. Monosaccharides are present when a brick-red precipitate forms at the bottom of the test tube.

## 4. Check for mucilages and gums:

- a) Since they are insoluble in alcohol, gums and mucilages precipitate when 95% alcohol is added.
- b) For Molisch's test, mix a 10% alcoholic alpha-naphthol solution with an aqueous or alcoholic substance solution in a test tube. Give it a good shake, and then add a few drops of concentrated sulfuric acid to the side of the test tube. The presence of carbohydrates, gums, and mucilages can be confirmed by the creation of a violet ring at the confluence of two liquids.

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#### 5. Protein and amino acid testing:

- a) The biuret test uses 2 millilitres of extract, 2 millilitres of 10% NaOH solution, and 2 to 3 drops of 1% CuSO4 solution, all of which are mixed together. If the sample turns a violet or purple colour, it means it contains proteins.
- b) Ninhydrin assay: Mix 0.5 ml of ninhydrin solution with 2 ml of extract. Bring to a boil and then let cool. The presence of protein is shown by the production of blue colour.
- c) Xanthoproteic test: 2 millilitres of extract, 1 millilitre of concentrated hydrochloric acid, boil, cool, and 40% NaOH, drop by drop. The presence of proteins is shown by the coloured solution's appearance.
- d) The Millon's test: 2 millilitres of extract with 2 millilitres of Millon's reagent, boiled, cooled, and then a few drops of NaNO2 solution. Proteins are present when a red precipitate or colouring is visible.

## 6. Analyse for phenolic compounds and tannins:

- a) The ferric chloride test can be used to detect phenols; it involves mixing 90% alcohol with a 5% W/V solution of ferric chloride.
- b) The tannin test, which involves precipitating tannins with lead acetate, is one method.
- c) Gelatin solution test: mix 0.5–1% tannins with 1% gelatin and 10% sodium chloride in an aqueous solution. Precipitation of a white to buff hue is produced.

## 7. Identify steroid and sterol levels:

- a) The Liebmann Burchard reagent consists of 2 millilitres of acetic anhydride and 2-3 drops of concentrated hydrochloric acid added to approximately 2 millilitres of chloroform-extracted solution in a dry test tube. Blend and wait a few minutes. The presence of steroids or sterois causes a shade of emerald green to appear.
- b) Salkowski's test: Pour 5 ml of a chloroform extract solution into a dry test tube. Carefully add an equal volume of concentrated sulfuric acid, along the edges of the tube. Look at the acid layer on the bottom and the chloroform layer on top. A greenish-yellow light appears on the acid layer. The chloroform layer causes a colour shift from bluish-red to a more subtle violet-red.

#### 8. Triterpenoids test

a) The Noller's test involves dissolving the extract in chloroform and adding a piece of metallic tin to a test tube. Include a single drop of thionyl chloride. The presence of triterpenoids is indicated by the development of a pink colour.

# 9. Saponin testing:

- a) Foam test: dissolve 1 millilitre of alcoholic extract in 10 millilitres of distilled water by shaking the mixture for 15 minutes. Set aside. Saponins are present when a layer of foam forms, which is approximately one centimetre thick and happens after standing.
- b) If saponins are present, the hemolysis test will turn out positive (3 drops of blood + 1 drop of extract).

#### 10. Flavonoid test:

a) The sodium hydroxide test can be used to determine the presence of flavonoids. After dissolving the extract in water, the filtrate is treated with sodium hydroxide and a yellow colour is noticed.

As a sulphuric acid test, the yellow colour is removed when a drop of concentrated sulphuric acid is added to the mixture.

Table 2 Preliminary phytochemical screening of ethanolic extract of *Terminaliapaniculata*.

Alkaloids	-
Glycosides	-
Carbohydrates	+
Gums and mucilages	+
Proteins and amino acids	+
Tannins and phenolic compounds	+
Steroids and sterols	-
Triterpenoids	+
Saponins	-
Flavonoids	+

(+): Present; (-): Absent.

## 2. Results And Discussion

Table 2 shows that ethanolic leaf extracts of Terminalia paniculata contained carbs, tannins, phenolic compounds, terpenoids, and flavonoids, as revealed by the preliminary phytochemical screening.

**Characterization of isolated compound:** We will isolate and purify the chemicals using chromatographic techniques like thin-layer chromatography (TLC) and column chromatography. The next step is to use mass spectrometry, nuclear magnetic resonance, ultraviolet light, and infrared light to evaluate their spectra.

#### **Examination of the Ethanolic Extract:**

The Liebermann-Burchard reaction was negative and the ferric chloride and Shinoda assays for flavonoids were both positive in the ethanolic extract of Terminalia paniculata. Two distinct spots (solvent system: ethyl acetate: ethanol -1:0.25) were seen upon TLC analysis of silica gel (60-120 mesh particle size) after heating and spraying with 5% alcoholic H2SO4. The remaining ethanol was analysed using column chromatography on silica gel (Merck). The volume was measured in millilitres. The chromatogram's trajectory is detailed in table 2.

Table 3: Chromatography of the ethanolic extract of *T. paniculata*.

Eluent	Fractions	Compound
Benzene	1-4	Waxy

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Benzene: Chloroform (90:10)	5-11	Waxy
Benzene: Chloroform (80:20)	12-16	Waxy
Benzene: Chloroform (70:30)	17-22	Waxy
Benzene: Chloroform (60:40)	23-27	Intractable gum
Benzene: Chloroform (50:50)	28-33	Intractable gum
Benzene: Chloroform (40:60)	34-38	Intractable gum
Benzene: Chloroform (30:70)	39-42	Intractable gum
Benzene: Chloroform (20:80)	43-47	Intractable gum
Benzene: Chloroform (10:90)	48-52	Intractable gum
Chloroform	53-57	Intractable gum
Chloroform: Ethanol (90:10)	58-61	Intractable gum
Chloroform: Ethanol (80:20)	62-66	Intractable gum
Chloroform: Ethanol (70:30)	67-71	Intractable gum
Chloroform: Ethanol (60:40)	72-77	Intractable gum
Chloroform: Ethanol (50:50)	78-83	Intractable gum
Chloroform: Ethanol (40:60)	84-87	Intractable gum
Chloroform: Ethanol (30:70)	88-95	Compound-01 (PS 01)
Chloroform: Ethanol (20:80)	96-101	Intractable gum
Chloroform: Ethanol (10:90)	102-107	Compound-02 (PS 02)
Ethanol	108-116	Intractable gum

# **Examination of the fraction (ethanolic extract):**

#### Fractions 1-87

Fractions 1 to 87 were found to be waxy and gummy and were not pursued further.

# **PS-01** (3, 3',4', 5, 7-pentahydroxy flavone):

Fractions 88 to 95 were found to be similar and showed a single spot. Thus they were combined as they were similar in TLC and crystallized from acetone. On TLC, a purple spot was observed under UV light and darkened when exposed to ammonia. The compound had UV absorption maxima at 275 nm

# Characterization of Isolated compound (PS-01):

The isolated compound (PS-01) was characterized by using physical properties, chemical test, Rf value, and spectral data particularly IR,1H NMR,13C NMR, and Mass spectroscopy.

## **Physical properties:**

The purified compound (PS-01) was solid powder and yellowish in color.

#### **Chemical test:**

The qualitative chemical tests of purified compound (PS-01) gave positive ferric chlorideand Shinoda tests for flavonoid.

## **Melting point:**

The melting point of the isolated compound (PS-01) was 282-284 °C by using the melting point apparatus.

**Thin-layer Chromatographic (TLC) method:** The isolated PS-01 molecule had an Rf value of 0.73 when measured using a solvent system consisting of chloroform and ethanol in a ratio of 8:2.

# **Spectroscopic methods:**

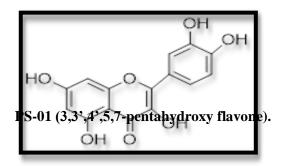
Infrared (KBr, 2max/cm-1):

In the infrared (v cm-1) spectrum of the compound PS-01, there were absorption bands at 3404.73 (str, Alcoholic OH at C-3), 3316 (str, =C-H of Aromatic ring), 1665.78 (str,  $\gamma$ -lactone 6 membered ring), 1562.21 (str, C=O of ketone in lactone ring at C-4), 1520.92 (str, non-conjugated C=C at C2 and C3), and 1382.16 (str, Phenolic OH at C9, C10, C13, Cr14).(IH-NMR) with DMSO-d6, 500 MHz, and  $\delta$  ppm:

The 1H-NMR spectrum of compound PS-01 showed distinct signals at  $\delta$ H12.48 (s, 1H, -OH at C3), 6.94 (s, 1H, Ar-H at C7), 6.93 (s, 1H, Ar-H at C9), 6.89-6.88 (d, Ar-H at C15), 6.47 (s, 1H, Ar-H at C12), 6.41-6.40 (d, Ar-H at C16), 5.52 (s, 1H, Phenolic OH at C9), 5.42 (s, 1H, Phenolic OH at C8), 5.11 (s, 1H, Phenolic OH at C13), and 5.04 (s, 1H, Phenolic OH at C14). An I3C-NMR experiment was conducted using DMSO-d6, 500 MHz, and  $\delta$  ppm.

Typical signals at δC 175.91 (C4), 163.95 (C8), 160.80 (C10), 156.22 (C16), 147.75 (C14), 146.87 (C2), 145.12 (C13), 122.05 (C12), 120.08 (C3), 155.69 (C11), 115.14 (C5), 103.10 (C9), 98.27 (C7), and 93.44 (C6) were seen in the 13C-NMR spectra of compound (PS-01).

**Mass spectroscopy:** The mass data showed that the chemical formula is C15H11O7+, with a mole ratio of 303.15 (M+H). Peaks at (m/z) 303.15 (M+H, 100%, C15H11O7+), 304.05 (M+2H, 18%, C15H12O7+2), and 305.05 (M+3H, 3%, C15H13O7+2) were seen in the mass data (ESI-MS) of (PS-01) molecule.



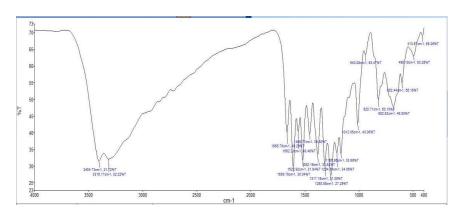


Figure 1: IR spectra of the 3,3',4',5,7-pentahydroxy flavone molecule PS-01.

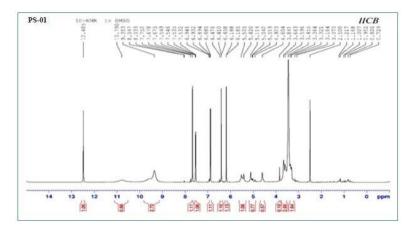


Figure 2. Compound (PS-01) (3,3',4',5,7-pentahydroxy flavone) 1H NMR spectra.

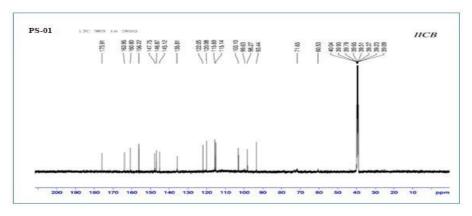


Figure: 3. The chemical's 13C NMR spectrum is (PS-01) (3,3',4',5,7-pentahydroxy flavone).

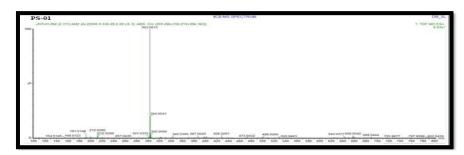


Figure 4. Compound (PS-01) (3,3',4',5,7-pentahydroxy flavone) mass spectra.

**PS-02 (3', 3, 6, 7-tetramethoxy -4', 5, 8-trihydroxy flavones**): Similarities and a single location were observed in fractions 102–107. Since they crystallized from ethyl acetate and showed similarity on TLC, they were so combined. If you look closely at the TLC, you can see a purple spot that was darker when exposed to ammonia and UV light. Particularly at 265 nm, the chemical exhibited the maximum ultraviolet absorption.

Characterization of Isolated compound (PS-02): Physical and chemical tests, as well as spectrum data (IR, 1H NMR, 13C NMR, and mass spectroscopy), were used to characterise the isolated compound (PS-02).

**Physical properties:** The substance that had been refined, known as PS-02, was a white, solid powder.

**Chemical test:** Positive results for flavonoids were obtained from the ferric chloride and shinoda qualitative chemical tests of the purified substance (PS-02).

**Melting point:** The melting point device was used to determine that the isolated chemical (PS-02) has a melting point ranging from 178 to  $180^{0}$  C.

**Thin layer Chromatographic (TLC) method:** The isolated chemical PS-02 has an Rf value of 0.40 when measured using a solvent system consisting of a 1:2 ratio of chloroform to ethanol.

## **Spectroscopic methods:**

**IR** (**KBr**,  $v_{\text{max}}$ /**cm**<sup>-1</sup>): The compound PS-02's infrared (v cm-1) spectra revealed absorption bands at 3608.9 to 3315.7 (O-H, free hydroxyl group), 2953.1 (Cyclic C-H, str), 2866.3 (Ali- C-H, str), 1660.7 (C=O), 1500.0-1400.3 (C-C ring stretch), 1284.6-1193.8 (C-C stretching), 1114.8-997.2 (O-H, out of plane bend), and 786.9-709.8 (monosubstituted in aromatic ring.

<sup>I</sup>H-NMR (DMSO-d<sub>6</sub>, 500 MHz, δ ppm): The distinctive signals at δH 7.80 (OH-6, s), 7.30 (OH-5, s), 6.67 (OH-4', s), 5.89 (H-5', s), 4.29 (H-2', s), 4.17 (OCH3-3, dd, H-6'), 3.85 (OCH3-3', t), 3.14 (OCH3-6, s), and 2.37 (OCH3-5) were shown in the 1H-NMR spectrum of compound PS-02.

<sup>13</sup>C-NMR (DMSO-d<sub>6</sub>, 500 MHz,  $\delta$  ppm): The compound PS-02's 13C-NMR spectra showed distinct signals at chemical shifts of 2-77.37, 3-126.99, 4-123, 5-135.55, 6-140.11, 7-145.84, 8-105.20, 3'-148.77, 1'-100.62, 2'-77.00, 5'-76.57, and 6'-64.21.

**Mass spectroscopy**: Chemical formula C19H18O9 was indicated by the mass data, which showed m/z = 390 (100) [M+]. The chemical, designated as PS-02, is a flavone that is 3',3,6,7-tetramethoxy -

# 4',5,8-trihydroxy.

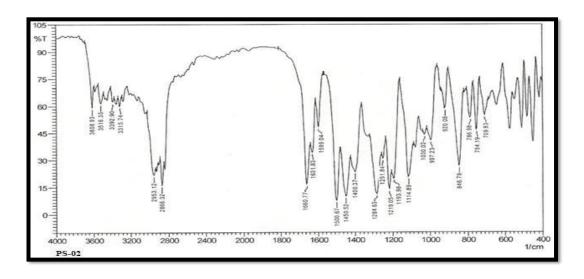


Figure 5: (PS-02) 3',3,6,7-tetramethoxy -4',5,8-trihydroxy flavone's infrared spectra.

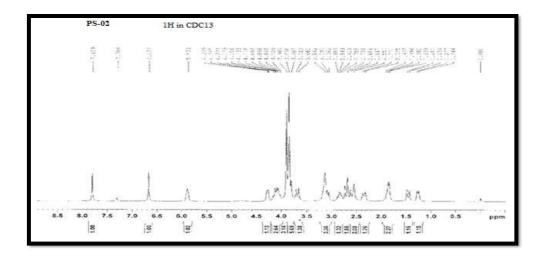


Figure 6: 13C NMR Formulation of the compound: (PS-02) 3',3,6,7-tetramethoxy -4',5,8-trihydroxy flavone.

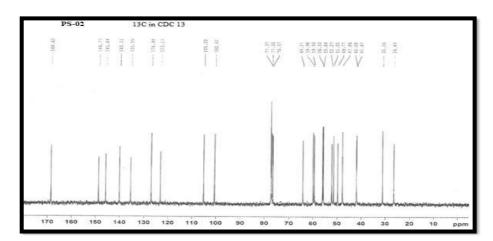


Figure 7: 1H NMR Formulation of the compound: (PS-02) 3',3,6,7-tetramethoxy -4',5,8-trihydroxy flavone.

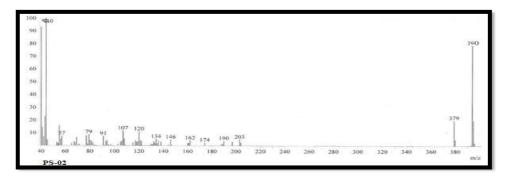


Figure 8: (PS-02) 3',3,6,7-tetramethoxy -4',5,8-trihydroxy flavone Mass spectral ratio.

## 3. Conclusion

Once the ethanolic extract of Terminalia paniculata barks was purified and analysed using column chromatography, two compounds were identified: 3,3',4',5,7-pentahydroxy flavone (PS-01) and 3',3,6,7-tetramethoxy -4',5,8-trihydroxy flavones (PS-02). To describe the chemicals, scientists consulted spectral data and chemical tests. Spectrum data and chemical testing revealed two compounds in the ethanolic bark extract of Croton bonplandianum: 3,3',4',5,7-pentahydroxy flavone (PS-01) and 3',3,6,7-tetramethoxy -4',5,8-trihydroxy flavones (PS-02).

#### 4. References

- [1] Farnsworth NR, Akerele O, Bingle AS, Soejarto DD, Guo ZG. Medicinal plants in therapy.
- [2] Bulletin of the World Health Organisation. 1985; 63: 965-981.
- [3] WHO. General guidelines for methodologies on research and evaluation of traditional medicine, World Health Organization, Geneva. 2000; 55-56.
- [4] WHO Fact Sheet No.134, Revised May 2003.

Health Perspect. 1999; 107 (10): 783-789.

[5] Anonymous, Ancient-modern concordance in Ayurvedic plants: some examples. Environ

- [6] Narayana D, Baba BA, Katiyar B. Herbal remedies through GMP / HACCP Technique, The Eastern Pharmacist, 1997; 477: 21.
- [7] Philipson JD, Anderson LA. Ethnopharmacology and Phytomedicine. Journal of Ethnopharmacology. 1989; 25: 61-72.
- [8] Jirge SS. Tatke PA. Gabhe S. Development and validation of a novel HPTLC method for simultaneous estimation of beta-sitosterol-d-glucoside and Withaferin A. Int J Pharm Pharmaceut Sci. 2011; 3(Suppl 2): 227-230.
- [9] Md. Asaduzzaman, Asao T. Phytochemical- source of antioxidant and role in disease prevention. Published: November 5th 2018
- [10] Narayan DP. Purohit SS. Arun KS. Tarun K. A handbook of Medicinal Plants. Agrobios publishers. 2003; 509.
- [11] Nair RV. Indian Medicinal Plants. Orient longman private limited. 1996; 5: 277-278.
- [12] Chopra RN. Nayer SL. Chopra IC. Glossary of Indian Medicinal Plants. 242.
- [13] Yesodharan K, Sujana KA. Ethnomedicinal knowledge among Malamalasar tribe of Parambikulam wildlife sanctuary, Kerala. Indian Journal of Traditional Knowledge. 2007; 6(3): 481-485.
- [14] Cock IE. The medicinal properties and phytochemistry of plants of the genus Terminalia (Combretaceae). Inflammo pharmacology. 2015; 1-28.
- [15] Srinivasan R, Dhanalekshmi UM, Gowri T, Duarah S. Terminalia paniculata bark extract for antidiabetic activity. IJPSR. 2016; 7(3): 1331-1337.
- [16] Eesha BR, Mohanbabu Amberkar V, Meena Kumari K, Sarath B, Vijay M, Lalit M. Hepatoprotective activity of Terminalia paniculataagainst paracetamol induced hepatocellular damage in Wistar albino rats. Asian Pac J Trop Med. 2011; 4: 466-469.
- [17] Ramachandran S, Rajasekaran A, Manisenthilkumar KT. Investigation of hyperglycemic, hyperlipidemic and antioxidant activity of aqueous extract of Terminalia paniculata bark in diabetic rats. Asian Pac J Trop Biomed. 2012; 2(4): 262-268.
- [18] Rajasekhar K, Venkata Raju RR. Phytochemical, Antioxidant and Antimicrobial Studies of Terminalia paniculata- A Potential Medicinal Plant. IJPPR. 2016; 8 (1): 14-17.
- [19] Ramgopal Mopuri, BalajiMeriga. In vitroanti oxidant activity and acute oral toxicity of Terminalia paniculatabark ethanolic extract on Sprague Dawley rats. Asian Pac J Trop Biomed. 2014; 4(4): 294-298.
- [20] Agrawal S, Kulkarni GT, Sharma VN. Acomparative study on the antioxidant activity of methanolic extracts of Terminalia panicualta and Madhuca longifolia. Free Radicals and Antioxidents. 2011; 1(4): 62-68.
- [21] Chaitra Narayan L, Ravishankar Rai V. Anti-HIV-1 Activity of Ellagic acid Isolated from Terminalia paniculata. Free Radicals and Antioxidants. 2016; 6 (1): 101-108.

ISSN: 1001-4055 Vol. 44 No. 6 (2023)

- [22] Ankita Durge, Pratiksha Jadaun, Ashish Wadhwani, Ashish A. Acetone and methanol fruit extracts of Terminalia paniculata inhibit HIV-1 infection in vitro, Natural Product Research. 2016; 1-4.
- [23] Sahil Talwar, Hitesh V Jagani, Pawan G Nayak, Nitesh Kumar. Toxicological evaluation of Terminalia paniculata bark extract and its protective effect against CCl4-induced liver injury in rodents. BMC Complementary and Alternative Medicine. 2013; 13(127): 1-11.
- [24] Fahmy NM, Al-Sayed E, Singab AN. Genus Terminalia: A phytochemical and Biological Review. (Montin.) Species. Med Aromat Plants. 2015; 4(5): 1-21.
- [25] Row LR, Raju RR. Chemistry of Terminalia species-XI: Isolation of 3,4,3'-O-trimethyl flavellagic acid from Terminalia paniculata Roth. Tetrahedron. 23: 879-884.
- [26] Kokate C.K., Purohit A.P. and Gokhale S.B. Pharmacognosy, Nirali Prakashan, Pune, 29th Edn., 2004;108-109.
- [27] Mukherjee P.K. Quality Control Herbal Drugs. Business Horizons Pharmaceutical Publishers, New Delhi, 1st Edn., 2002; 246-379.
- [28] Harborne J.B. Phytochemical Methods: A Guide to Modern techniques of Plant analysis. Chapman and Hall, New York, 1984; 37-214.