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Phytochemical and pharmacological investigations of *Graptophyllum pictum* leaves

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Abstract
The search for novel, effective anti-diabetic medications with fewer side effects and less costs has switched to medicinal plants. The dried powder of leaves of *Graptophyllum pictum* was subjected to physicochemical evaluations i.e. moisture content was found to be 14. 56% and foreign matter content & swelling index were nil. The cold extractive values of leaves obtained from different media i.e. petroleum ether, chloroform, ethyl acetate, alcohol & water were found to be 0.44, 2, 2.9, 3.37 & 4.73% respectively. The obtained results showed that total, acid-insoluble, water-soluble and sulfated ash values were 1, 1.7, 18.2 & 19.1% respectively. The extractive values of leaves were found to be 1.9 & 7.5% for petroleum ether and ethanolic solvent respectively. TLC analysis of ethanolic extract was performed with different solvents i.e. $CHCl_3$:CH_3OH (5:1), $CHCl_3$:CH_3OH (5:2), C_6H_6:$CHCl_3$:CH_3OH (6:4:0.5) (v/v). The calculated R_f values ranged from 0.16-0.80. Chemical investigation of benzene fraction, obtained from ethanolic extract of leaves of G. pictum led to the isolation of two fatty acid esters namely n-octanyl capriate and n-decanyl cetoleate. GC-MS analysis of petroleum ether extract identified eight phytoconstituents namely Phytol, Tetracosane, Tetratertacontane, Tocopherol, Squalene, Vitamin E, Tetratriacontane and Stigmasterol. Administration of the extract at doses 250mg/kg and 500 mg/kg caused significant reduction in SGPT, SGOT, cholesterol and triglyceride levels. These investigations revealed that the extract possess potent antidiabetic activity.

Keywords: *Graptophyllum pictum*, extract, phytoconstituents, Antidiabetic activity

Introduction
Herbal medicine has evolved alongside the advancement of science and technology to become an alternative medicine. It has lately acquired popularity due to its increasing safety, effectiveness, and efficiency. According to the World Health Organization (WHO), up to four billion people (about 80% of the world's population) in poor nations rely on herbal medicines for basic health care [1].

Graptophyllum pictum (L.) Griff. (Family-Acanthaceae) is a tropical evergreen shrub with oval to elliptic leaves (up to 6 inches long) that are deep green and varyingly blotched with cream along the midveins. The Caricature plant is indigenous to New Guinea and its nearby islands, although it is also found in India, Mexico, the United States, Ghana, and Bolivia. *G. pictum* was traditionally used as folk medicine to improve fertility, as a poultice for cuts, wounds, and all types of swellings, and to cure ulcers, abscesses, hemorrhoids, constipation, rheumatism, urinary infections, scabies, hepatomegaly, and ear ailments. It possesses anti-inflammatory, anti-plaque, and anti-diabetic properties. Here updated Phytochemistry and pharmacological activity of G. Pictum was presented. Furthermore, the current information, derived primarily from experimental investigations, was critically evaluated to give proof and justification for local traditional applications of G. Pictum, as well as to propose future research possibilities and prospective therapeutic uses for this plant [2, 3].

Methodology
Collection of Plant: The fresh plant was collected from Krishnendra Nursery, Lalbagh, and Bangalore, India

Identification and Authentication of Plant
The plant was identified and authenticated by Dr. Anjula Pandey, Pusa campus, National Bureau of Plant and Genetic Resources (NBPGR), New Delhi. The voucher specimen no. (NHCP/NBPGR/2015-2) was preserved in NBPGR and also deposited in TMCOP, TMU, and Moradabad.

Drying and Size Reduction
The plant material (leaves) was dried under shade, mechanically reduced to a moderately coarse powder, and stored in an airtight container. The coarse powder of plant drug was used for extraction and determination of physiochemical parameters and phytochemical studies.

Physicochemical Studies
Foreign Matter Determination
Weigh and spread out 100-500 g of the drug sample to be analyzed or the minimal quantity recommended in the monograph in a thin layer. The foreign substance should be noticed with the naked eye or with the use of a lens (6X). Separate, weigh, and compute the percentage present.

Determination of Moisture (Loss on Drying)
Weigh 1.5g of the powdered medication into a flat and thin Porcelain dish. Dry it in an oven at 100°C or 105°C. Cool in desiccators and monitor weight loss, which is commonly reported as moisture.

Determination of Swelling Factor
The husk was weighed and placed in a graduated cylinder, where its volume (V_1) was measured. A sufficient amount of filtered water was poured into the graduated cylinder containing the PO husk and allowed to stand for 3 hours. The volume of the swelled PO husk was measured (V_2), and the swelling index was computed as follows.

Swelling index = $[(V_2 - V_1)/V_1] \times 100$, where V_1 is the volume of the dry husk and V_2 is the volume of the swollen husk. The swelling index was computed in triplicate, along with the mean and standard deviation.

Determination of Extractive Values
Extraction values are important for identifying crude pharmaceuticals and providing information about the nature of the chemical ingredients present. The plant drug samples were determined by using different solvents i.e. petroleum ether, chloroform, ethyl acetate, ethanol, and water.

Determination of alcohol soluble extractive value
About 5gms of air dried coarse powdered medication was weighed and macerated in a closed flask for 24 hours with 100ml of 90%alcohol, shaking often during the first 6 hours, then standing for 18 hours. Following that, it was quickly filtered in order to avoid solvent loss. In a tarred flat bottomed swallowed dish, 25ml of the filtrate was evaporated to dryness, dried at 105°C, and weighed. The percentage of alcohol soluble extractive values was obtained using the air-dried medication. Similar procedure was applied for determination of petroleum ether, chloroform, ethyl acetate and water-soluble extractive values.

Ash Values
- **Total Ash:** A tarred silicon crucible was filled with approximately 2gm of powdered medication. The crucible was cooled and weighed after being incinerated at 450°C in a muffle furnace. The percentage of total ash was estimated using air-dried material.
- **Acid Insoluble Ash:** The ash produced from the whole ash was heated for a few minutes in 25ml of 2N HCl. Filtered with ash-free filter paper. The filter paper was placed in a tarred silica crucible. Incinerated at 450°C in a muffle furnace until carbon-free. The crucible was weighed and cooled. The percentage of acid insoluble ash was determined using air-dried material.
- **Water Soluble Ash:** The ash collected from the total ash was heated for a few minutes in 25ml of distilled water and filtered via ash-free filter paper. The filter paper was placed in a tarred silica crucible. The crucible was cooled and weighed after being incinerated at 450°C in a muffle furnace. The percentage of water-soluble ash was estimated using an air-dried material.
- **Sulfated Ash:** Two grams of sample were placed in a porcelain dish that had been cleaned, dried, and preweighed. 1ml of strong sulfuric acid was then added. It was also cooked over a low flame until the odors dissipated. This process was performed twice, and the China dish was then placed in the muffle furnace for 30 minutes at a temperature of 600 ±25°C. The China dish was weighed after it had cooled. The proportion of sulfated ash was calculated using the equation below: Sulfated Ash (%) = (weight of sulfated ash/weight of powder ash) × 100.

Soxhlet Extraction
Hot extraction was carried out using the Soxhlet apparatus. The extraction was carried out by using the solvents in increasing order of polarity, i.e., petroleum ether, and ethanol. (4-7)

Preliminary Phytochemical Screening
Test for carbohydrates
- **Molisch test:** Add a few drops of alcoholic alpha-naphthol to the extract. Add 0.2ml of concentrated sulfuric acid progressively down the edges of the test tube until a purple to violet ring emerges at the junction.
- **Fehling's Test:** The Fehling A and Fehling B reagents were combined, and a few drops of extract were added before boiling. A brick red cuprous oxide precipitate appears, confirming the presence of carbohydrates.
- **Benedict's test:** 5ml of Benedict's solution was added to 0.5 mg of extract and heated in a water bath. The presence of reducing sugars is indicated by the formation of red, yellow, or green precipitate.
- **Barfoed's Test:** In a test tube, one milliliter of a sample solution is added. Three milliliters of Barfoed's reagent are added. After two minutes in a boiling water bath, the solution is allowed to cool. Keep track of the color and the time it takes to form a red precipitate.

Test for steroids
- **Salkowski test:** 2ml of plant extract, 2ml of chloroform, and 2ml of concentrated H_2SO_4 were mixed and well shaken. The chloroform layer was red, while the acid layer was greenish yellow fluorescent. This validates the presence of sterols.
- **Liebermann-Burchard Test:** Chloroform was combined with 2ml of extract. In the mixture, 1-2ml of acetic anhydride and 2 drops of concentrated H2SO4 from the test tube's side were added. The presence of sterols is indicated by the presence of red, blue, and eventually green color.
- **Libermann's test:** 3ml extract was mixed with 3ml acetic anhydride. It has been heated and chilled. A few drops of concentrated H_2SO_4 were added and the blue color was noted.

Tests for Cardiac Glycosides
- **Baljet's test:** With sodium picrate, a test solution for yellow to orange color was detected.

- **Legal's test:** 1ml pyridine and 1ml sodium nitroprusside were added to an aqueous or alcoholic test solution and the pink to red color was noted.
- **Kellar Killani test**: Glacial acetic acid, one drop of 5% $FeCl_3$, and concentrated H_2SO_4 were added to 2ml extract to examine for reddish brown color at the junction of the two liquids and upper layers bluish green.

Tests for anthraquinone glycosides

Borntrager's test: For 5 minutes, boil powdered medication with 5 cc of 10% sulfuric acid. Filtered while hot, then cooled and gently shaken with an equivalent volume of benzene. The benzene layer was isolated and then treated with a 10% ammonia solution. The presence of anthraquinones gave the ammonical layer a rose-pink color.

Tests for flavonoids

- **Shinoda Test:** 5ml 95% ethanol, a few drops of concentrated HCl, and 0.5 g magnesium turnings were added to the dry powder or extract. The color pink was detected.
- **Lead Acetate Test:** A small amount of residue was treated with lead acetate solution, and a yellow precipitate was detected. The addition of increasing amounts of sodium hydroxide to the residue produced yellow coloration, which was decolorized with the addition of acid.

Tests for saponins

Foam test: When the test solution was shaken, foam formed and remained stable for at least 15 minutes.

Tests for tannins and phenol compounds

To 2-3ml of alcoholic or aqueous extract, add few drops of the following reagents:

- **One drop NH_4OH, excess 10% $AgNO_3$ solution:** Heated for 20 min in boiling water bath. White precipitate was observed, then a dark silver mirror deposited on wall of test tube.
- **5% $FeCl_3$ solution:** Deep blue-black color.
- **Lead acetate solution:** White precipitate.
- **Bromine water:** Discoloration of bromine water.
- **Acetic acid solution:** Red coloaddr solution.
- **Dilute iodine solution:** Transient red color

Test for alkaloids

- **Dragendorff's test:** A few milligrams of extracts sample were dissolved in 5ml of water. After that, 2 M hydrochloric acid was added until an acid reaction occurred. 1ml of Dragendorff's reagent (potassium bismuth iodine solutions) was added to this combination. If alkaloids were found in sample extracts, orange red precipitate was generated.
- **Wagner's test**: In the test tube, acidify the plant extract sample with hydrochloric acid (1.5% v/v) and add a few drops of Wagner's reagent (iodine potassium iodide solution). It produced reddish brown precipitates, indicating the presence of alkaloids.
- **Mayer's test**: 2ml of plant extracts sample was obtained, and 2-3 drops of Mayer's reagent (Potassium Mercuric Iodine Solution) was added to the test tube. If alkaloids were found in the sample, a dull white precipitate appeared.
- **Hager's test:** It was treated with a saturated picric acid solution. The presence of a yellow precipitate showing alkaloids was present. (8-13)

Test for inorganic elements

The total ash of the drug material was treated with 50% HNO_3. It was kept for 1 hour and filtered. With the filtrate following tests were performed.

- **Calcium:** Ca is precipitated as carbonates in the presence of NH_4Cl and NH_4OH using $(NH_4)_2CO_3$. Due to the formation of soluble calcium acetate, the white precipitate of calcium carbonate produced in hot dilute acetic acid.
- **Magnesium:** Mg reacts with ammonium phosphate in presence of NH_4Cl and NH_4OH to form white precipitate of magnesium ammonium phosphate.
- **Iron:** To a drop of unknown solution, add several drops of NH_4SCN. Bloody red color appears if Fe is present.
- To a drop of unknown solution, add several drops of K3Fe (CN) 6. The deep blue color is an indication of Fe.
- **Phosphate:** Salts react with ammonium molybdate solution to form a deep yellow precipitate of ammonium phosphate molybdate.
- **Chlorides:** Salts on reaction with silver nitrate solution to form a white precipitate of silver chloride which is soluble in ammonium hydroxide [14, 17].

Isolation of phytoconstituents

Before isolation, the ethanolic extract was subjected to TLC analysis for separation and identification of individual phytoconstituents.

Fractionation

The extract was dissolved in distilled water (120ml) until the crude extract dissolved. The water solution was transferred into a separatory funnel and fractionated by 30ml and 20ml of hexane, petroleum ether, benzene, chloroform, ethyl acetate, n-butanol, and water. After extraction, all fractions were kept inside the fume hood for evaporation of the mother solvents to give hexane, petroleum ether, benzene, chloroform, ethyl acetate, n-butanol and water. Finally, the remaining water fraction was refractioned while the benzene fraction was subjected to column chromatography.

Thin Layer Chromatography

Preparation of Chromaplate

In a hot air oven, the glass slides were cleaned and dried. In a sterile beaker, slurry was made by combining silica gel with twice as much distilled water. Using a different slide edge, one drop of slurry was applied to the slide and dispersed throughout to create a thin layer. The slides remained like a way for a little while. The chromo plates were then heated for 30 minutes at 120°C in a hot air oven to activate them.

Loading of sample

The origin of the slides was noted around 2 cm from the bottom after they had been allowed to cool to ambient temperature. Every slide above had its working suspensions loaded from the edge in the middle.

Visualization

Thin layer chromatography for ethanolic extract of leaves was performed on precoated Silica gel 60 F 254 GLP plates with the help of Chloroform: Methanol (5:1 & 5:2) and benzene:

Chloroform: Methanol (6:4:0.5) as mobile phase, the spots were visualized under iodine vapor for detection.

Determination of R_f value

The spot was observed on the TLC plates and R_f value was calculated by using the following formula.

R_f = distance traveled by center of component / distance traveled by the solvent front (18-21)

Column Chromatography

Chromatographic methods play an important role in the chemistry of natural products and have a major impact on the development of new and inventive substances with pharma-cological and therapeutic value. This work concentrated on providing a step-by-step visual representation of the column-chromatographic methods utilized for the fractionation and separation of physiologically active plant secondary metabolites. The steps involved in utilizing column chromatography to isolate bioactive chemicals are sample preparation; packing of the column, pouring of the sample into the column; elution of fractions and thin layer chromatography analysis of each fraction. However, substances can be further purified utilizing nuclear magnetic resonance (NMR) spectrum analysis and high-performance liquid chromatography (HPLC), depending on the type of study being done. A glass column with a cylinder form and a stationary phase (silica gel) is gradually approached from the top. The mobile phase descends the column with the aid of gravity or an external pressure source. Compounds can be purified using this method from a mixture. The sample is placed into the column's top once it is prepared. After that, the mobile solvent is let to pass through the column. The distinct chemicals are gathered as fractions and subjected to further analysis to clarify their structures.

- **Test sample:** Depending on the nature of the task, an appropriate solvent was used to extract the air-dried sample powder, which was then vacuum-dried before being loaded into the column. Measuring the sample amount is necessary to determine the target extract yield.
- **Gradient solvent system:** The optimal elution and separation of different organic components from any plant-based organic extract are achieved using a gradient solvent system, which is a non-polar to high-polar solvent system. The quantity of the material that has to be purified, however, may affect the solvent's volume.
- **Isolation and purification of bioactive compounds:** Based on the volume of the sample, a long, cylindrical glass column of the appropriate size should be firmly supported by a column-chromatography stand. To facilitate sample dispersion in a silica gel column that has previously been packed, a completely dried plant extract sample should be combined with silica gel to create a finely powdered form. The powdered mass sample has to be topped with a layer of cotton and placed on top of the pre-packed silica column. To fractionate the sample extract, solvents with varying polarity were then fed through a column at a consistent pace while subjected to gravity. Each fraction was collected independently in a test tube and given a sequential number in preparation for further thin-layer chromatography examination. Using thin-layered chromatographic plates, thin layer chromatography (TLC) allows for the partial separation of both organic and inorganic materials. TLC is particularly helpful for verifying the quality of fractions. Using a capillary tube to apply each fraction to activated TLC plates, space them 1/2 inch apart from the lower edge. The plates are then developed in a chamber with an appropriate solvent system for a predetermined amount of time, or until the developing solvent reaches the top of the TLC plate. After removing the plate from the developing chamber and drying it, the solvent front is indicated with a lead pencil. Compound bands or spots seen on a TLC chromatoplate's can be identified visually, in an iodine chamber, under UV light (254 nm), and by employing a spray reagent (vanillin-sulfuric acid) to check for the presence of certain compounds. The chromatoplate's visible spots for each component are labeled, and each spot's R_f value is determined using the following formula.

R_f = Distance traveled by the solvent (cm) / distance traveled by the sample (cm).

High-performance liquid chromatography may be used to further purify TLC plates displaying the number of bands (compounds) for each fraction (HPLC). To clarify the chemical structure of target compounds, further spectrum studies including infrared (IR), mass spectrometry, and nuclear magnetic resonance (NMR) can be carried out depending on the nature of the substances [22].

Packing of column (adsorbent/adsorbate weight 30:1)

Stationary phase used	Siica gel G (60-120 mesh)
Solvent used	Petroleum ether

Mobile phase used

Solvents	**Ratio**
Benzene	100%
Benzene: Chloroform	99:1

Gas Chromatography-Mass Spectroscopy

Gas chromatography mass spectrometry (GC-MS) is a combination of two highly distinct analytical techniques: mass spectrometry (MS) and gas chromatography (GC), which is hyphenated (i.e., uses a hyphen rather than a forward slash). The analytical tool is typically a gas chromatograph that is hyphenated to the mass spectrometer through a heated transfer line; the two procedures are performed in sequence. Nonetheless, certain specialized instruments-which are often small or portable-contain the entire GC-MS in a single box.

The process of identification involves comparing the retention duration of peaks in a sample to standards of recognized chemicals that are examined using the same technique. Hyphenation to an MS is an effective method for identifying unknowns, as GC alone is not always sufficient for this purpose. The column effluent might be divided between the MS and GC detector (s) or utilized exclusively with MS. Three-dimensional data from a GC-MS includes a chromatogram that may be utilized for both qualitative and quantitative analysis, as well as mass spectra that can be used for identification confirmation or to identify new chemicals.

Working

Using the THERMO MS model (TSQ 8000-Triple quadrapol) working in electron impact mode (EI, 70 eV) and the THERMO GC model (TRACE 1300), GCMS analysis of the physiologically active chemicals included in the extracts was

carried out. It made use of a TG-5 MS Column (30 m x 0.25 mm x 0.25 µm). It made use of a TG-5 MS Column (30 m x 0.25 mm x 0.25 µm). The temperature program was set to raise the temperature to 280°C at a rate of 15 C per minute for two minutes, then keep it there for another fourteen. 250°C was the injector's temperature. One milliliter per minute was the carrier gas's (helium) flow rate. They injected 1 µl of the liquid. The compounds were recognized by comparing their mass spectral fragmentations to standard reference spectra obtained from the NIST database using the AMIDS 2.64 program [23, 24].

Pharmacological activity

Anti-Diabetic Activity

Anti-diabetic activity of ethanolic extract of leaves of *Graptophyllum pictum* was evaluated in Streptozotocin-induced diabetes male albino mice.

Acute toxicity study

According to OECD-423 criteria, an acute oral toxicity investigation was carried out. Acute toxicity investigation using five Wistar rats (n = 6) of either sex chosen by random sampling procedure. The animals were given just water during their overnight fast, and then they were examined for 14 days while the extracts were given orally by gastric intubation at a dosage level of 250 & 500 mg/kg body weight.

Animals and treatment

Albino Wistar rats, both male and female, weighing between 150 and 250 grams were chosen and housed in typical settings. They were divided into groups at random, kept in cages, and allowed free access to food and water while being kept in normal circumstances at 26±2°C. The institutional animal ethics committee provided direction for the execution of all the protocols [25].

Induction of Diabetes Mellitus

Mice were given intraperitoneally (i.p.) injections of 60 mg/kg of streptozotocin (STZ), diluted in 0.1M cold citrate buffer (pH =4.5), to develop diabetes. The diagnosis of diabetes was established by measuring the fasting blood glucose level three days after streptozotocin was administered.

Oral glucose tolerance test

An Antihyperglycemic effect of the extract was done on overnight fasted mice (14 h). After fasting, mice were randomly divided into 5 groups (6 mice per group). Baseline BGL was measured (just immediately before giving each agent based on their grouping). Thereafter DW, extract and standard drug were administered. Thirty mins post administrations of each agent; animals were loaded with 2.5 g/kg of glucose solution orally. Blood glucose levels were then measured after 30, 60 and 120 min.

Streptozotocin induced diabetic mice

Anti-hyperglycemic activity of repeated dose of the extract was carried out in STZ induced diabetic mice. Based on their grouping, diabetic mice were given DW, standard drug or extract for 3 weeks. The non-diabetic group (NOC) was also administered DW for the same time. The blood glucose lowering effects of extract was then determined by measuring FBG every seven days for three weeks. BGL of diabetic mice were measured just before starting treatment on the 1st day of treatment (3 days after STZ injection) as baseline, and then on the first, second and third week following fasting for 8 h [26, 27].

Estimation of Biochemical Parameters

Biochemical parameters i.e. Aspartate transaminase (AST) [SGPT], Alanine aminotransferase (ALT) [SGOT], cholesterol, and triglycerides, were analyzed using an automated biochemical analyzer. (28)

Statistical analysis

The results were expressed as Mean ± Standard error mean (SEM). The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Dunnnet's comparison test and $p<0.05$ was considered significa.

Results

Physicochemical Studies

Various physicochemical parameters such as foreign organic matter content, moisture content, swelling index, extractive values and ash values were determined five times and mean values were calculated as recorded in following tables (1-3).

Table 1: Physical parameters for leaves of *Graptophyllum pictum*

Parameters	% yield (w/w)
Foreign organic matter	Nil.
Loss on drying	14.56
Swelling factor	Nil.

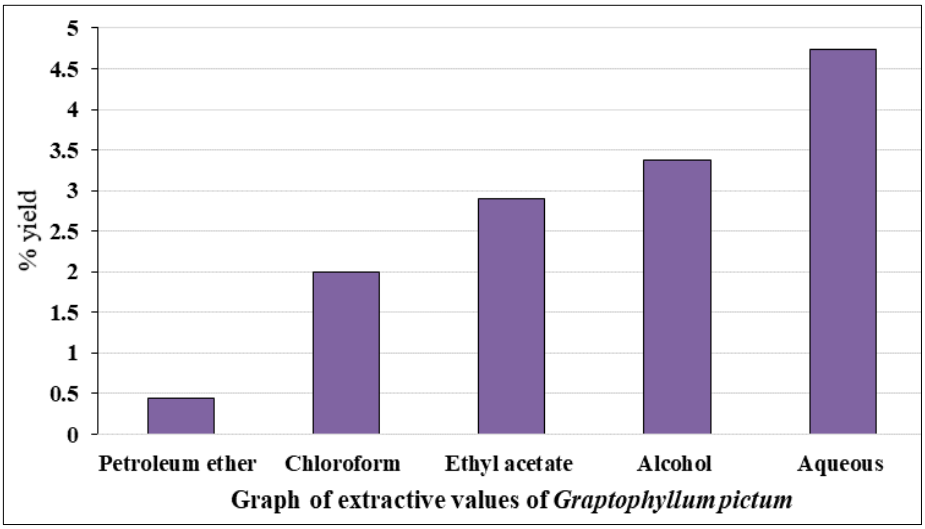


Fig 1: Graph of extractive values of *Graptophyllum pictum*

Table 2: Extractive values of leaves of *Graptophyllum pictum*

Extracts	Leaves		
	% Yield (w/w)	Color	Consistency
Petroleum ether	0.44	Yellowish	Smooth
Chloroform	2	Dull green	Smooth
Ethyl acetate	2.9	Yellowish- green	Smooth
Alcohol	3.37	Dark green	Smooth
Aqueous	4.73	Dark brown	Smooth

The percentage yield of aqueous extract of leaves (4.73%) was found to be highest and that of petroleum ether extract (0.44%) was lowest.

Table 3: Ash value of leaves of *Graptophyllum pictum*

Parameters	Leaves% yield (w/w)
Total ash	10
Acid insoluble ash	1.7
Water soluble ash	18.2
Sulphated ash	19.1

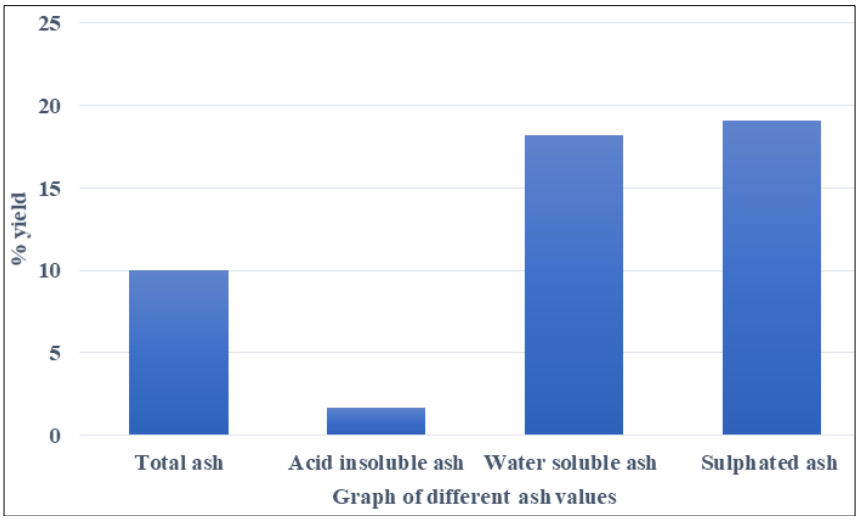


Fig 2: Graph of different ash values of *Graptophyllum pictum*

The result of ash values concluded that the % of sulphated ash was highest as compared to other ash values.

Phytochemical Investigations

Extractive values using Soxhlet extraction

Soxhlet extraction was performed with petroleum ether & ethanol and percentage yield were calculated.

Table 4: Successive extractive values (Soxhlet extraction) of leaves of *Graptophyllum pictum*

Extracts	Leaves		
	% Yield	Color	Consistency
Petroleum ether	1.9	Yellowish	Waxy
Ethanol	7.5	Dark green	Sticky semisolid mass

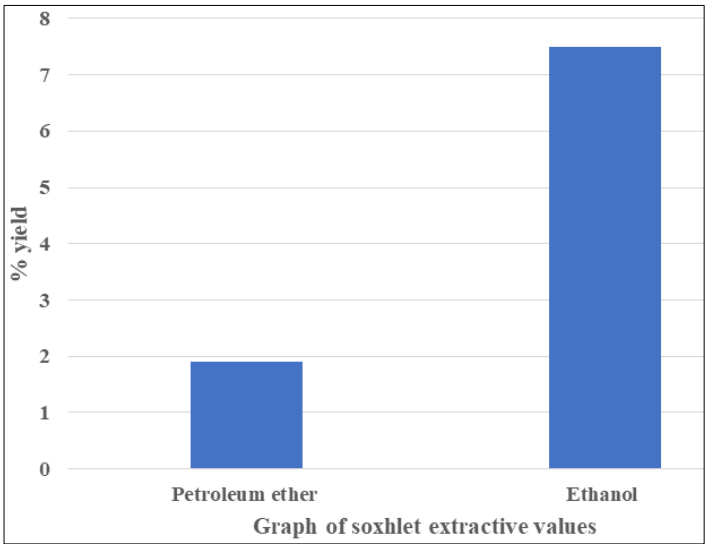


Fig 3: Graph of different Soxhlet extractive values of *Graptophyllum pictum*

The obtained percentage yield of successive extraction of leaves of *Graptophyllum pictum w*as found to be 1.9 & 7.5% for petroleum ether and ethanolic extracts respectively.

Detection of organic phytoconstituents

Preliminary Phytochemical Investigations

Petroleum ether and ethanolic extracts were subjected to preliminary phytochemical screening through qualitative chemical tests.

Table 5: Preliminary qualitative analysis of leaves of *Graptophyllum pictum*

S. No.	Reagents	Petroleum ether extract	Ethanolic extract
Alkaloids			
1	Dragendroff reagent	-	+++
2	Hager reagent	-	+
3	Wagner reagent	-	++
4	Mayer's test	-	+
Flavonoids			
5	Shinoda test	-	+
6	Lead acetate	-	+++
Tannins			
7	5% $FeCl_3$	-	+
8	Lead acetate	-	+++
9	Gelatin	-	+++
10	Br_2 water	-	+
11	Acetic acid	-	-
12	$K_2Cr_2O_7$	-	+++
13	Iodine	-	++
14	Nitric acid	+	++
15	NH_4OH & potassium ferricyanide	-	-
16	Dil. $KMnO_4$	-	+++
Glycosides			
17	Legal test	-	-
18	Killer-Killani test	++	+
19	Borntrager test	-	-
20	Baljet test	+	++
21	Foam test	-	++
Steroids			
22	Salkowski test	-	-
23	Liebermann Burchard test	-	-
24	Liebermann reaction	+	-
Sugars			
25	Molisch test	-	-
26	Fehling test	-	-
27	Benedict test	-	-
28	Barfoed test	-	-
29	Tollen's test	-	-
30	Tollen's phloroglucinol test	-	-
31	Iodine	-	-
32	Tannic acid	-	-

Where '+' = Present, '-' = Absent

The study revealed the presence of alkaloids, flavonoids, tannins & glycosides in alcoholic extract whereas petroleum ether extract showed presence of steroids, tannins and glycosides only.

Detection of Inorganic Phytoconstituents

Various inorganic constituents i.e. calcium, iron, phosphate & chloride were identified & the obtained results were shown in table below.

Table 6: Inorganic phytoconstituents of leaves of *Graptophyllum pictum*

S. No.	Inorganic content	Result
1	Calcium	++
2	Magnesium	-
3	Iron	+++
4	Chloride	++
5	Phosphate	+++

Where '+' = Present, '-' = Absent

Table 7: TLC analysis of ethanolic extract of leaves of *Graptophyllum pictum*

S. No.	Extract fraction	Mobile phase	No of spots	R_f values
1	Aqueous fraction	$CHCl_3$:CH_3OH (5:1)	1	0.24
2	Aqueous fraction	$CHCl_3$:CH_3OH (5:2)	2	0.41
				0.38
3	Benzene fraction	C_6H_6:$CHCl_3$: CH_3OH (6:4:0.5)	4	0.16
				0.22
				0.33
				0.48
4	Ethyl acetate fraction	C_6H_6:$CHCl_3$: CH_3OH (6:4:0.5)	1	0.51
5	Butanol fraction	C_6H_6:$CHCl_3$: CH_3OH (6:4:0.5)	2	0.32
				0.80

Table 8: Details of TLC Analysis of Column Chromatography

S. No.	Eluent	Mobile phase for TLC	No. of spots
1	100% Benzene	C_6H_6:$CHCl_3$: CH_3OH (6:4:0.5)	2
2	Benzene: Chloroform	C_6H_6:$CHCl_3$: CH_3OH (6:4:0.5)	1

Isolated Compounds

After fractionation of ethanolic extract with n-hexane, petroleum ether, benzene, dichloromethane, chloroform, ethyl acetate, n-butanol, and water, the aqueous fraction was refractionated with similar solvents. The refractionated aqueous portion on treatment with methanol gave a compound (C1), which was recrystalized with methanol yielded 50 mg (1.66% yield) mass. Benzene fraction was subjected to column chromatography with silica gel and eluted with benzene and chloroform in various combinations giving compound 2 (C2) yielding 20 mg (0.6% yield) mass.

Code	Name	Columneluant	R_f value (Mobile Phase)	Yield (% w/w) Solvents used for recrystallizat on	M.P. ºC	Mol. Wt (mol. For.)	Nomenclature
C1	N-decanyl cetoleate	-	0.41 C:M (5:2)	1.66 (Methanol)	250ºC	478 $C_{32}H_{62}O_2$	n-decanyl-n-docosanoate
C2	N-octanyl capriate	B:C (99:1)	0.33 C: B:M (6:4:0.5)	0.6 (Methanol)	260ºC	284 $C_{18}H_{36}O_2$	n-octanyl-n-decanoate

Characterization of the isolated compound

Compound- C1

Physical state: Solid

Color: White

Solubility: DMSO, Chloroform

Melting point: 250ºC

R_f: 0.41 (Chloroform: methanol, 5:2)

Structure elucidation of compound

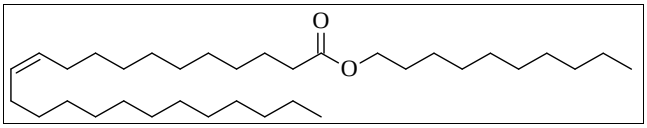


Fig 4: N-decanyl cetoleate

Table 9: IR spectrum assigned to compound C-1

Functional group	Wave number
Ester group	1726 cm^{-1}
Long aliphatic chain	801 cm^{-1}
	754 cm^{-1}

IR υ_{max} (KBr): 2916, 2850, 1726, 1469, 1377, 1255, 1173, 1102, 983, 908, 801, 754 cm^{-1}

^{1}H NMR ($CDCl_3$): δ 5.20 (1 H, m, H-11), 4.86 (1 H, m, H-12), 4.47 (2 H, brs, H_2-1'), 2.31 (1 H, d, J= 7.2 Hz, H_2- 2a), 2.26 (1 H, d, J = 7.2 Hz H_2- 2b), 1.63 (2 H, m, H_2-10), 1.61 (2 H, m, H_2-13), 1.56 (4 H, brs, 2 x CH_2), 1.25 (46 H, brs, 23 x CH_2) 0.87 (3 H, t, J= 6.1 Hz, Me-10'), 0.84 (3 H, t, J = 6.5 Hz, Me-22)

+ Ve ion TOF MS m/e (rel. int.): 956 [2 xM] $^+$ (2 x $C_{32}H_{62}O_2$) (100), 478 $[M]^+(C_{32}H_{62}O_2)^+$, 321 (11.3).

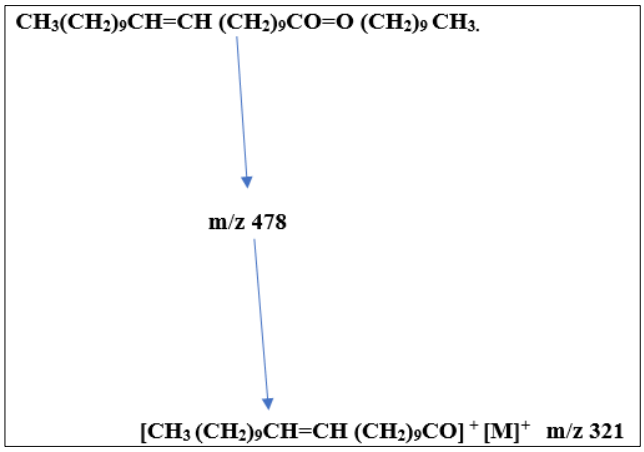


Fig 5: Mass Fragmentation Pattern Of n-decanyl cetoleate (Compound 1)

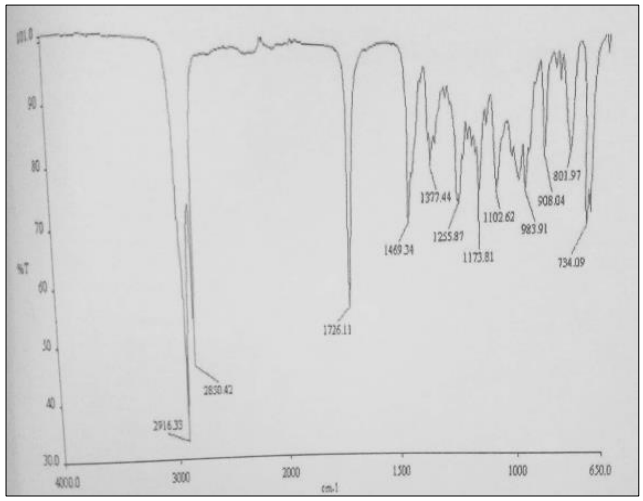


Fig 6: IR spectra of n-decanyl cetoleate (Compound 1)

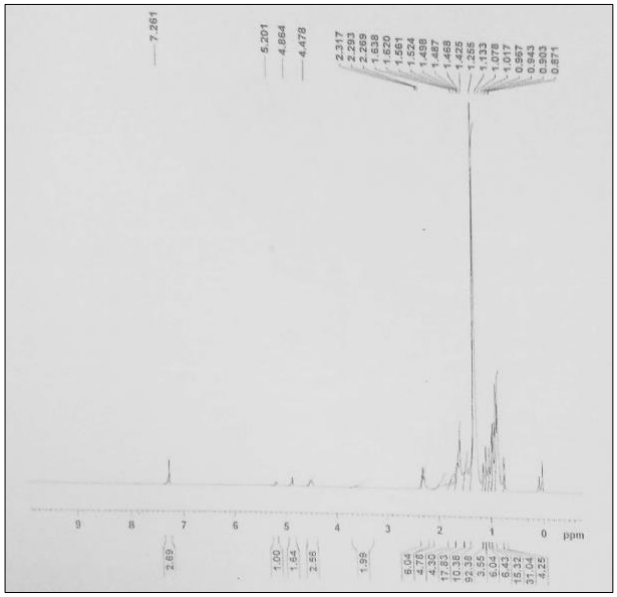


Fig 7: ^{1}H NMR spectra of n-decanyl cetoleate (Compound 1)

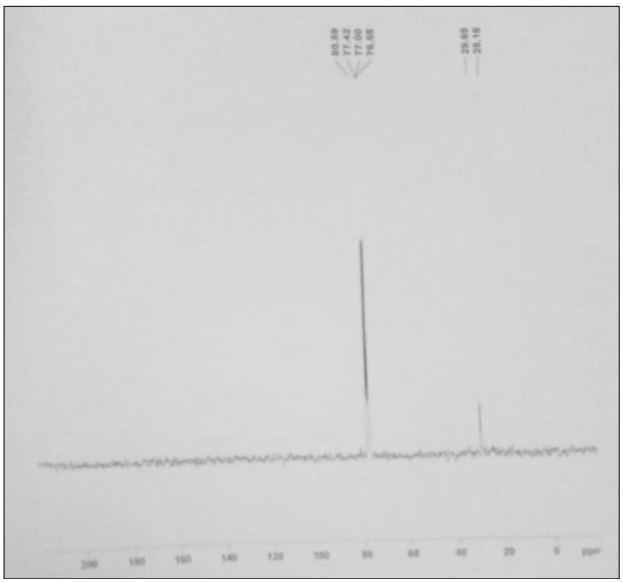


Fig 8: ^{13}C NMR spectrum of n-decanyl cetoleate (Compound 1)

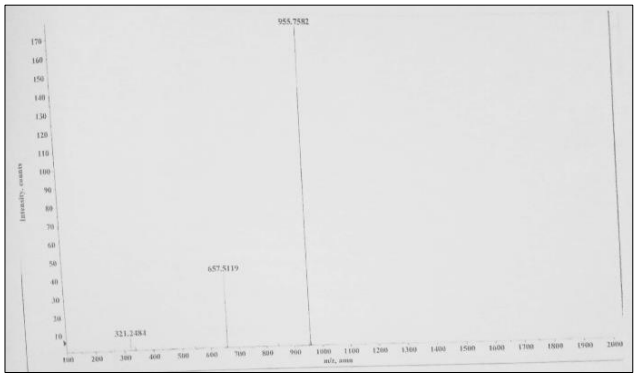


Fig 9: Mass spectrum of n-decanyl cetoleate (Compound 1)

Compound 2
Physical state: Solid

Color: White

Solubility: Chloroform

Melting point: 260ºC

R_f: 0.33 (Benzene: Chloroform: Methanol, 6:4:0.5)

Structure elucidation of compound

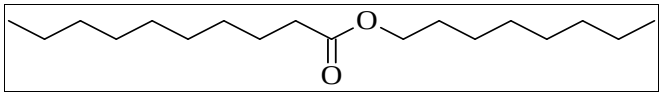


Fig 10: N-octanyl capriate

Table 10: IR spectrum assigned to compound C-2

Functional group	Wave number
Ester group	1740 cm^{-1}
Long aliphatic chain	720 cm^{-1}

IR υ_{max} (KBr): 2918, 2850, 1740, 1464, 1376, 1168, 720 cm^{-1}
^{1}H NMR ($CDCl_3$): δ 4.13 (2 H, m, H-11), 2.35 (2 H, brs, H-2), 2.03 (2 H, brs, CH_2), 1.60
(4 H, brs, 2 x CH_2), 1.25 (24 H, brs, 12 x CH_2), 0.88 (6 H, brs, Me-10, Me-8')
^{13}C NMR ($CDCl_3$): δ 31.91 (CH_2), 22.71 (17 x CH_2), 29.16 (CH_2), 22.70 (CH_2), 14.12 (2 x CH_3), 33.48 (CH_2), 31.15 (CH_2), 29.70 (24 x CH_2), 26.52 (CH_2), 22.83 (CH_2), 14.21 (2 x CH_3)
+ Ve ion TOF MS m/e (rel. int.): 284 $[M]^+$ ($C_{18}H_{36}O_2$) (59.8), 55 (25.2)

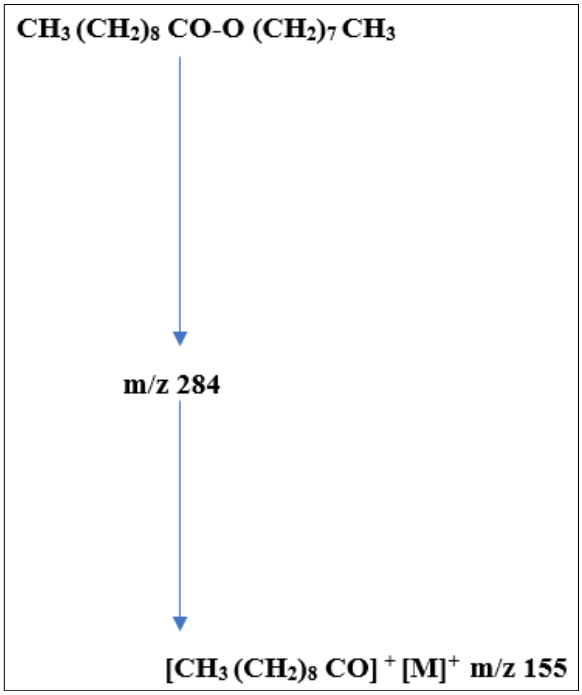


Fig 11: Mass Fragmentation pattern of n-octanyl capriate (Compound 2)

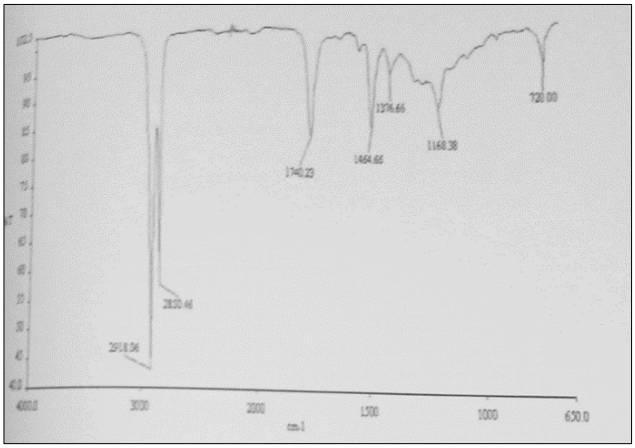


Fig 12: IR spectra of n-octanyl capriate

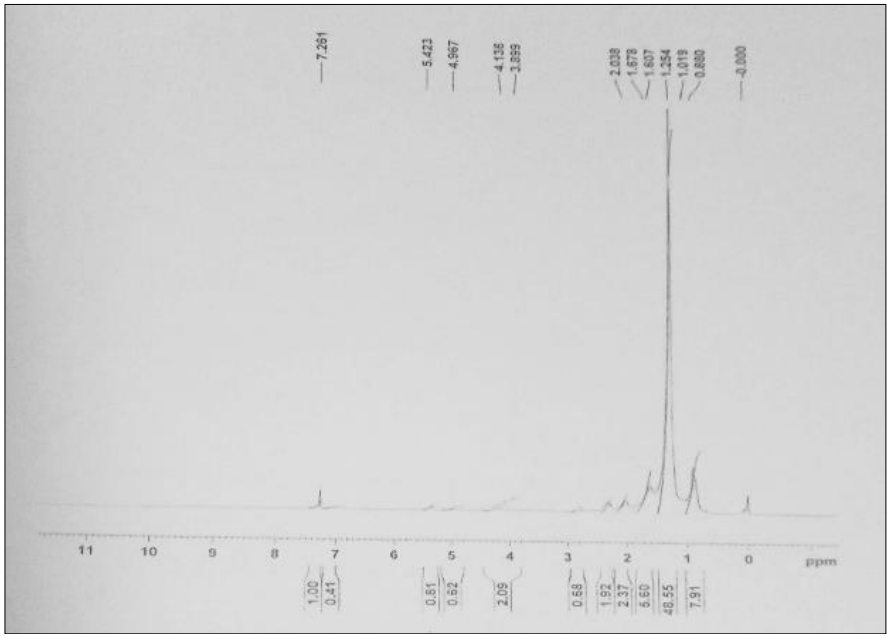


Fig 13: ^{1}H NMR spectra of n-octanyl capriate

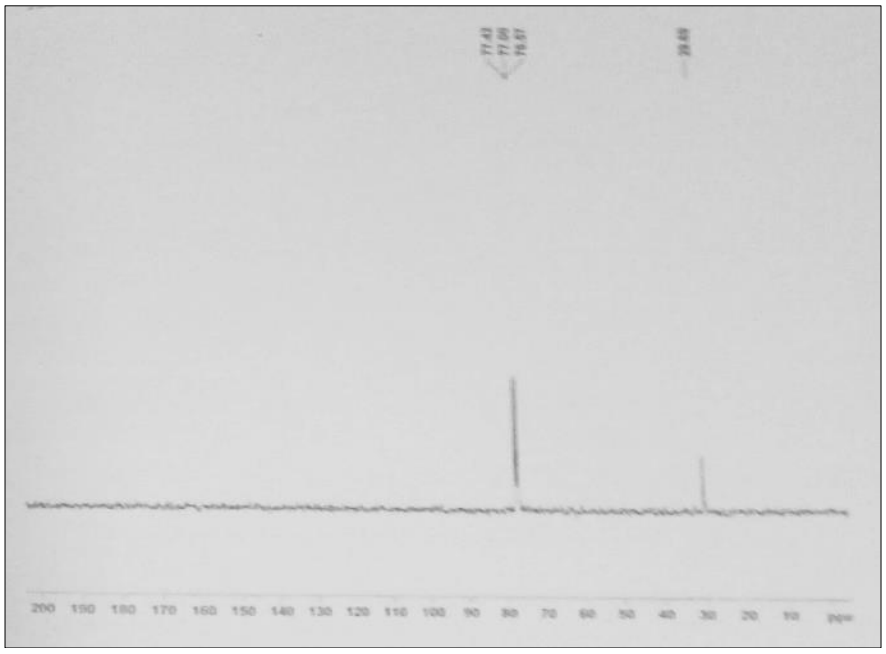


Fig 14: ^{13}C NMR spectra of n-octanyl capriate

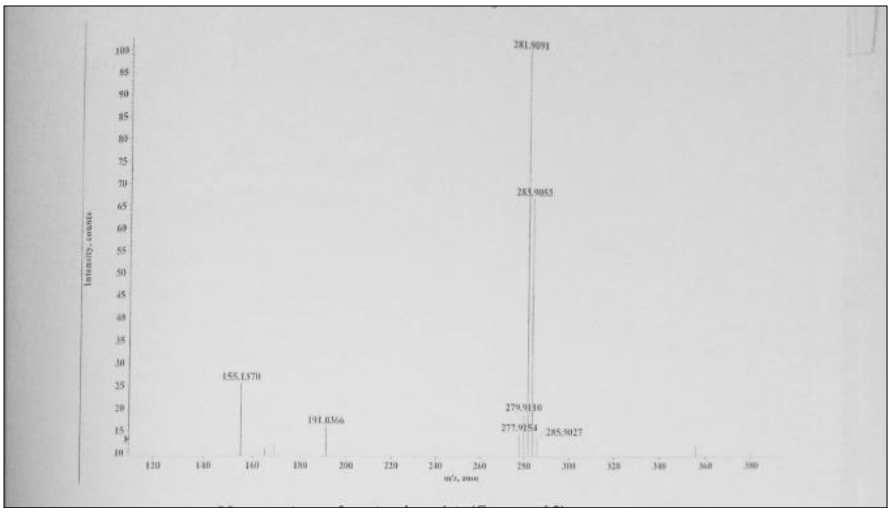
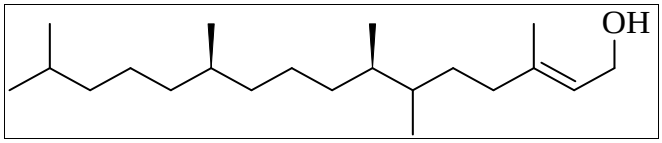
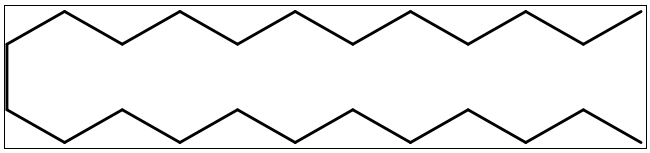


Fig 15: Mass spectra of n-octanyl capriate

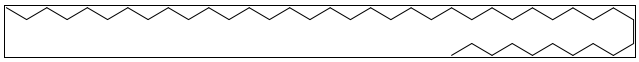
Table 11: Compounds Identified in the petroleum ether extract of leaves

RT	Name of compounds	Molecular formula	Molecular weight	Peak area%
22.05	Phytol	$C_{20}H_{40}O$	296.53	27.06
22.54	Tetracosane	$C_{34}H_{70}$	478.91	3.29
22.61	Tetratertacontane	$C_{44}H_{90}$	619.18	3.76
24.97, 29.46	Tocopherol	$C_{28}H_{48}O_2$	416.68	12.58
32.99	Squalene	$C_{30}H_{50}$	410.71	8.85
34.24	Vitamin E	$C_{29}H_{50}O_2$	430.71	7.46
35.17	Tetratriacontane	$C_{34}H_{70}$	478.91	9.63
37.50	Stigmasterol	$C_{29}H_{48}O$	412.69	27.33

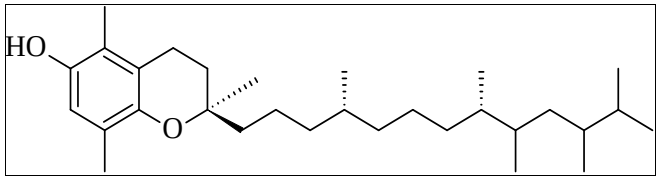




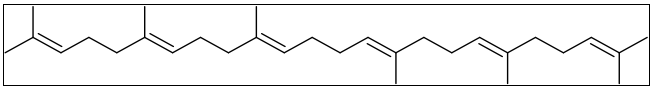
Phytol Tetracosane



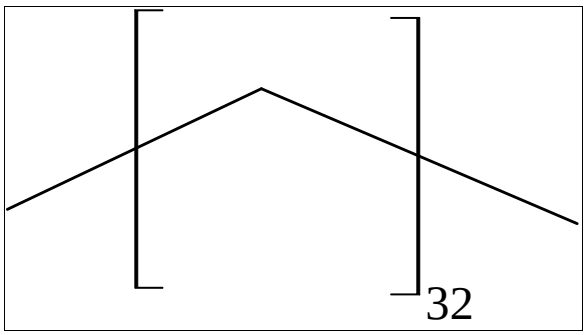
Tetrateracontane

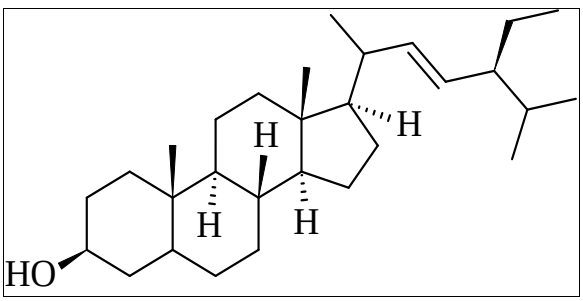


Tocopherol



Squalene





Tetratriacontane Stigmasterol

GC-MS analysis of petroleum ether extract identified eight phytoconstituents namely Phytol, Tetracosane, Tetratertacontane, Tocopherol, Squalene, Vitamin E, Tetratriacontane and Stigmasterol.

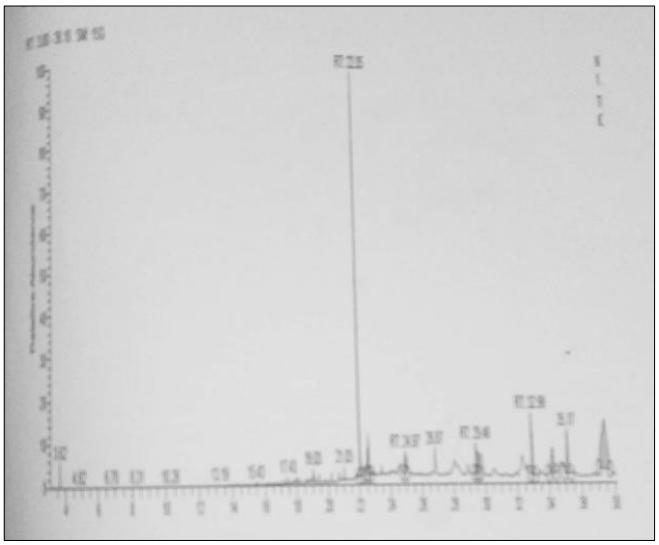


Fig 16: GC-MS chromatogram of petroleum ether extract of *Graptophyllum pictum* leaves

Pharmacological Activity

Acute Toxicity Study

In an acute toxicity study, the oral administration of graded doses of ethanolic extract to the rats was found to be non-lethal up to the dose of 2000 mg/kg body weight. Therefore, the extract was safe for long-term administration.

Anti-Diabetic Activity

Ethanolic extract of *Graptophyllum pictum* leaves showed significant antidiabetic activity in mice. The obtained results were compared to that of standard Glibenclamide (10 mg/kg) as shown below.

Table 12: Effect of Ethanolic extract of *Graptophyllum pictum* leaves on body weight of STZ induced diabetic mice]

Body weight				
Group	**Day 1**	**Day 7**	**Day 14**	**Day 21**
Control	25.50±2.38	25.5±2.38	25.7±2.60	25.7±2.55
Diabetic control	25.50±2.38	22.6±2.60	20.4±2.51	16.50±2.10
Standard	25.9±2.80	24.2±2.34	24.85±2.56	25.8±2.64
EEGP (250 mg/kg)	25.7±2.34	23.5±2.16	24.1±2.14	24.6±2.38
EEGP (500 mg/kg)	25.6±2.56	23.8±2.24	24.4±2.18	25.0±1.66

All value represented as mean ± SEM (n=6), EEGP= Ethanolic extract of *Graptophyllum pictum* leaves, SEM: Standard error of the mean

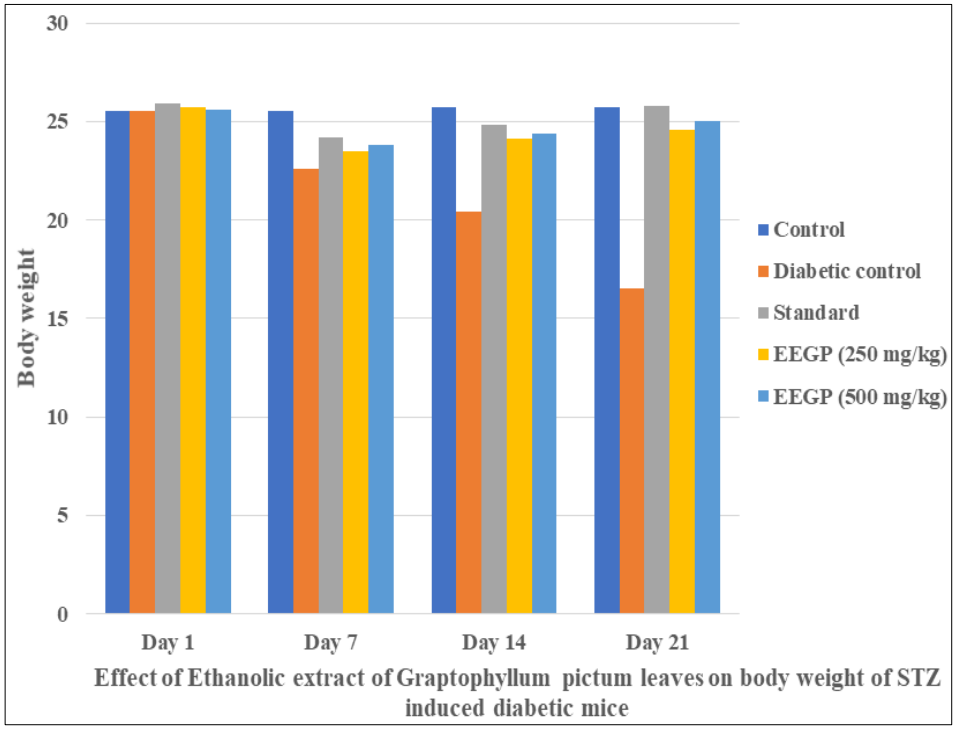


Fig 17: Graph showing the effect of ethanolic extract of *Graptophyllum pictum* leaves on body weight of STZ induced diabetic mice

Table 13: Effect of ethanolic extract of *Graptophyllum pictum* leaves on oral glucose tolerance in normal mice

Group	Blood glucose level (mg/dl)				
	0 h	30 min	60 min	90 min	120 min
Control	78.5±0.38	130.5±0.58	158±0.5	132.5±0.5	120±0.5
Standard	60±0.5	106±0.33	123.5±0.58	108±0.3	97±0.3
EEGP (250 mg/kg)	69.5±0.25	118.4±0.68	134.5±0.75	116.9±0.21	106.6±0.42
EEGP (500 mg/kg)	66.6±0.52	114.2±0.54	129±0.34	112±0.32	101.5±0.52

All value represented as mean ± SEM (n=6), EEGP= Ethanolic extract of *Graptophyllum pictum* leaves, SEM: Standard error of the mean

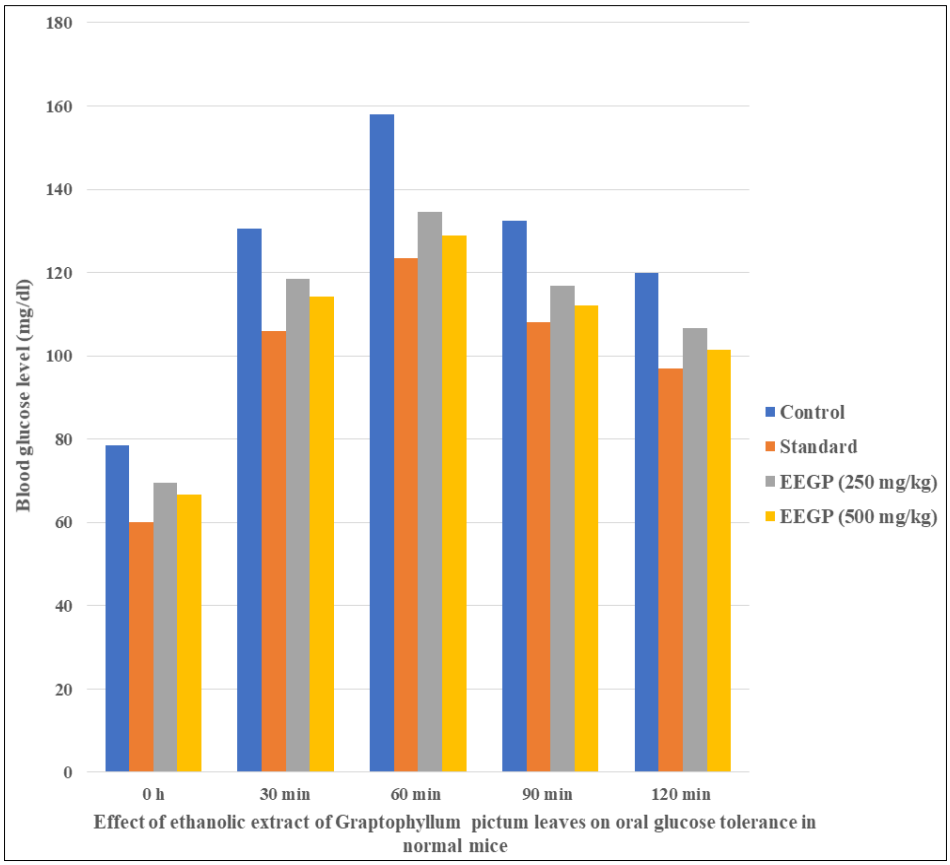


Fig 18: Graph showing the Effect of ethanolic extract of *Graptophyllum pictum* leaves on oral glucose tolerance in normal mice

Table 14: Effect of ethanolic extract of *Graptophyllum pictum* leaves on fasting blood glucose level in Strepzotocin induced diabetic mice

Groups	Days			
	0 day	7 day	14 day	21 day
Control	77.66±5.95	82.33±6.59	77.33±3.20	78.16±6.49
Diabetic control	317.5±18.42$^{\#}$	331.16±19.92$^{\#}$	395.16±19.65$^{\#}$	364.5±22.46$^{\#}$
Standard	318.83±19.93$^{\#}$	251.16± 24.76***	196.33±23.44***	133.66±17.88***
EEGP (250 mg/kg)	319.00±11.56$^{\#}$	276±21.54***	217±14.21**	175.15±15.50**
EEGP (500 mg/kg)	321.2±14.5$^{\#}$	269.95±17.55***	211±14.36***	167.45±19.25***

Each value representing mean ±SEM, n= 6. $^{**}p<0.01$ compared to diabetic control, $^{***}p<0.001$ compared to diabetic control, $^{\#}p<0.05$ compared to control, SEM: Standard error of the mean, EEGP: Ethanolic Extract of *Graptophyllum pictum* leaves

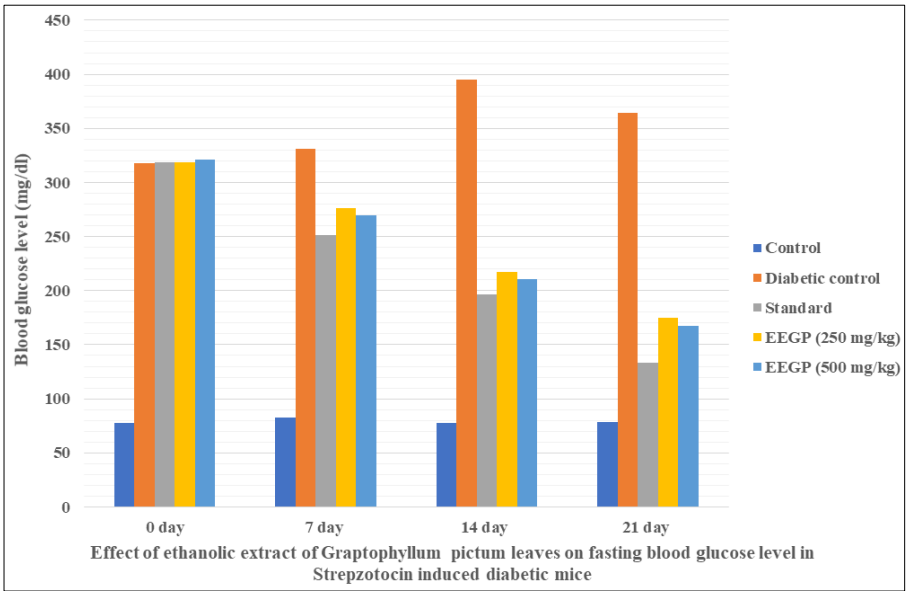


Fig 19: Graph showing the Effect of ethanolic extract of *Graptophyllum pictum* leaves on fasting blood glucose level in Strepzotocin induced diabetic mice

Table 15: Effect of ethanolic extracts of *Graptophyllum pictum* leaves on biochemical parameters in Strepzotocin induced diabetic mice

Group	Biochemical Parameters			
	SGPT (IU/L)	SGOT (IU/L)	Cholesterol (mg/dl)	Triglyceride (mg/dl)
Vehicle	38.5±2.73	40.83±3.97	108±11.45	84.16±7.56
STZ	79.66±5.81$^{\#}$	88.33±4.5$^{\#}$	178±7.66$^{\#}$	174.66±14.85$^{\#}$
Standard	44±2.09***	47±5.17***	112.5±11.79***	95.66±6.15***
EEGP (250 mg/kg)	52.25±3.12***	53.35±1.69***	121.21±5.65***	107.10±22.25***
EEGP (500mg/kg)	50.08±1.56**	50.2±2.14***	119.2±5.22***	102.6±5.80***

Each value representing mean ± SEM, n=6. $^{**}p<0.01$ compared to diabetic control, $^{***}p< 0.001$ compared to diabetic control, $^{\#}p<0.05$ compared to control, SGPT: Serum glutamate-pyruvate transaminase, SGOT: Serum glutamic oxaloacetic transaminase, SEM: Standard error of the mean, EEGP: Ethanolic Extract of *Graptophyllum pictum* leaves

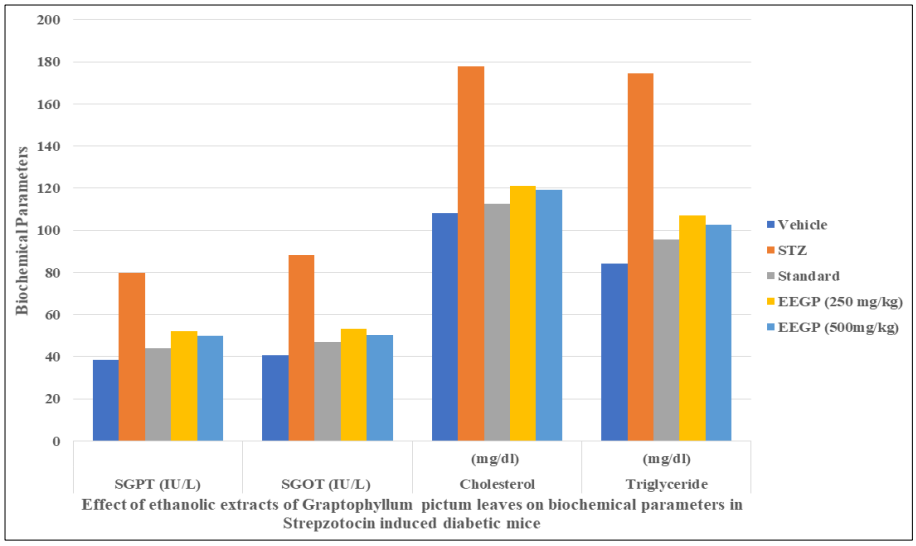


Fig 20: Graph showing the effect of ethanolic extracts of *Graptophyllum pictum* leaves on biochemical parameters in Strept ozotocin-induced diabetic mice

The antidiabetic activity of ethanolic extract of leaves of *Graptophyllum pictum against* streptozotocin-induced diabetes was studied. The extent of diabetes was estimated by increased blood glucose level, SGPT, SGOT, cholesterol, and triglycerides.

Induction of STZ to mice caused significant reduction in body weight as compared to control group 16.50±2.10 (before 25.50±2.38) as compared with the normal group 25.7±2.55 (before 25.50±2.38). Administration of ethanolic extract at dose levels of 250 mg/kg & 500 mg/kg significantly increased body weight (24.6±2.38) and (25.0±1.66) respectively, while with standard drug Glibenclamide, it was (25.8±2.64).

The blood glucose level rose to a maximum in 60 min after glucose loading. The extract (250 mg/kg and 500 mg/kg b.w.) and Glibenclamide (10 mg/kg) treated groups showed a significant decrease in level of glucose as compared to the control. The extract treated group showed a marked fall in glucose level 90 min to 120 min intervals.

Administration of EEGP at the dose of 250 mg/kg b.w. to diabetic mice for 21 days revealed a fall in plasma glucose level from 319 mg/dl to 175. 15 mg/dl on 22nd day when compared to 0-day value. EEGP at the dose of 500 mg/kg b. w. showed significant (p<0.001) fall in plasma glucose level from 321.2 mg/dl to 167.45 mg/dl on 22nd day.

The administration of EEGP and Glibenclamide reversed level of SGPT, SGOT, total cholesterol and triglycerides in diabetic mice significantly (p< 0.01 and p<0.001) compared to the control.

Discussion

The present research work was aimed at physiochemical, preliminary phytochemical and pharmacological evaluation of the leaves of *Graptophyllum pictum*.

The dried powder of leaves of *Graptophyllum pictum* was subjected to physicochemical evaluations i.e. foreign organic matter, moisture content, swelling index, extractive values and ash values. Moisture content was found to be 14.56% and foreign matter content &swelling index were nil. The cold extractive values to be extracts obtained from different media i.e. petroleum ether, chloroform, ethyl acetate, alcohol and water were found to be 0.44, 2, 2.9, 2.37 & 4.73% respectively. The obtained results showed that total, acid insoluble, water soluble and sulphated ash were 1, 1.7, 18.2 & 19.1% respectively. Further coming to phytochemical studies successive Soxhlet extraction revealing the yield of the leaves of the plant drug in different solvents was calculated. The obtained extractive values were found to be 1.9 & 7.5% for petroleum ether and ethanolic solvent respectively. The qualitative chemical tests revealed the presence of alkaloids, flavonoids, tannins and glycosides with some inorganic constituents i.e. Cl^-, Fe, Ca and PO_4^{3-}. TLC analysis of various fractions was performed with different solvent systems. i.e. $CHCl_3$: CH_3OH (5:1), $CHCl_3$: CH_3OH (5:2), C_6H_6: $CHCl_3$: CH_3OH (6:4:0.5) (v/v). The calculated R_f value ranged from 0.16-0.80. Chemical investigation of benzene fraction, obtained from ethanolic extract of leaves of G. pictum led to isolation of two fatty acid esters namely n-octanyl capriate and n-decanyl cetoleate. GC-MS analysis of petroleum ether extract identified eight phytoconstituents namely Phytol, Tetracosane, Tetratriacontane, Tocopherol, Squalene, Vitamin E, Tetratriacontane and Stigmasterol.

Phytol, an acyclic diterpene alcohol, reported to possess anti-inflammatory, antispasmodic, antimycobacterial and cytotoxic activities and used as a precursor for the manufacture of synthetic forms of Vitamin E and Vitamin K1.

Stigmasterol, an unsaturated plant sterol, possessing anti-hypercholesterolemia, neutralizing snake venom induced actions, inhibit tumor promotion, anti-inflammatory and antimicrobial activities and also participates in the synthesis of semisynthetic progesterone, corticoids and Vitamin D_3.

Tocopherol, a methylated phenol, reported to have antioxidant and nephroprotective activities.

Tetracosane, an alkane hydrocarbon, reported to possess which shows anticancer activity.

Squalene, essential omega-2 oil potentiating the effects of some cholesterol lowering drugs and also possessing antioxidant, emollient, anticancer, immunostimulant and lipoxygenase inhibitor activity.

In addition, the ethanolic extract of *Graptophyllum pictum* was monitored for Antidiabetic activity using STZ induced diabetes model in mice. Administration of the extract at doses 250 mg/kg and 500 mg/kg caused significant reduction in SGPT, SGOT, cholesterol and triglyceride levels. These investigations revealed that the extract possess potent Antidiabetic activity.

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