STUDY OF PHYTOCHEMICAL PROFILLING AND IN VITRO STUDIES ON ANTIOXIDANT PROPERTIES OF ETHANOLIC EXTRACT OF *CLEMATIS TRILOBA*

Vishal Ramesh Rasve[1]*, Anup Kumar Chakraborty[2], Sachin Kumar Jain[3], Sudha Vengurlekar[4]

ABSTRACT

The primary aim of this research is to assess the phytochemical composition, isolation, characterization, and antioxidant potential of extracts from *clematis triloba (Ranunculaceae)* using various experimental assays. Different solvents, namely aqueous, petroleum ether, chloroform, and ethanol, were employed to extract the medicinal properties of *clematis triloba*. The study highlights the ethanolic extract of *clematis triloba* as having the highest antioxidant activity. This investigation emphasizes the significance of medicinal plants, such as *clematis triloba*, in serving as valuable reservoirs of bioactive compounds for potential use in drug development for human health. The phytochemical screening identified the presence of alkaloids, carbohydrates, sterols, saponins, tannins, flavonoids, phenolic compounds, and terpenoids, underscoring the diverse range of beneficial compounds present in *clematis triloba.* The *Clematis triloba* yielded 30.67 ± 0.75 mg gallic acid equivalent/g phenolic contents and 49.36 ± 0.68 mg quercetin equivalent/g flavonoid contents. According to the ethanolic extract different concentrations like 20,40,60,80 and 100 µg/ml were carried out by three methods such as DPPH assay, Hydrogen Peroxide (H_2O_2) Radical Scavenging Effect and iron (Fe^{2+}) Chelating Activity. As per the antioxidant properties of CTLE of DPPH scavenging showed the highest percentage of activity compared to the other methods of antioxidant. Among the three methods, DPPH showed excellent antioxidant activity compared with other methods and ascorbic acid as a standard. The reductive ability of the extracts increased with a rise in sample concentration. Because of bioactive components, antioxidant activities play an important role in pharmacological functions. Nonetheless, there are several health issues caused by macronutrient deficits that establish an effective approach. This study article explains the current state of herbal therapeutic items across the world, as well as properties ideas for making *clematis triloba* a better tomorrow in mankind. Therefore, the current study advises against using *Clematis triloba* as traditional medicines or therapeutic agents for illnesses caused by oxidative stress.

Keywords: *Clematis triloba*, Phenolic content, DPPH, Antioxidant Activity, Folins Ciocalteau reagent etc.

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INTRODUCTION

The creation of new drugs will continue to rely heavily on natural medicinal plants, which are frequently referred to as the foundations of drug discovery and development[1].Since ancient times, traditional medical systems have used medicinal plants to treat a range of diseases, and this practise is still in use today. A significant number of the pharmaceuticals that are currently on the market were either straight or indirectly produced from plant sources. In addition, throughout history, plants have consistently served as a good source for the production of medicinal medications. The most recent research by provides an up-to-date look at the rigorous interplay between the most significant physiologically active compounds present in plants and botanicals. This is achieved by giving a brief overview of the properties of botanicals, a definition of the study, examples of creative research (such as an analysis of the interactions between bioactive substances, chemo metrics, and the new objective of bio refineries), and a description of current databases. (i.e., plant metabolic pathways). Numerous plant species have been recognized as a crucial source of potent anti-diabetic drugs for many millennia. This might be seen as the first step in comprehending the biological processes and beneficial actions that these chemicals carry out.[2] The main methods now employed to examine the interactions of phytochemicals can generally be divided into the various categories like the creation of model systems for the investigation of interactions, examines both extractable and non-extractable substances and characterization of extracts with high levels of physiologically active compounds. Till now no reports were found in this research, so it is now planned to discover new herbal remedy from indigenous plant, which are potent and nontoxic agents for the prevention and to treat the diabetes mellitus.[3]

The plant *Clematis triloba* is a woody climber belongs to the family *Ranunculaceae* (buttercup family). Traditional practitioners employ *clematis triloba* to treat liver diseases, heal wounds and reduce inflammation, as well as an antipyretic drug, according to an ethno-medicinal survey.[4] *Clematis triloba* roots are used to cure malarial fever and headaches in Ayurveda medicine, while the root and stem paste is used to treat psoriasis, itching, and skin allergies. The leaf and stem juices are used by traditional medical practitioners to treat infectious old wounds, psoriasis, dermatitis, blood illnesses, leprosy, and liver and cardiac ailments.[5]

The objective of this research is to determine the qualitative, quantitative, isolation and antioxidant activity of the fresh and dried leaves of *Clematis triloba* by investigating the DPPH radical scavenging activity, H_2O_2 assay, and reducing power assay.

MATERIAL AND METHODS

Authentication of Plant

Fresh leaves of the plant were collected in large quantities from Nashik District in Maharashtra. The botanical identification and authentication of the plant material were conducted by Dr. Sayyad I.G., Head of the Department of Botany at Gandhi College, Kada, Ashti, Beed, Maharashtra, India.

Morphological study

The macroscopic examination of the plant encompassed both the identification and authentication of the medicinal properties. By comparing the distinctive features outlined in different floras, *Clematis triloba* could be conclusively identified and authenticated. Following the established protocols detailed in numerous pharmacognosy publications, the macroscopic and organoleptic traits of Clematis triloba leaves were evaluated.

Preparation of plant Extract

The process began with dried leaves, mechanically ground and sieved using a No. 10/44 sieve, followed by storage for a duration of 2 to 3 months. The resulting powdered material from Clematis triloba leaves was individually packed into one-liter thimbles within a Soxhlet apparatus. Extraction took place over 72 hours in four separate 250g batches, employing water, ethanol (80%), petroleum ether (40-60°C), and chloroform (50-70°C). After each extraction, the residue underwent air-drying at

room temperature before the next solvent was used. The extracts obtained then underwent vacuum filtration and concentration via a rotary evaporator. The unmarked, refrigerated crude extracts obtained from each solvent were preserved for future utilization.[6]

Qualitative phytochemical analysis of plant extract

The extracts from *Clematis triloba* were subjected to standard methods of preliminary phytochemical analysis. The screening involved identifying the presence or absence of various active principles, including phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, proteins, amino acids, and tannins.[7-15]

Quantification of secondary metabolites

Quantitative analysis serves as a vital method for determining the quantity of phytoconstituents within plant extracts. This involves the assessment of both Total Phenolic Content (TPC) and Total Flavonoids Content (TFC).[16-19]

Total phenol determination

The total phenolic content was assessed using the Folins-Ciocalteu colorimetric technique, which measures the quantity of material needed to prevent reagent oxidation. The total phenolic content (TPC) of the extract was then determined using a standard graph constructed with gallic acid, and the results were expressed as milligrams of gallic acid equivalents per gram (mg/g).

Total flavonoids determination

The aluminum chloride colorimetric method was employed for the determination of total flavonoid content. The concentration of flavonoids in the extract was quantified by referencing a standard graph constructed with quercetin. The Total Flavonoid Content (TFC) was expressed in milligrams of quercetin equivalents per gram (mg/g).[20,21]

Characterization of purified molecules[22-28]

Silica Gel Column Chromatography

The concentrated crude ethanolic leaves extract of Clematis triloba was introduced into a silica gel column with a particle size of 100-200 mesh, packed in a low-polar solvent. The elution of the column was carried out using solvents, such as ethanol/chloroform. Isolated fractions were examined for metabolite profiling using Thin-Layer Chromatography (TLC). Bands with similar characteristics were grouped together within each fraction and subsequently concentrated. The purity of the active substances was assessed using both TLC and High-Performance Liquid Chromatography (HPLC).

Thin Layer Chromatography (TLC) is a chromatographic technique involving several key steps:

1. Container Development

This refers to the creation of a suitable environment for the chromatographic process. The container should facilitate the movement of the solvent, allowing for effective separation of the components in the sample.

2. Preparation of TLC Plate

In this step, a thin layer of adsorbent material, often silica gel or alumina, is uniformly coated onto a flat and rigid support, creating the TLC plate. The choice of adsorbent and support material is crucial for the success of the chromatographic separation.

3. Spotting of TLC Plate

The sample to be analyzed is applied as small spots onto the TLC plate. This is typically done using a capillary tube or micropipette. Care must be taken to ensure accurate and consistent placement of the sample spots.

4. Development of Plate

The TLC plate is then placed in a solvent system, and the solvent is allowed to move through the adsorbent layer via capillary action. As the solvent travels up the plate, it carries the sample components along with it. The varying affinities of the sample components for the adsorbent result in their separation along the plate.

5. Visualization of Spots

Once the development is complete, the TLC plate is removed and the separated

components are visualized. Visualization techniques vary but commonly involve exposing the plate to UV light or treating it with a chemical reagent that reacts with the components to produce visible spots. This step allows for the identification and quantification of the separated substances.

Thin Layer Chromatography involves container development, preparation of the TLC plate, spotting the plate with the sample, developing the plate in a solvent system, and finally, visualizing the separated components for analysis. Each step is crucial to the overall success and accuracy of the chromatographic separation process.

UV–Vis Spectra

The UV-Vis spectra for the individual pure compounds were obtained utilizing a UV-Vis spectrophotometer (Shimadzu, Japan) within the wavelength range of 200 to 800 nm, with ethanol employed as the blank. Each purified compound was dissolved separately in ethanol at concentrations ranging from 5 to 25 g/ml.

Determination of R*f* Values

The "Rf value" in thin-layer chromatography (TLC) is the ratio of the solute's travel distance to the solvent's transit distance on the TLC plate, often referred to as the solvent front. These distances are measured from the common Origin or Application Baseline, where the sample was initially applied to the plate.

Infra-red Spectroscopy

Infrared (IR) spectroscopy is a technique used to analyze the vibrational modes of molecules. This spectroscopic method is based on the absorption of infrared radiation by chemical bonds within a molecule, leading to the generation of an infrared spectrum.

The IR spectra of the purified chemicals were recorded on a Perkin-Elmer 1600 series using KBr pellets.

NMR

The ^{1}H NMR spectra were obtained for purified compounds using a Bruker instrument with a 400 MHz magnetic field strength. Deuterated DMSO was the solvent, and TMS served as the internal standard for referencing the chemical shifts in the spectra. These details are important for ensuring the accuracy and reliability of the NMR data obtained during the experimental analysis of the compounds.

Mass Spectroscopy

The mass spectrometry (MS) data was obtained utilizing a high-resolution, double-focusing instrument, specifically the JEOL GCMATE II MS with a data system. The instrument has a maximum resolution of 6000 pixels, and the calibrated mass reaches up to 1500 Da. Two sources, namely chemical ionization (CI) and electron impact (EI), were employed (JEOL, Akishima, Tokyo, Japan).

In-vitro antioxidant activity

DPPH Radical Scavenging Assay

The DPPH radical scavenging activity of the *clematis triloba* extracts was assessed in vitro. A compound's capacity to donate an atom of hydrogen, the number of electrons it can contribute, and the mechanism behind its antioxidant action may all be revealed by this assay. The negative control was DPPH solution plus methanol, while the blank was methanol plus sample solution. A little amount of dry extract was dissolved in 50 ml of methanol to create extract solutions.

A blend of 5 ml of a 0.004% DPPH solution in methanol and 1 ml of plant extract in methanol, at different concentrations (20, 40, 60, 80, and 100 µg/mL), was combined and allowed to incubate at 25°C for 30 minutes under room temperature conditions in a relatively dark environment. Subsequently, the decreased absorbance was gauged at 517 nm using a Shimadzu UV-Visible spectrophotometer, referencing it against a control solution containing 1 ml of methanol in lieu of the extract.

The color of the stable free radical DPPH is purple.[27] Yellowing occurs when diphenyl picryl hydrazine is decreased. Three times each study was conducted to determine the average result. Ascorbic acid (Vitamin C) was used as a benchmark for comparison. The % inhibition or DPPH Scavenged was calculated.[29-31]

DPPH Scavenged (%) = (A_{blank} - A_{sample} / A_{blank}) × 100

Hydrogen Peroxide (H_2O_2) Radical Scavenging Assay

The technique was employed to assess the ability of the Clematis triloba extract to neutralize free radicals derived from hydrogen peroxide. The procedure relies on the extract's capability to transform hydrogen peroxide into water. The extract's effectiveness in scavenging hydrogen peroxide was evaluated using a method recommended in previous studies.[12, 13]. A stock solution containing 1 mg/ml of *clematis triloba's* ethanolic extracts is created by diluting the extracts in ethanol. Different amounts of stock solution (20, 40, 60, 80, and 100 µg/ml) were combined with 0.2 ml of H_2O_2 solution in 4 ml of 0.1 M phosphate buffer solution. (pH 7.4). After 10 minutes, the reaction mixture's absorbance at 230 nm was measured using a Shimadzu UV visible spectrophotometer. As a blank, the reaction mixture was employed without the sample. Ascorbic acid was used as a control. Three times the experiment was conducted. Through the use of linear regression analysis, the IC_{50} was determined [32-35].

Iron (Fe^{2+}) Chelating Activity Assay

This spectrophotometric approach is grounded on the concept that as the concentration of the reaction mixture increases, so does its absorbance, indicating a rise in antioxidant activity. The assay involves the conversion of ferrous ions from potassium ferricyanide to produce potassium ferrocyanide (1%) by the sample, and subsequently forming Prussian blue with ferric chloride. In the ethanolic extract solution (20, 40, 60, 80, and 100 µg/ml), potassium ferricyanide [K3Fe (CN)6] was introduced, and the mixture was incubated for 20 minutes at 50°C. Following this, the mixture underwent centrifugation for 10 minutes at 3000 rpm with 1 cc of trichloroacetic acid (TCA acid). The absorbance at 700 nm was measured using a UV-visible spectrophotometer, employing 2 mL of the supernatant solution, 2 mL of distilled water, and 0.5 mL of ferric chloride ($FeCl_3$). Ascorbic acid served as the reference solution, while phosphate buffer functioned as the control. The average of three observations from three repetitions in each experiment was utilized to generate the graph. Increased absorbance in the reaction mixture indicated higher reducing power. The antioxidant activity was quantified in terms of ascorbic acid equivalents per milliliter. [36-38]

STATISTICAL ANALYSIS

Experiments were carried out in triplicate, and the results are expressed as mean values with standard deviation.

RESULTS

Organoleptical evaluation

Table 1: Organoleptical observation of the leaves of *clematis triloba.*

Properties	**Results**
Color	Green
Odour	Characterstic
Taste	Astringent
Shape	Elliptic-ovate, toothed or lobed.

Yield of extract from leaves of *Clematis triloba*

The results showed that maximum yield was found in alcoholic extract among all the extracts.

Figure 1: Soxhlet extraction of *Clematis triloba* using different solvents.

Table 2: percentage yield of *clematis triloba* extrcation

Sr. No.	Solvents	Percentage yield
1.	Ethanol	08.48%
2.	Water	03.24%
3.	Petroleum ether	02.37%
4.	Chloroform	02.83%

ethanol extraction yielded the highest quantity, while the chloroform extraction resulted in the lowest yield. Given the superior yield in ethanol, the ethanolic extract was selected for further structural elucidation through isolation and subsequent pharmacological antioxidant investigation.

Table 3: Phytochemical studies of *clematis triloba* leaves extracts.

Sr. No.	Phyto-constituents	Tests	Various solvent Extracts			
			Ethanol	water	Petroleum ether	Chloroform
1	Carbohydrates and Glycosides	Fehling's test	+	-	-	-
		Molisch's test	+	-	-	-
		Borntrager's test	-	-	-	-
		Barfoed's test	+	-	-	-
2	Proteins and Amino Acids	Biuret Test	-	-	-	-
		Million's test	-	+	-	-
		Ninhydrin test	-	+	-	-
3	Alkaloids	Dragendroff's test	+	+	-	-
		Mayer's test	+	+	-	-
		Wagner's test	+	+	-	-
		Hager's test	-	+	-	-

4	**Sterols**	Liebermann-Burchard test	-	-	+	-
		Salkowski's test	-	-	-	-
5	**Tri-terpenoids and saponins**	Liebermann-Burchard test	+	+	-	-
		Foam test	+	+	-	+
6	**Flavones and flavonoids**	Aq. NaOH	+	-	-	-
		Conc.H_2So_4	+	-	-	-
		Shinoda test	-	-	-	-
7	**Phenols and Tannins**	$FeCl_3$ test	+	-	-	-

+ Indicates Presence, - Indicates Absence

Quantitative analysis of *CTLE*

Table 4 lists the phenolic and flavonoid content of *clematis triloba* leaves. The *clematis triloba* yielded 49.36 ± 0.68 mg quercetin equivalent/g flavonoid contents and 30.67 ± 0.75 mg gallic acid equivalent/g phenolic contents. (figures 2 and 3)

Table 4: Total phenolic and flavonoid content *clematis triloba* extract

Sr. No.	Extract	Total Phenol (mg GAE/g)	Total flavonoid (quercetin equivalent/g)
1.	*CTLE*	30.67 ± 0.75	49.36 ± 0.68

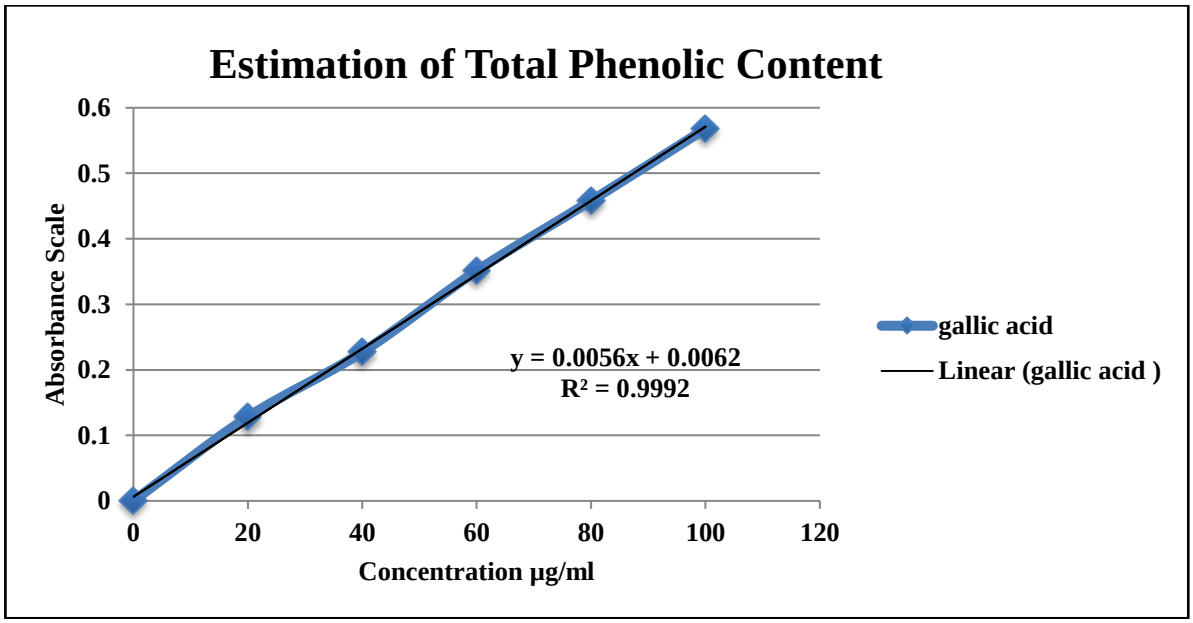

Figure 2: Graph of estimation of total phenolic content

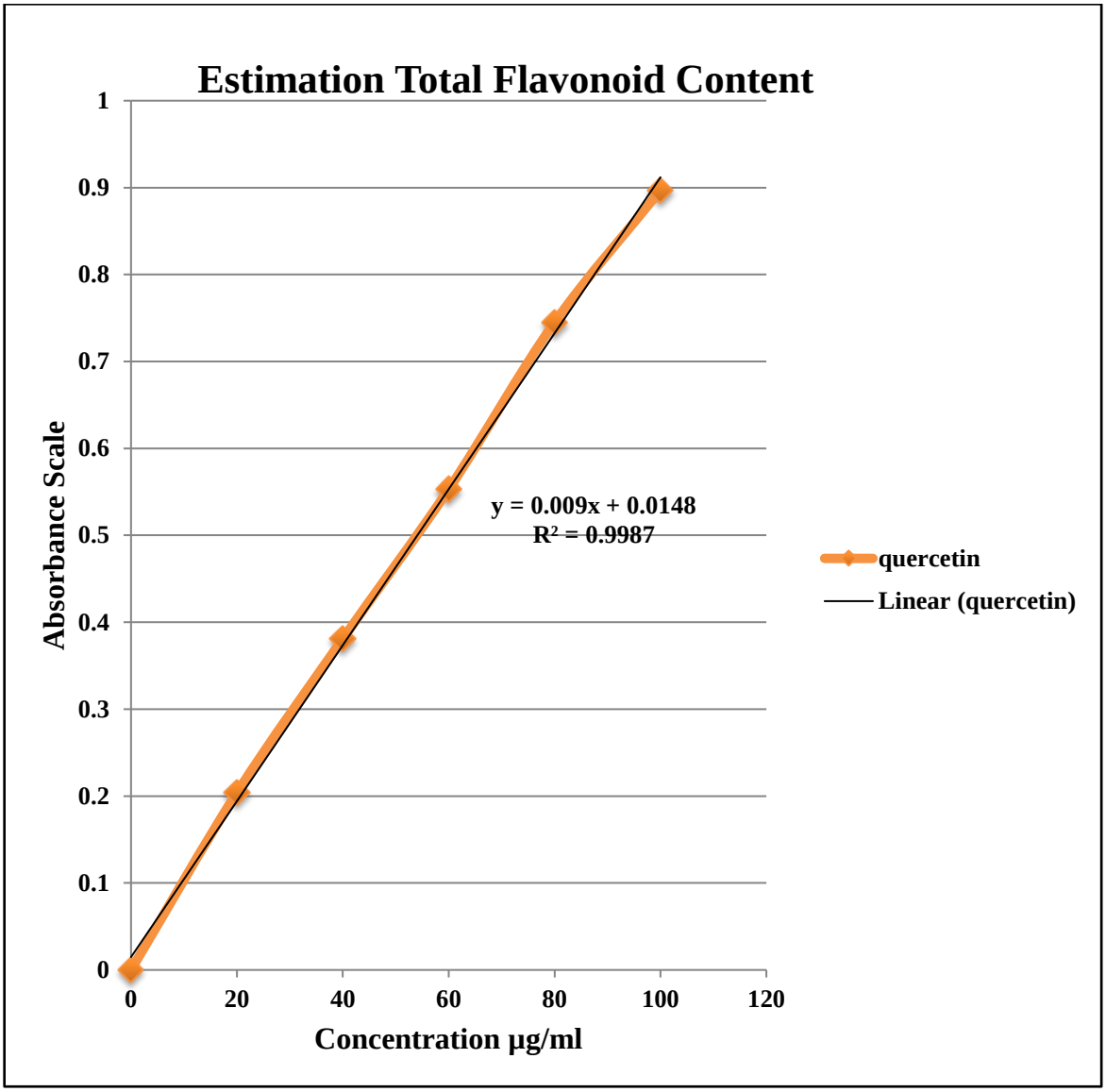

Figure 3: Graph of estimation of total flavonoids content

Column chromatography

The ethanolic leaves extract of *clematis triloba* were subjected and to perform the isolation from *Clematis triloba* leaves by using column chromatography. The solvent systems that were used for column chromatography was ethanol and chloroform.

Compound isolation and characterization from CTLE

The substance, which was isolated from the ethanolic leaf extract of *Clematis triloba*, was sparsely soluble in water but soluble in ethanol, chloroform, and methanol. The compound's chromatogram was created using ethanol, ammonium hydroxide solution, and water at a ratio of 8:1:1. On the TLC plate, the compound had a single orange colour spot. The chemical in the aforementioned solvent system had an Rf value of 0.45 at ambient temperature. The substance tested positive for the Liebermann-Burchard reagent, indicating that it is steroidal. The isolated compound's melting point was discovered to be between 286 and 290^{0}C. Additionally, the signals of the UV spectrum in ethanol, the infrared spectrum in KBr, and the nuclear magnetic resonance (1H-NMR) spectrum in $CDCl_3$ were studied in order to further analyze the structure of the compounds.

HPLC

The powder mixture solution of CTLE extract shows a 94% purity with drug.

T. L. C. study of CTLE

R_f value = Distance travelled by solute / Distance travelled by solvent.

Rf value = 3.2/ 7

= 0.45

Optimization of TLC

The best resolutions of spots for ethanolic extract of *clematis triloba* leaves were found in solvent system Chloroform (ethanol: ammonium hydroxide solution: water with ratio of 8:1:1 by single spot were found (Rf values 0.45). Results of chromatography were shown in figure no.4. The standard Rf value of ursolic acid is matched with our isolated sample. So, were confirmed the isolated compound was ursolic acid.

Physical Properties

Clematis triloba was purified of a light orange oil component on TLC with the Rf value of 0.45 (ethanol: ammonium hydroxide solution: water (8:1:1)).

Solvent system (ethanol: ammonium hydroxide solution: water (8:1:1))
Figure 4: TLC profile of the purified compound from *clematis triloba*

Structure elucidation using various spectral analysis

UV Spectrum

The purified chemical solution in ethanol's UV absorption spectra showed a maximum absorption at 210 nm. The visible region did not exhibit any absorption.

Table 5: Spectrophotometric data of concentration and absorbance for ursolic acid in ethanol.

Sr. No.	Conc. (microgram/ml)	Absorbance (nm)
01	0	0
02	5	0.1532
03	10	0.3023
04	15	0.4293
05	20	0.5730
06	25	0.7266

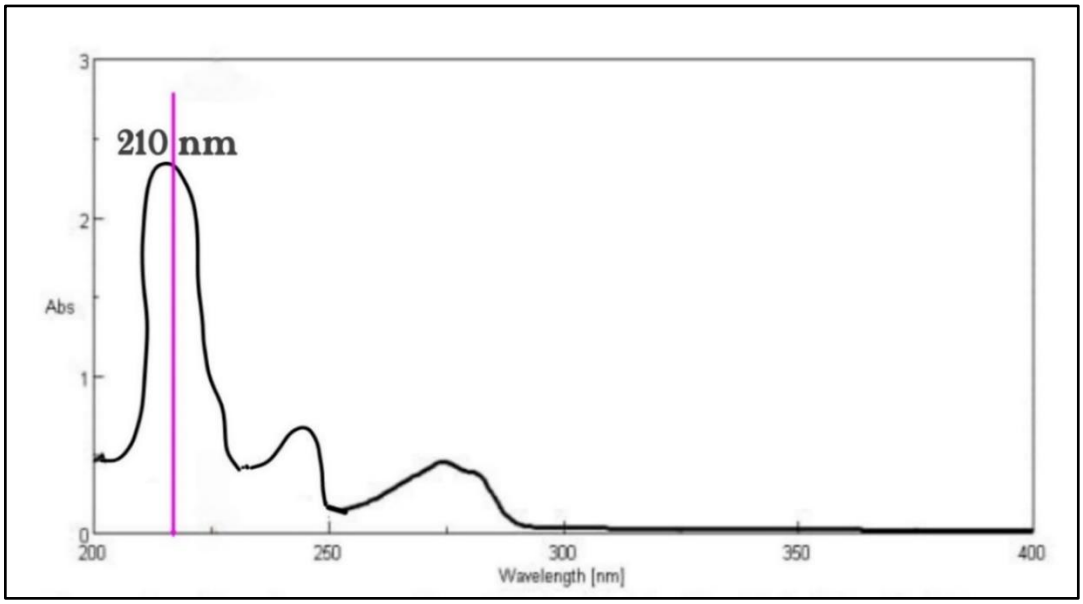

Figure 5: Calibration curve of ursolic acid in ethanol

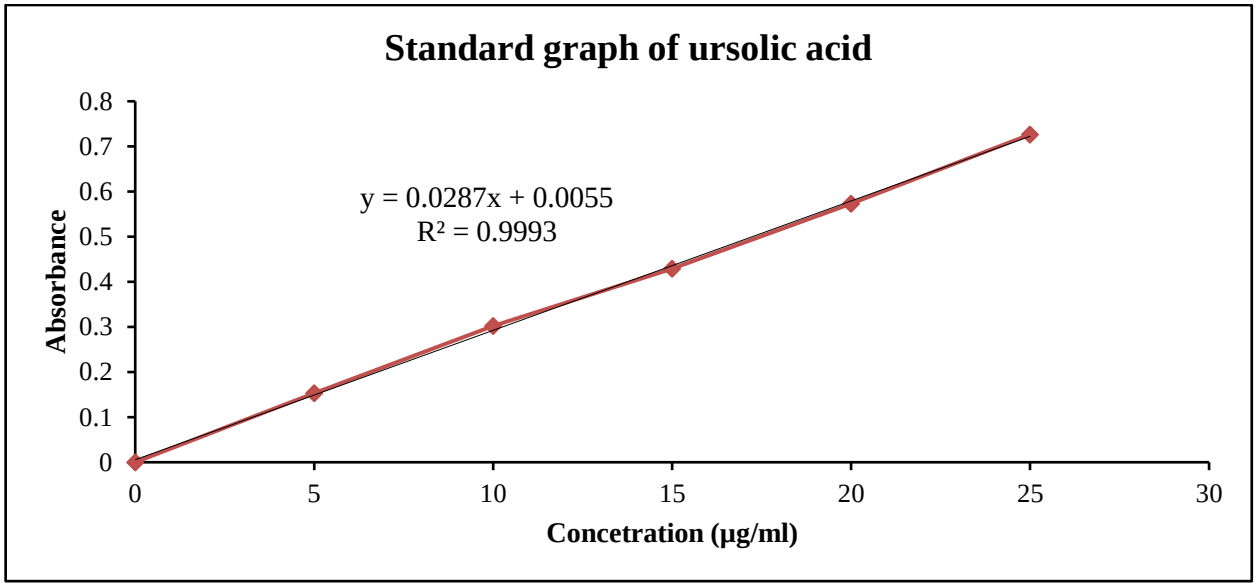

Figure 6: Standard graph of ursolic acid in ethanol

Table 6: Data for calibration curve parameters of ursolic acid in ethanol

Sr.No.	Parameters	Values
1	Absorbance maximum (λmax)	210 nm
2	Correlation coefficient (r)	0.999
3	Slope(m)	0.0287
4	Intercept(c)	0.0055
5	Regression equation	y = 0.0287x+0.0055

IR SPECTRUM

The purified compound's IR spectra showed an absorption band at vmax 2870-2530 cm^1. (carboxylic acid). A carbonyl group is shown by a prominent band at vmax 1670 cm^1. Strong bands between methyl group vmax 1485-1320 cm^1 and at 752 cm^1 indicate the ortho di-substitution of the benzene ring. (Figures 07)

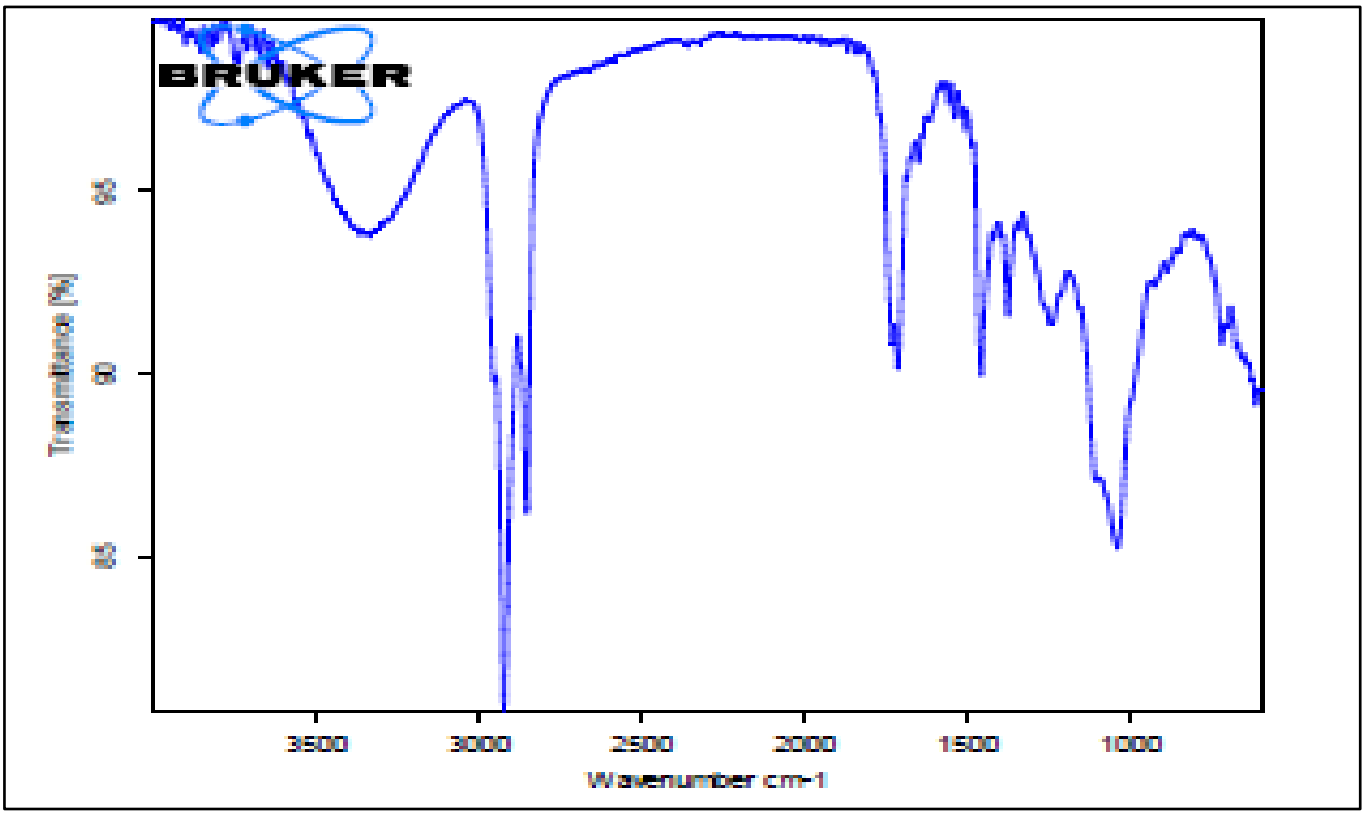

Figure 7: IR spectra of isolated compound (Ursolic Acid)

Table 7: Characteristics frequencies in IR spectrum of Ursolic acid

Sr. No.	Wave number (cm^1)	Inference
1	3334.2644	O-H stretching
2	2922.6864	R-O-H stretching
3	2853.2474	CH_3 stretching
4	1731.8823	C=O stretching
5	1039.6581	CH_2 stretching
6	720.0568	C-H bending
7	615.9081	OH bending

^{1}H NMR SPECTRUM

The ^{1}H-NMR spectra of UA show a peak at 5.35ppm of –OH group, 5.46 ppm of –OH group of –COOH, 2H of –CH=CH at 2.06-2.17 ppm, 0.84-1.95 ppm shows 34H pentacyclic triterpenoid in the purified compound (Figure 8)

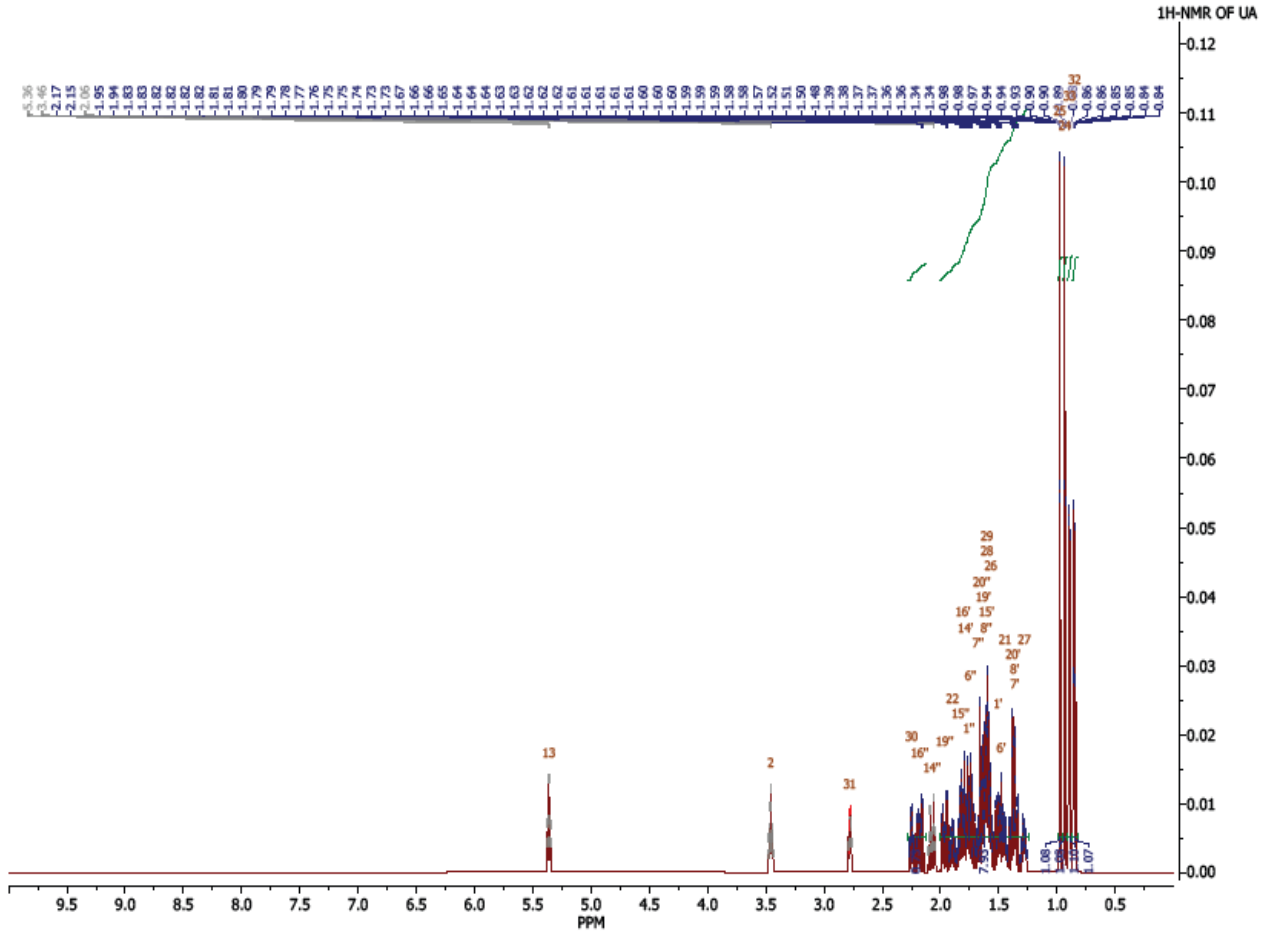

Figure 8: NMR spectra of isolated compound (Ursolic Acid)

MASS SPECTRUM

The purified Clematis triloba compound's MS spectrum revealed a molecular ion peak at m/z 457.368 (100, M+), demonstrating the purified compound's distinctive fragmentation peak. The presence of ursolic acid is shown by the M+1 peak in the MS spectrum at 457.37.(see figure 9&10)

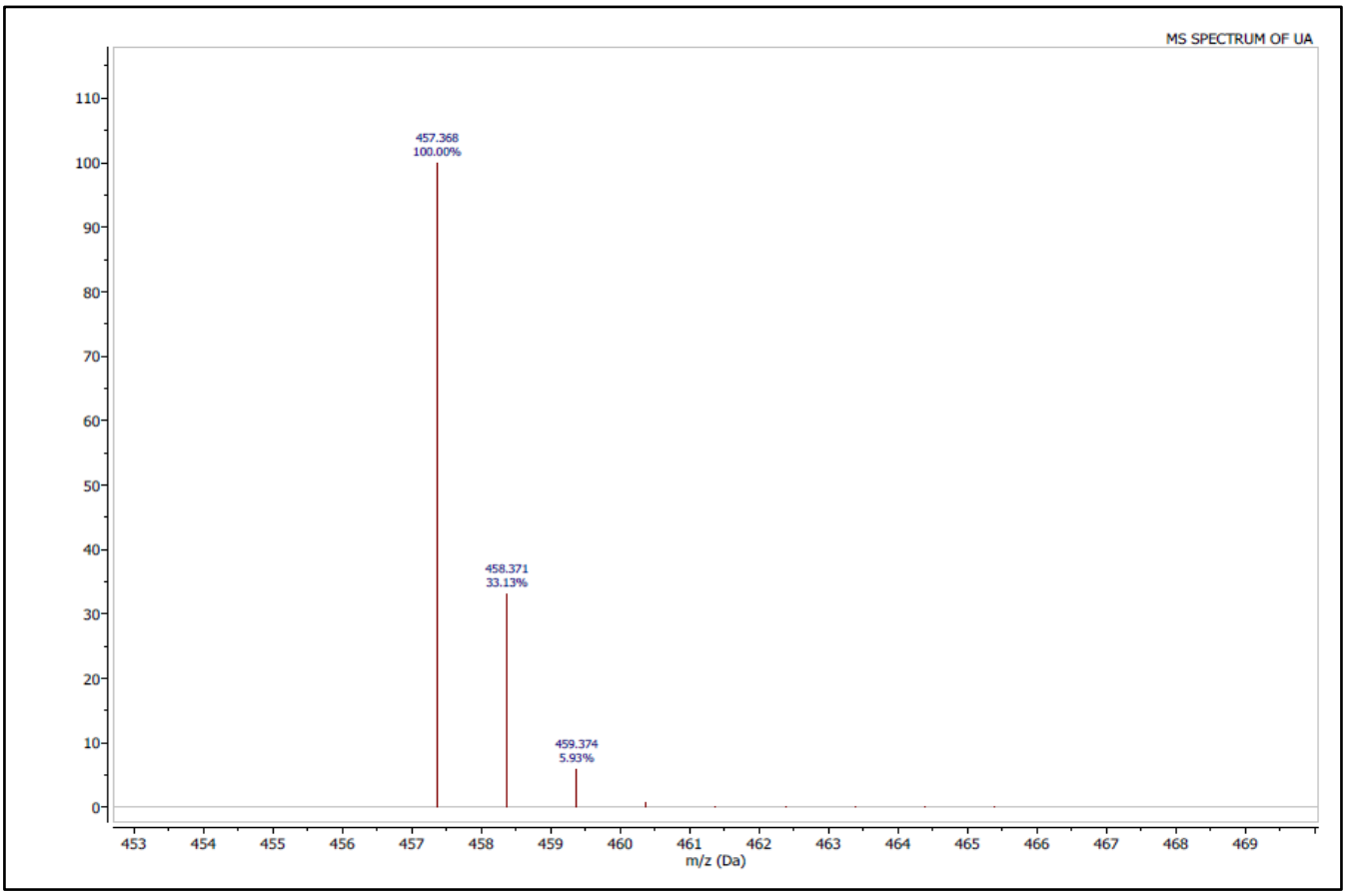

Figure 9 :Mass spectra of isolated compound (Ursolic Acid)

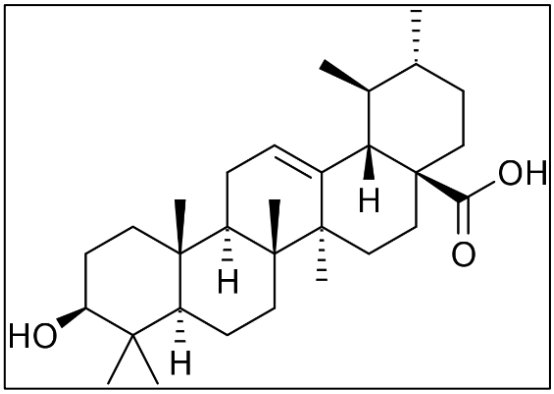

Figure 10: Ursolic acid

Melting point

The melting point of ursolic acid was determined by capillary tube method found to be 286-290 ^{0}C.

Solubility profile

Table 8: Solubility of isolated compound in different solvents

Sr. No.	Solvent	Solubility
1.	Ethanol	Soluble
2.	Methanol	Soluble
3.	DMSO	Freely soluble
4.	Water	Sparingly soluble

Pharmacological In-vitro Antioxidant Evaluation

DPPH Scavenging Activity

Based on the scavenging activity of the stable free radical DPPH, the potential antioxidant activity of the ethanolic leaf extract was assessed. The examined dry powder extract's capacity to decrease DPPH through antioxidant activity was only moderate. The ethanolic extract of *Clematis triloba's* percentage suppression of DPPH scavenging activity is shown in Table 9. *Clematis triloba* extract had a good free radical scavenging effect of 66.12 ±0.86% at 100 µg/ml compared to ascorbic acid's antioxidant efficacy of 84.89 ±0.93%.

Table 9: Free radical scavenging activity of *CTLE* by DPPH assay.

Sr. No.	Concentration (µg/ml)	Percentage inhibition	
		CTLE	Ascorbic Acid (Standard)
1.	20	18.27 ± 0.75	27.42 ± 0.23
2.	40	29.78 ± 0.24	42.98 ±0.47
3.	60	41.57 ± 0.78	57.24 ±0.36
4.	80	53.83 ±0.53	69.87 ±0.89
5.	100	66.12 ±0.86	84.89 ±0.93
6.	**IC_{50} (µg/ml)**	73.49 ± 0.69	50.86 ± 1.48

The data represented as the mean±SEM of each test run in triplicate.

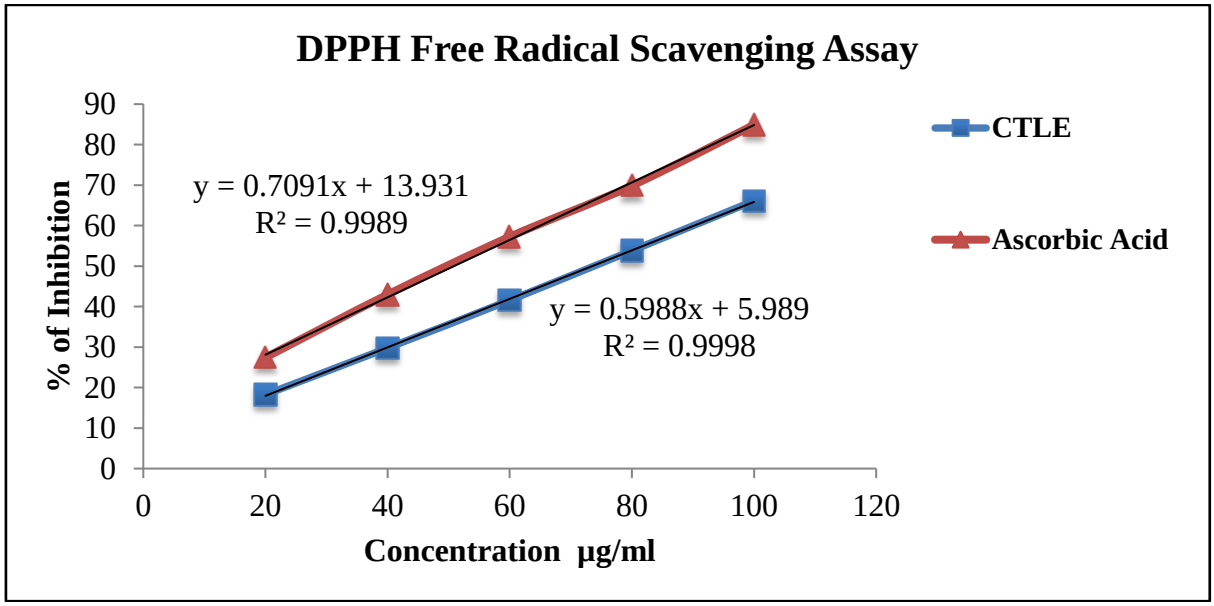

Figure 11: Percentage inhibition in DPPH free radical scavenging assay of *CTLE* at different concentration.

Hydrogen Peroxide (H_2O_2) Radical Scavenging Effect

The ethanolic extract of *Clematis triloba's (CTLE)* percentage suppression of H_2O_2 scavenging activity is shown in Table 10. The antioxidant effect of ascorbic acid was 86.93±0.69% at 100 µg/ml compared to the moderate free radical scavenging impact of 59.82±0.76% for the *CTLE*. Ascorbic acid and *CTLE* extract were found to have IC_{50} values of 52.84±0.48 µg/ml and 82.67 ±0.89 µg/ml, respectively. The extract has a strong reducing ability if the absorbance of the extracts and standard ascorbic acid increases as the

concentration of the extracts and ascorbic acid increases. The results showed that the extracts had only weak antioxidant action against hydrogen peroxide free radicals.

Table 10: H_2O_2 scavenging activity of ethanolic extract of *Clematis Triloba*

Sr. No.	Concentration (µg/ml)	Percentage inhibition	
		CTLE	Ascorbic Acid (Standard)
1.	20	13.42±0.46	23.46±1.17
2.	40	25.08±0.84	41.02±0.63
3.	60	37.83±0.58	55.27±1.08
4.	80	48.15±0.27	72.46±0.87
5.	100	59.82±0.76	86.93±0.69
6.	**IC_{50} (µg/ml)**	82.67 ±0.89	52.84±0.48

The data represented as the mean±SEM of each test run in triplicate.

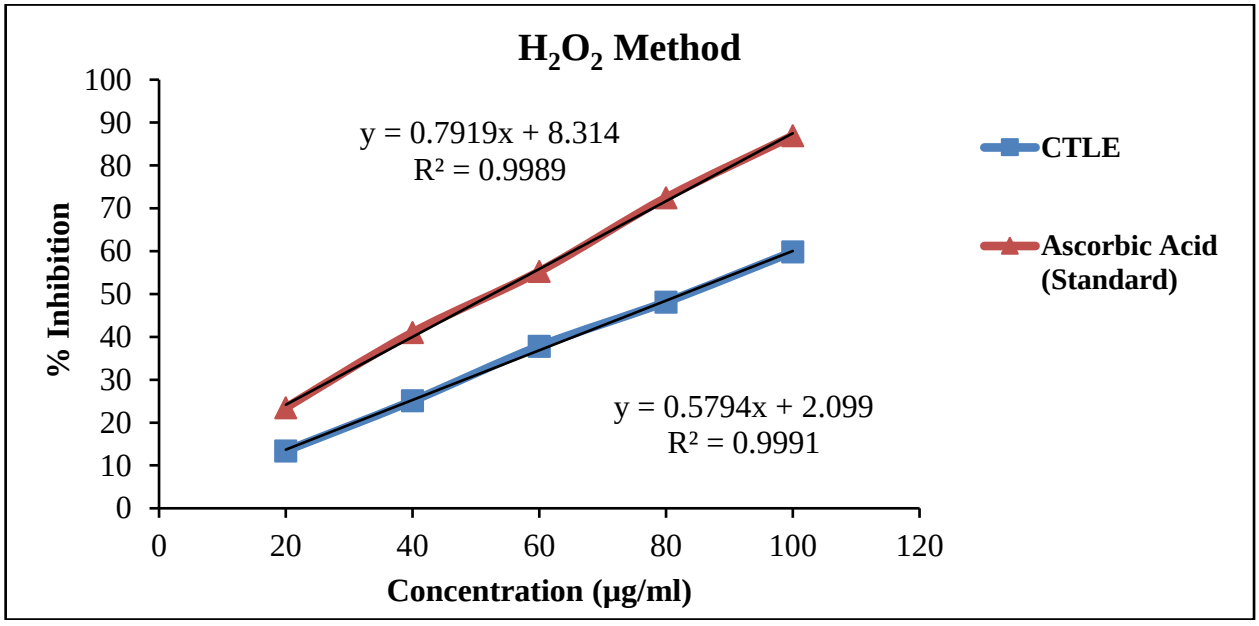

Figure 12: Percentage inhibition in H_2O_2 scavenging activity assay of CTLE at different concentration

Iron (Fe^{2+}) Chelating Activity (Reducing Assay)

The outcomes demonstrate the extracts' ability to donate electrons. The anti-oxidative potential of reduction's, which break the free radical chain by giving a hydrogen atom, is what determines a compound's reducing capability. The table below shows the ethanolic extract of *clematis triloba's* percentage inhibition of the iron (Fe^{2+}) Chelating Activity. The antioxidant effect of ascorbic acid was 81.38 ±0.63% at 100 µg/ml compared to the moderate free radical scavenging impact of 62.82 ±1.24% for the *clematis triloba* extract. Ascorbic acid and CTLE extract were found to have IC_{50} values of 57.15 ±0.83µg/ml and 79.35 ±0.42 µg/ml, respectively.

Table 11: iron (Fe^{2+}) Chelating Activity assay of ethanol extract of *Clematis Triloba*

Sr. No.	Concentration (µg/ml)	Percentage inhibition	
		CTLE	Ascorbic Acid (Standard)
1.	20	13.49 ±0.72	21.59 ±0.28
2.	40	26.24 ±0.57	37.26 ±1.37
3.	60	38.75 ±0.86	52.89 ±0.72
4.	80	49.63 ±0.93	67.54 ±1.21
5.	100	62.82 ±1.24	81.38 ±0.63
6.	**IC_{50} (µg/ml)**	79.35 ±0.42	57.15 ±0.83

The data represented as the mean±SEM of each test run in triplicate.

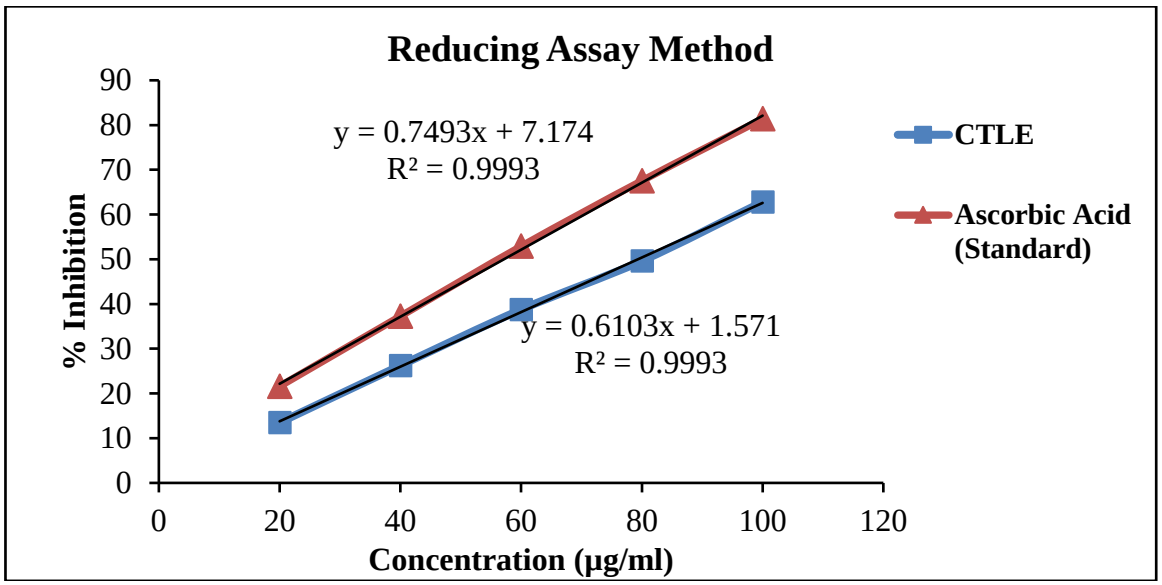

Figure 13: iron (Fe^{2+}) Chelating Activity assay of ethanol extract of *Clematis Triloba*

The selection of Ethanolic extracts for in-vitro antioxidant activity screening involved employing the DPPH technique, Hydrogen peroxide method, and assessing iron (Fe2+) Chelating Activity. The % inhibition of clematis triloba leaf extracts were determined. Comparative analysis with ascorbic acid revealed that the ethanolic extracts of *CTLE* exhibited the most significant scavenging activity, indicating higher antioxidant potential. Consequently, the CTLE were chosen for further pharmacological studies.

DISCUSSION

The *Clematis triloba* was extracted using the Soxhlet extraction method, which involved sequential extractions with chloroform, petroleum ether, water, and ethanol. The phytochemicals of the *clematis triloba* plant were examined using a petroleum ether extract, which yielded positive results for sterols and tannins. Saponins and alkaloids were found in the aqueous extract. Only tri-terpenoids and saponin are detected in the chloroform extract. Alkaloids, glycosides, flavonoids, saponins, phenolic chemicals, and triterpenoids were found in ethanol extract. The compound, isolated from the ethanolic leaf extract of *clematis triloba* was soluble in ethanol, chloroform, methanol, freely soluble in DMSO and sparingly soluble in water. The chromatogram of compound was developed in ethanol: ammonium hydroxide solution: water at the ratio of 8:1:1. The compound showed single orange color spot on TLC plate. The R_f value of the compound in above solvent system at room temperature was 0.45. The powder mixture solution of *CTLE* extract

shows a 94% purity with drug. The purified chemical solution in ethanol's UV absorption spectra showed a maximum absorption at 210 nm. The visible region showed no signs of absorption. A carbonyl group is shown by a prominent band at vmax 1670 cm^1. Strong bands between methyl group vmax 1485-1320 cm^1 and at 752 cm^1 indicate the ortho di-substitution of the benzene ring. The MS spectrum of the purified compound of *Clematis triloba* showed a molecular ion peak at *m*/*z* 457.368 (100, M+) indicating the very characteristic fragmented peak of the purified compound. The *CTLE* extracts showed free radical scavenging activity against DPPH, hydrogen peroxide, and the reducing power tests in the in-vitro antioxidant examination. The *CTLE* extract polyphenolic content, flavonoids, and other phytochemical components may be responsible for its overall antioxidant action. The results of the present investigation suggested that *CTLE* extracts may be a potential source of natural antioxidants that may be helpful as therapeutic agents in halting or slowing the advancement of reactive oxygen species and related oxidative stress-related degenerative diseases. The extract's antioxidant activity substantially correlated with its overall phenolic content, demonstrating that phenolic compounds play a significant role in the antioxidant activity of the extract. The mechanism of action may be the high polyphenolic contents, particularly flavonoids, which are responsible for antioxidant activity. Despite being taken orally for its anti-bacterial, antiviral, and anti-cancer properties, *clematis triloba's* ethanolic leaves extract has poor solubility, stability, and oral absorption.

CONCLUSION

Terpenoids, phenolic chemicals, and flavonoids are the main bioactive components of these plant species, and they are typically in charge of giving the plant species their distinctive and potent scents. These secondary metabolites are also in charge of supplying the antioxidant and anti-diabetic properties that help to advance the general well-being of humanity. This makes it especially important to extract, isolate, and characterize the bioactive components of *Clematis triloba* species.

The present investigation strongly supports the ethno-medical use of *Clematis triloba*. The present study investigated that, phytochemical composition, poly-phenolic content and antioxidant properties of the plant Clematis *triloba* which are used for therapeutic purposes. From the preliminary phytochemical screening it can be concluded that the Petroleum ether extract showed positive test for steroids and flavonoids. The aqueous extract showed the presence of tannins, saponins and alkaloids and ethanolic extract has potential secondary metabolites like Alkaloids, Carbohydrates, Sterols, Saponins, Tannins, flavonoids, Phenolic compounds, and terpeniods etc. From phytochemical screening, it was determined that the ethanolic extract of *Clematis triloba* contains potential secondary metabolites, i.e., bioactive chemical, ursolic acid which is responsible as potent pharmacological agent give antioxidant activity.

The pharmacology of *clematis triloba* has been studied in pre-clinical and clinical tests, and this area needs additional focus in the future. To ensure the safety, effectiveness, and mechanism of action of *clematis triloba* before therapeutic use, scientific validation of the traditional knowledge is crucial.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest to publish this manuscript.

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