PREPARATIVE ISOLATION, CHARACTERIZATION, AND ACUTE TOXICITY STUDY OF QUERCETIN FROM THE WHOLE PLANT OF BASELLA ALBA

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Abstract

Basella alba L.is a frequently utilised plant that belongs to the Baselaceae family. The purpose of the present research is to isolate and characterize the bioactive flavonoid quercetin from the hexane, ethyl acetate, and ethanol extracts of the whole plant of B. alba. Conventional qualitative techniques were employed for the purpose of conducting a phytochemical examination. The analysis of TFCs (total flavonoid contents) was conducted on the extracts of the entire plant. Following its isolationvia Preparative HPLC (High-performance liquid chromatography), quercetin underwent evaluation through various standard analytical techniques such as mass spectrometry (MS), HPLC, ^{1}H NMR (proton NMR), ^{13}C NMR (carbon NMR), among others. It was revealed that the ethyl acetate extract exhibited a higher concentration of flavonoids in comparison to the other extracts. The present investigation documented the very first extraction of quercetin from a comprehensive B. alba plant using ethyl acetate. Furthermore, it was observed that no potential toxicological effects were detected in the B. alba extract at doses of 300 milligrammes per kilogramme of body weight and 2000 milligrammes per kilogramme of body mass in female Swiss albino mice.

Keywords: Quercetin, Basella Alba, Total Flavonoid Content, Isolation, Preparative HPLC, Acute Toxicity Study.

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1. Introduction

The secondary metabolites found in nature are of great interest not only due to their medicinal properties that have evolved to combat various ailments, but also because of their potential as an essential element in the discovery of new medicines. Throughout history, nature has consistently provided us with various natural products that contain high levels of drugs such as quinine and taxol, which have been utilized to combat various disorders (Moundipa et al. 2005). Basella alba L. is currently considered a highly promising candidate for pharmaceutical use, owing to its rich content of pharmacologically significant compounds such as vitamin C, flavonoids, carotenoids, saponins, minerals, and amino acids(Premakumari et al. 2010).The naturally occurring substances derived from B. alba possess significant medicinal properties such as antibacterial, anti-inflammatory, antiulcer, anti-cancer, and wound healing effects. This substance exhibits properties such as central nervous system depression, antioxidant activity, androgenic effects, as well as cardioprotective and renal protective effects. In addition to alkaloids, plants contain a wide variety of other bioactive chemicals, including glycosides, saponins, tannins, sterols, phenolic components, quinines, terpenoids, flavonoids, carotenoids, and polyphenols. Nature contains three main classes of polyphenols that exhibit chemical diversity, namely chalcones, xanthones, and flavonoids. Medicinal herbs contain a plethora of naturally occurring active molecules such as sugars, amino acids, and minerals, which serve as fundamental constituents for primary drug analogues (Anandarajagopal et al. 2011, Selvakumaran et al. 2003). Recently, there has been a growing interest among scientists in exploring natural sources of biologically active molecules with therapeutic potential(Banerjee and Banerjee 2023, Banerjee et al. 2021a, Banerjee et al. 2021b, Banerjee et al. 2019, Padhy et al. 2022). Medicinal plants have emerged as a crucial platform for the development of life-saving drugs. The desire of such exploratory and investigative endeavours has resulted in an advancement in the evolution of novel pharmaceuticals. A plethora of plants are distributed worldwide, displaying significant levels of activity. Although chemically synthesized ultramodern drugs offer numerous benefits, the search for new medications derived from renewable resources remains a highly discussed topic due to their low cost and minimal adverse effects. Recent developments in phytochemical research have expanded the potential for novel drug development, attracting increased attention towards enhancing the efficacy of traditional pharmaceuticals and phyto-therapeutic interventions (Rahmatullah et al. 2011, Reddy et al. 2014). The synthesis of natural products poses an interesting challenge in organic chemistry due to their unique natural activities and chemical structures, which can only be isolated in limited quantities. Basella alba is currently considered to be one of the most intriguing herbs due to its numerous properties. The Basellaceae family encompasses two distinct species, namely B. alba L. (also known as Basella rubra Roxb.) and Basella rubra L.

The geographical distribution of this phenomenon encompasses a broad area in the tropical region of Asia. There are discernible distinctions between the flora of the African continent and those of tropical America. Basella alba L. is a botanical specimen that serves as a reservoir of secondary metabolites such as phytosterols, phenolic acids, and triterpenes. Additionally, it contains essential nutrients such as thiamine, riboflavin, and niacin, as well as minerals such as calcium, magnesium, and iron. Furthermore, it is a rich source of vitamins A, C, and B_9, amino acids, flavonoids, saponins, organic acids, and diverse antioxidants. The herb is employed in the management of diverse conditions such as purging, erythema, dermatological ailments, ingestion, ulceration, wound healing, gastrointestinal motility enhancement, anti-inflammatory effects, diuretic properties, and nephron protection (Kumar et al. 2018).The present investigation illustrates the process of producing diverse B. alba extracts, their phytochemical constituents, and developing an analytical methodology for discovering the qualitative and quantitative aspects of the flavonoid quercetin in the entire plant. Additionally, the study endeavours to establish the safety profile of quercetin and identify a

threshold level that is deemed safe and devoid of any adverse effects. The toxicity investigation is a non-clinical safety analysis method utilised to furnish data on the toxicological properties of test substances. Before being employed as experimental agents in clinical trials, chemical constituents must undergo toxicity screening of each component to establish their safety (Arome and Chinedu 2013). One expeditious approach for determining the toxicological profile of a medicinal plant is to conduct an acute toxicity assessment on it.

The safety and non-toxicity of Quercetin were established through investigations on acute toxicity, repeated dosage toxicity, and mutagenicity, as reported in literature(Aggarwal et al. 2016). The goal of toxicity impact analysis is to establish a reliable database for drawing conclusions about the toxicology of a substance. As per the literature study, acute toxicological assessment of quercetin was conducted in compliance with the OECD 423 guideline. The study established a sufficient safety margin and furnished comprehensive experimental data, indicating that the substance is safe, harmless, and non-toxic at doses of 300 milligrammes per kilogramme of body weight and 2000 milligrammes per kilogramme of body mass(Ruiz et al. 2006).

2. Material and Methods

Collection and authentication of plant materials: The entire plant of Basella alba L. was harvested when it was in the vegetative stage from pesticide-free crops in the vicinity of Kolkata, West Bengal, India. Shibpur, Howrah's Acharya Jagadish Chandra Bose Indian Botanic Garden certified the specimen's authenticity. The Plant material is authenticated from BSI and Authentication no is CNH/Tech.II/2019/9.

Preparing Plant Extracts: With the use of Soxhlet equipment, extracts of entire plants were made in a sequential method. Before being crushed, plant components were washed and dried in air at ambient temperature. To facilitate extraction, a total of 900 millilitres of three different solvents, namely hexane, ethyl acetate (ETA), and ethanol, were combined with 100 grammes of desiccated powdered material separately and allowed to soak for a period of 48 hours, based on their respective polarities. At the final stage of each extraction, the extracts underwent filtration using Whatman filter paper (Iwamoto et al. 1985). The filtrate was then condensed in an automatic rotary evaporator (Superfit-ROTAVAP, India) for 25 minutes at 40 °C underneath decreased vacuum as well as pressure. Thereafter, an estimate of the extracts' yield was determined.

Phytochemical Screening: Standard qualitative methodologies were used to conduct phytochemical screening for secondary constituents. The Harborne (Moundipa et al. 2005) and Eega (Premakumari et al. 2010) methods were used to screen for tannins, carbohydrates, cardiac glycosides, saponins, flavonoids, phlobatannins, triterpenoids, terpenoids, alkaloidas, anthraquinones, coumarins, proteins, glycosides, quinones, phenols, steroids, and phyto-steroids (Premakumari et al. 2010).

Estimation of Total Flavonoid Content (TFC): The TFC present in whole plant extracts, namely hexane, ethyl acetate, and ethanol, was quantified using the methodology outlined by Sakanakaet al. (Anandarajagopal et al. 2011). The quantification of flavonoid concentrations was conducted using the $AlCl_3$ method with quercetin as a standard reference (Siddique et al. 2010). The production of ethanol at a concentration of 1 milligramme per millilitre has been achieved through the utilization of extracts and quercetin. A solution was prepared by combining 0.1 ml of quercetin extract with 0.9 ml of distilled water in test tubes, followed by the addition of 75 µl of 5% sodium nitrite solution. In addition to a six-minute interval, 150 µl of a 10% solution of aluminium chloride was introduced into the reaction solution. Following a further 5-minute incubation period, the solution was subjected to treatment with 0.5 millilitre of a 1M solution of sodium hydroxide (NaOH). 2.5 ml of distilled water was added to the reaction solution and homogeneously blended. The wavelength of the absorbance at 510 nm was

expeditiously identified through employment of a spectrophotometer. The experiment was performed thrice, wherein different concentrations of quercetin (ranging from 20-140 µg) were utilized to determine the absorbance levels (Shantha et al. 2016). The data was presented as milligrammes of flavonoid quercetin equivalent or its corresponding value per gramme of dry extract weight.

Isolationof Quercetin: The process of purification serves as a fundamental basis for the synthesis of novel molecules and the exploration of new drugs. The accurate assessment of a drug's efficacy can only be achieved subsequent to its purification from extraneous contaminants.

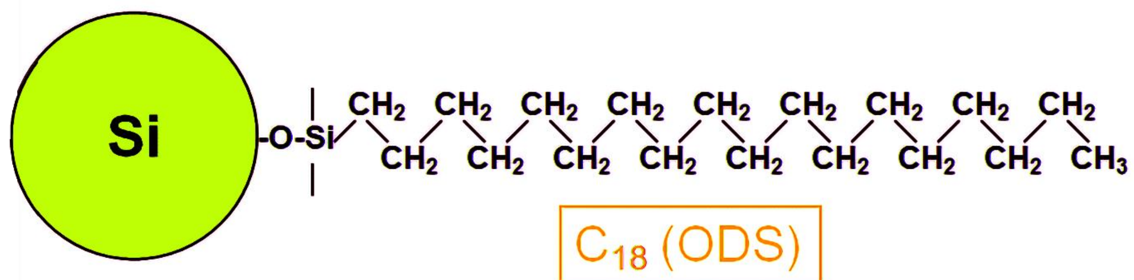
Thin layer chromatography (TLC): A twin through chamber was employed to develop thin layer chromatography (TLC) using various solvent systems, including toluene (methylbenzene), ethyl acetate (ethyl ethanoate), chloroform (trichloromethane), and methanol (methyl alcohol) at ratios of 9.5:0.5, 9:1, 7:3, and 1:1. Each fraction was subjected to analysis on a pre-coated aluminium plate of silica gel 60 F_{254}, with a thickness of 0.2 millimetres (Bláha et al. 2004). The adjustment of solvent proportions in order to develop an optimal solvent system led to the identification of a suitable solvent for compound detection. The R_f value was determined subsequent to immersion of the plate in a vanillin-sulfuric acid reagent for visualisation purposes (Salahuddin et al. 2010, Sharma et al. 2008).

The following formula was used to compute the retardation factor (R_f).

$$R_f = \frac{Distance\ travelled\ by\ the\ solute}{Distance\ travelled\ by\ the\ solvent}$$

Reverse Phase Preparative-HPLC (Modern isolation technique): The isolation of the flavonoid quercetin from an ethylacetate crude plant extract posed significant challenges when utilizing conventional column chromatography techniques. The isolation of pure quercetin was a highly intricate and time-consuming process, requiring significant attention to specifies in order to ensure accuracy. In this particular scenario, a prompt preparative isolation was deemed necessary in order to obtain a highly pure compound. Due to the presence of numerous undesired compounds in the crude ethylacetate extract alongside the target compound quercetin, a contemporary preparative isolation technique involving reverse phase chromatography was deemed necessary to obtain the target molecule in its purest form within a specified timeframe. Preparative reverse phase chromatography is a technique used for the separation and purification of compounds based on their hydrophobicity. This is a separation technique that relies on the differential partitioning of solutes between a liquid mobile phase and a liquid stationary phase that is coated on a solid support. Typically, support material comprises silica gel and was initially coated with a liquid stationary phase that exhibited low solubility in the mobile phase.

- Stationary phase: Low polarity(Hydrophobic)
  Octadecyl group-bonded silica gel (ODS)
- Mobile phase: High polarity
  Acetonitrile as solvent and 20 mM ammonium bicarbonate as a modifier.
  Column selection for quercetin isolation and ideal separation in reverse phase.
- C_{18} (ODS) type: Phenyl type



Effect of Chain Length of Stationary Phase:

- C_{18} (ODS), as long alkyl chain as strong for good separation.
- Rugged and highly retentive.

Figure 1.A Reverse Phase Preparative-HPLC

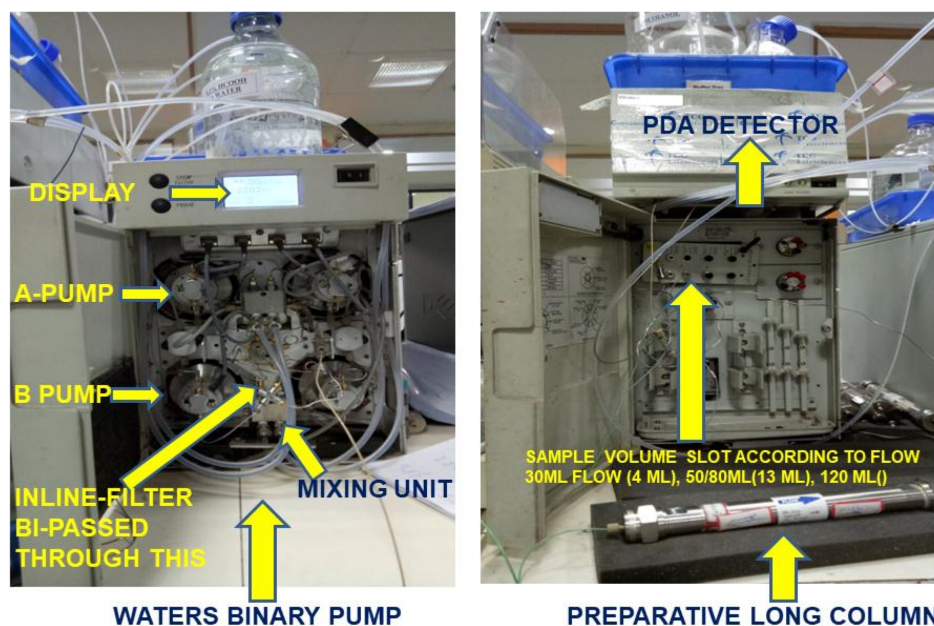


Figure 1.B complete Instrumentation of a Preparative-HPLC unit

Prior to commencing the preparative HPLC technique, an analytical pilot-scale development trial was conducted. The development of pre-purification analytical methods typically involves conducting small-scale pilot studies or trial-and-error experiments. In this study, a maximum of 10 milligrams of test sample quercetin was utilized to prepare a sample solution for analysis in a long YMC-Actus C_{18} analytical column (YMC; 4.6x150 mm, 5 µm particle size). The mobile phase consisted of Acetonitrile as the solvent and 20mM Ammonium Bicarbonate as the modifier, with a flow rate of 1 ml. The sample was dissolved in 1 milliliter of Dimethyl sulfoxide (DMSO) prior to analysis. In order to transition from analytical pilot scale to large scale preparative isolation and select an appropriate preparative column, it was necessary to undergo a scale up transformation that involved adjusting the flow rate, column internal diameter, and length. This was achieved by utilizing the following formula:

$$Scale\ up\ Factor = \frac{(Diameter\ Preparative)^2\ x\ Length\ \Pr eparative}{(Diameter\ Analytical)^2\ \ x\ Length\ Analytical}\ x\ Analytical\ flow$$

Upon scaling up from pilot to large scale, it emerged that the appropriate flow rate for isolation is 16 ml (using above mentioned formula).The preparative HPLC utilized for the isolations was a Waters 2545 series instrument (Waters (India) Private Limited, 36A, 2ndPhase, Peenya Industrial Area, Bangalore), which was equipped with a binary pump and a YMC-Actus C_{18} column (YMC; 250 x 20 mm, 5 µm particle size). A sample volume of 200 µL with a concentration of 25 mg/mL was subjected to repeated injections for the purpose of isolation. The mobile phase utilized in the experiment comprised of a combination of aqueous 20mM ammonium bicarbonate (pH: 8.7, solvent-A) and acetonitrile (solvent-B) with a flow rate of 16 mL/min. The mobile phase was initially composed of 60% A and 40% B solvent. This was followed by a transition to 20% A and 80% B over a period of 22 minutes. Subsequently, the composition was altered to 5% A and 95% B within 3 minutes, and this composition was maintained for 2.5 minutes for the purpose of column washing. The mobile phase was then returned to its initial composition over a period of 25 minutes and held constant until 27 minutes. The process of partitioning the quercetin molecule involved utilizing a hydrophobic stationary phase

composed of a long alkyl chain known as C_{18}(ODS or octadecylsilyl). Due to its polar nature, quercetin exhibits a higher affinity towards non-polar or hydrophobic stationary phases. Consequently, it competes with the polar, aqueous mobile phase, resulting in its rapid movement through the bed and early elution. Conversely, the non-polar component of the molecule exhibits a longer retention time in the column due to its weaker affinity towards the non-polar stationary phase. This results in a slower elution rate, as demonstrated in the chromatogram (Figure **6**). Each injection, consisting of 200 µL of sample solution at a concentration of 25 mg/ml, was administered at 27-minute intervals with a run time of 27 minutes for each consecutive run. The mobile phase was utilized to conduct the sample through an inline filter and into a sample loop with a volume of 5 ml. The study utilized the HPLC-2998 photo diode array detector (PDA) to monitor the constituents and fractions collected through the PDA threshold, targeting specific peaks and sorting them into the fraction lynx collector. The detector was set to wavelengths ranging from 190-400 nm to ensure accurate monitoring of individual components. The study involved the investigation of solvent front delay (SFD) in relation to the utilization of a specific flow rate of 16 mL. The sample was transferred from the PDA to the fraction collector within a predetermined time interval (in seconds) via a designated pathway, following detection of the specific peak wavelength. Subsequently, the desired fraction was subjected to lyophilization in order to obtain the dehydrated state of the pure compound. The fraction lynx collector is comprised of a total of four test tube racks, with each rack possessing an optimal capacity of 78 test tubes, each with a volume of 20 milliliters.The total numbers of test tubes in the fraction lynx collector was 312, representing its net capacity. The method software was utilized to manually incorporate the fraction collection volume prior to injection submission. This process was based on the crude profile of LCMS method development, which takes into account the close proximity of impurities merging with the desired compound in order to achieve an ultra-pure form. The preparative purification cycle employed a continuous, automated fraction re-analysis process with high throughput and yielded a significant sample recovery collection (generally 5 ml on each test tubes). This approach outperformed conventional column chromatography and other separation techniques.Finally, the sample was subjected to lyophilization in order to achieve dehydration and obtain a powdered specimen suitable for subsequent analysis and experimentation.

Figure 2. Collection of pure eluents in the test tubes

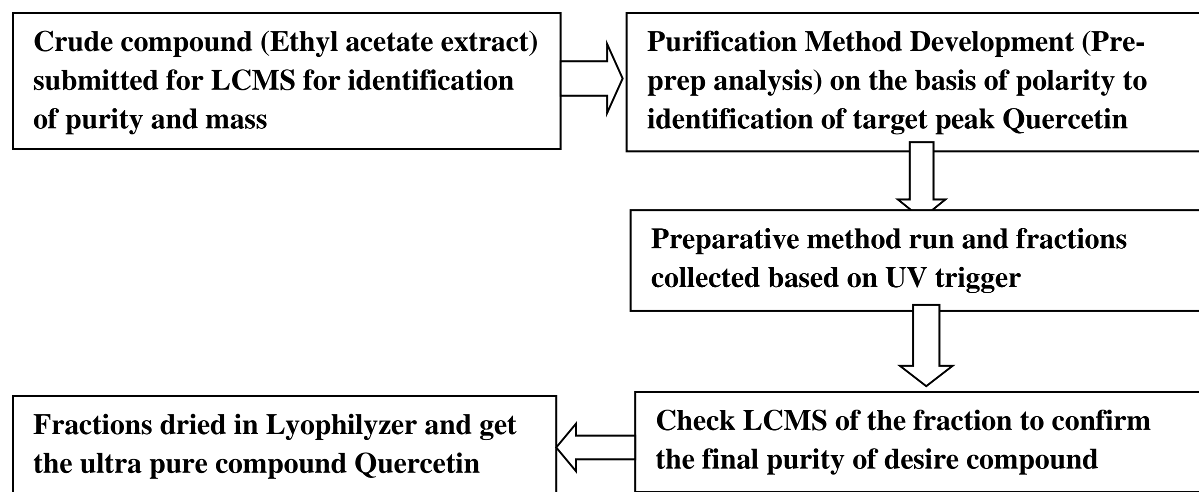


Figure 3. Schematic representation of Preparative HPLC

HPLC analysis of isolated Quercetin: A rapid and specific RP-HPLC method was developed utilizing an isocratic solvent system consisting of acetonitrile (CH_3CN) and 2% v/v acetic acid in a ratio of 40:60, with a pH of 2.6. The column temperature was maintained at 35 °C, and the flow rate was set at 1.3 ml per minute. Detection was performed at a wavelength of 370 nm using a UV detector. The HPLC analysis employed a Shimadzu-LC system (Manufacturer: Shimadzu, Japan), which consisted of an LC-Pump 20AT, a controller 20A, a detector 20AV, an auto sampler 20A, a DGU-20A5 prominence degasser, and a CTO10ASvp column oven. The authors utilized a ThermoHypersil Gold column, which is a 5-micron particle with an internal diameter of 4.6 millimetres and a length of approximately 250 millimetres, to achieve chromatographic separations (Ashraf et al. 2012). In order to ensure the protection of the analytical column, specifically the Zorbax Eclipse Plus, a guard column has been implemented. This guard column utilizes a replaceable or alternative cartridge with a C18 composition. The dimensions of this guard column were 12.5 millimetres in length, 4.6 millimetres in internal diameter, and a particle size of 5 microns. The present study utilized an isocratic elution technique in a reverse phase HPLC analysis employing gradient elution. The column temperature was maintained at 35 °C, while the flow rate was set at 1.3 ml per minute. The λ_{max} was determined to be 370 nm, and the mobile phase consisted of acetonitrile and 2% v/v acetic acid in a ratio of 40:60. Each solution was introduced via injection having volume of 20 µL (Paramasivam et al. 2009). The total run time for each injection was 18.5 minutes. The data was collected and processed using the LC-Solution Software. The solvents and distilled water were filtered using a nylon membrane (0.45 µm) in glass bottles with a vacuum pump (Model: Fisher brand FB 70155; Manufacturer: Fisher Scientific, UK) before use.

Characterization of quercetin

Fourier transforms infrared (FT-IR) spectroscopy: The analytical technique employed for the examination of quercetin was the potassium bromide (KBr) pellet method (Ashraf et al. 2012). Infrared spectra were obtained at a temperature of 25°C using an FT-IR spectrometer (Thermo Scientific, Waltham, USA) with a NICOLET NEXUS 470 model and DTGS detector. A total of 256 interferograms were obtained with a resolution of 4 cm^{-1}. The data was collected through the use of sixty-four scans and a 2 cm^{-1} gap, spanning from 4000 to 400 cm^{-1}. The system underwent periodic purging with dry air (Yang and Ren 2008).

UV spectroscopy (selection of λ_{max})

The measurement of absorbance was conducted using a Shimadzu 1800 dual beam ultraviolet-visible (UV-VIS) spectrophotometer, equipped with a pair of matched quartz cells with a width of 1 cm. In addition, the Shimadzu digital balance was employed to measure weight, while the Ultra sonicator (Manufacture: PCI Analytics

instruments) was utilized for the sonication of both the drug and sample solution (Chen et al. 2010).In order to generate a specimen of 10 μg/ml concentration, the quercetin compound was diluted utilizing methanol. The solution was subjected to a scan against methanol as a blank within the wavelength ranges of 200-400 nm. The maximum absorption value was determined and documented as λ_{max}(Fahlman and Krol 2009).

Nuclear magnetic resonance (NMR) spectroscopy: The study employed a Bruker AV NMR apparatus equipped with 5 mm ^{1}H and ^{13}C probes, capable of operating at 500MHz, to obtain the ^{1}H- and ^{13}C NMR spectra. Tetramethyl silane (TMS) was utilized as a standard in the analysis.

Mass spectroscopy: The evaluation of fraction compositions was carried out using an electrospray ionisation (ESI)—Liquid Chromatography-Mass Spectrometry (LC-MS) Triple Quadruple (TQ) system obtained from the Agilent 6400 series (Manufacture:Agilent Technologies, Beijing, China). The present study outlines the ideal identification parameters, wherein a chromatographic condition has been established to effectively ascertain the identity of individual substances (Mullen et al. 2004). The process of wavelength scanning was executed over a range spanning from 190 to 400 nm, while maintaining a flow rate of 0.4 millilitres per minute, utilizing a sample volume of 5 μl and a column temperature of 30 °C. The mass spectrometric conditions employed in the study involved the use of negative ion mode, with a dry gas velocity of 9 litres per minute, atomization gas pressure of 40 pounds per square inch, and a drying temperature of 350 °C. Electrospray ionisation was utilized with an ionisation voltage of 3000 volts, and a mass-to-charge scanning range of 200 to 800 was employed. The anion detection method was utilized, and an automobile MS^n approach was employed(Ishii et al. 2003).

Experimental animals: The protocol for this experiment was authorized by the NSHM Knowledge Campus' institutional ethics council under Regd. No. 1458/PO/E/11/CPCSEA-10. The OECD 423 guideline was followed for conducting the oral toxicity investigation. The experimental subjects were young adult female Swiss albino mice sourced from Kolkata. The mice were in a state of good health, were not pregnant, and had a weight range of 25-30 g. The experimental subjects were housed in polypropylene cages measuring 55 x 32.7 x 19 cm, which were maintained at a temperature of 23 ± 2ºC. The cages were furnished with sawdust bedding. The lighting system was programmed to alternate between 12 hours of light and 12 hours of darkness within a 24-hour cycle. Each cage was identified using a card. The aforementioned card documented various parameters related to the animal experiment, including the weight of the animal, the cage number, the code for the test substance, the route of administration, and the administered dose level. The animals were provided with standard laboratory animal food pellets and ample water (Lipnick et al. 1995).The animals were divided into two distinct groups comprising of six mice each, through a process of random or arbitrary selection. The markers were utilized in conjunction with a yellow dye to differentiate them. One of the mice was devoid of any markings, while the remaining mice exhibited distinct markings on their heads, bodies, and tails, thereby facilitating the process of observation (Halim et al. 2011).

A single dose of the experimental substance was administered to eachmouse. A specifically designed mice oral needle was used to gavage the test drug in a single dosage. Animals were fasted for 3 hours preceding to treatment (only food, not water, was withheld throughout this time). Two different dosage levels were administered, namely 300 milligram/kilogram and 2000 milligram/kilogram. The animals underwent daily toxicity symptom assessments over a period of 14 days. During the initial half-hour period and subsequently within the first 24 hours, with particular attention to the initial 4 hours, and on a daily basis thereafter for a duration of 14 days, individual animals were subjected to observation. The mice were subjected to regular monitoring, at a minimum of twice daily, to detect any signs of illness or deviations in behaviour, clinical manifestations, and other characteristics, like,

body weight, urine, food, and water consumption, as well as respiration, convulsion, tremor, and constipation.Female mice were preferred due to their higher sensitivity to therapy(Oduola et al. 2007).

3. Results

Determination of total yield and performing phytochemical study ofthe extracts

In addition to the process of harvesting, the plant samples were subjected to desiccation. Various solvents were employed to extract plant extracts. Table **1** presents the calculated yield of whole plant extracts, across different solvents. Table **2** delineates the attributes or traits of phytochemicals that are present and deficient in whole plant extracts.

Table 1.Final yields(gm) of the whole plant extract in different solvents

Extracts	**Yield(%)**
Hexane (C_6H_{14})	0.50
Ethyl acetate($CH_3COOC_2H_5$)	**22.752**
Ethanol(CH_3CH_2OH)	5.64

Table 2.Phytochemical analysis of whole plant extracts in various solvents

Analysis	**Ethanol** (CH_3CH_2OH)	**Ethyl acetate** ($CH_3COOC_2H_5$)	**Hexane** (C_6H_{14})
Saponin analysis/investigation	–	+	–
Carbohydrates analysis/investigation	+	+	+
Flavonoid analysis/investigation	+	+	+
Glycoside analysis/investigation	–	–	–
Alkaloid analysis/investigation	+	+	+
Triterpenoids analysis/investigation	–	–	–
Terpenoids analysis/investigation	–	–	–
Coumarins analysis/investigation	–	+	–
Phenols analysis/investigation	+	+	+
Steroids and Phyto-steroids analysis/investigation	–	–	–
Proteins analysis/investigation	–	–	–

(–) = Absence, (+) = Presence

TFCCalculation: The estimation of TFC was conducted by utilising the absorbance of the flavonoid quercetin Quercetin, a flavonoid compound (Figure **4**), was detected in all three solvent extracts. The absorbance results obtained from the flavonoid quercetin indicated that hexane and ethanol extracts displayed approximately equivalent TFC values, while ethyl acetate extracts exhibited a TFC value that was more than four times greater.

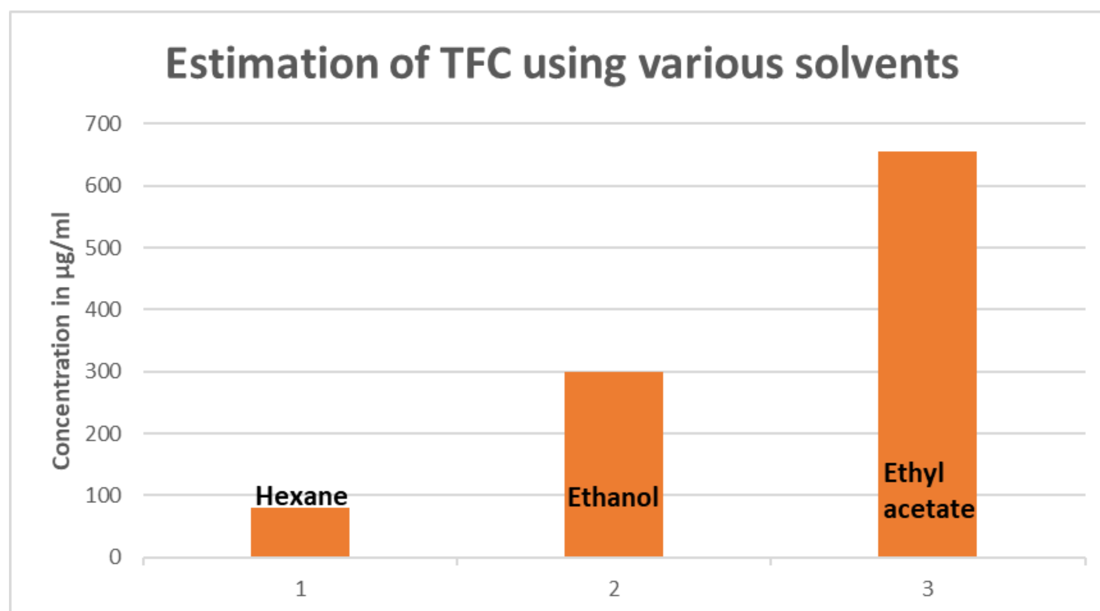


Figure4.TFC assessment employing various solvents

Table 3.Results of calibration curve

SL No	Concentration of plant Extract (µg/ml)	Absorbance at 415nm
1	403	0.094
2	1007	0.243

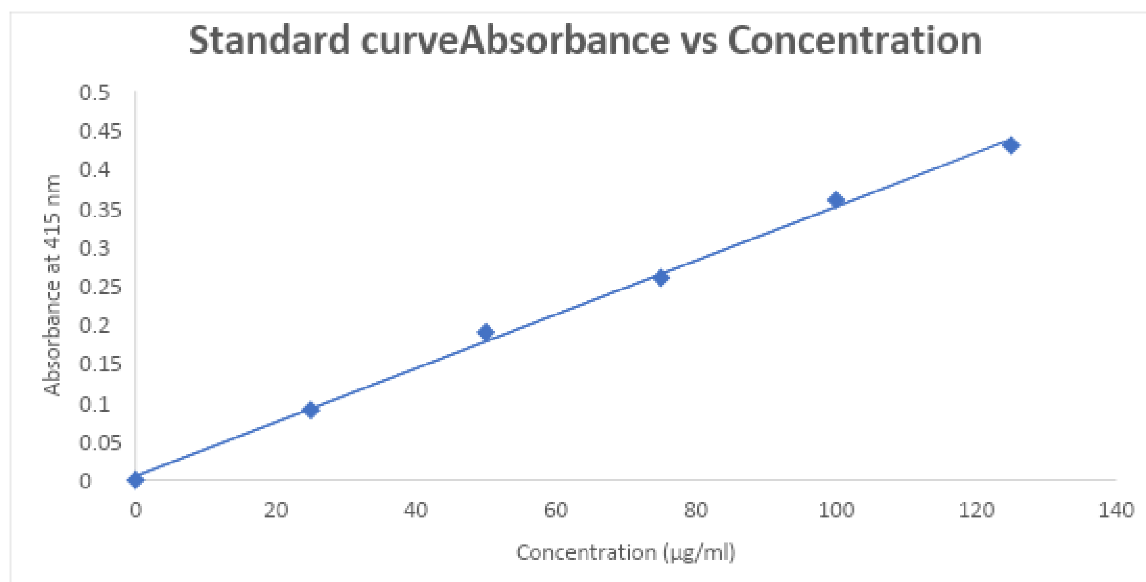


Figure5. Quercetin standard curve

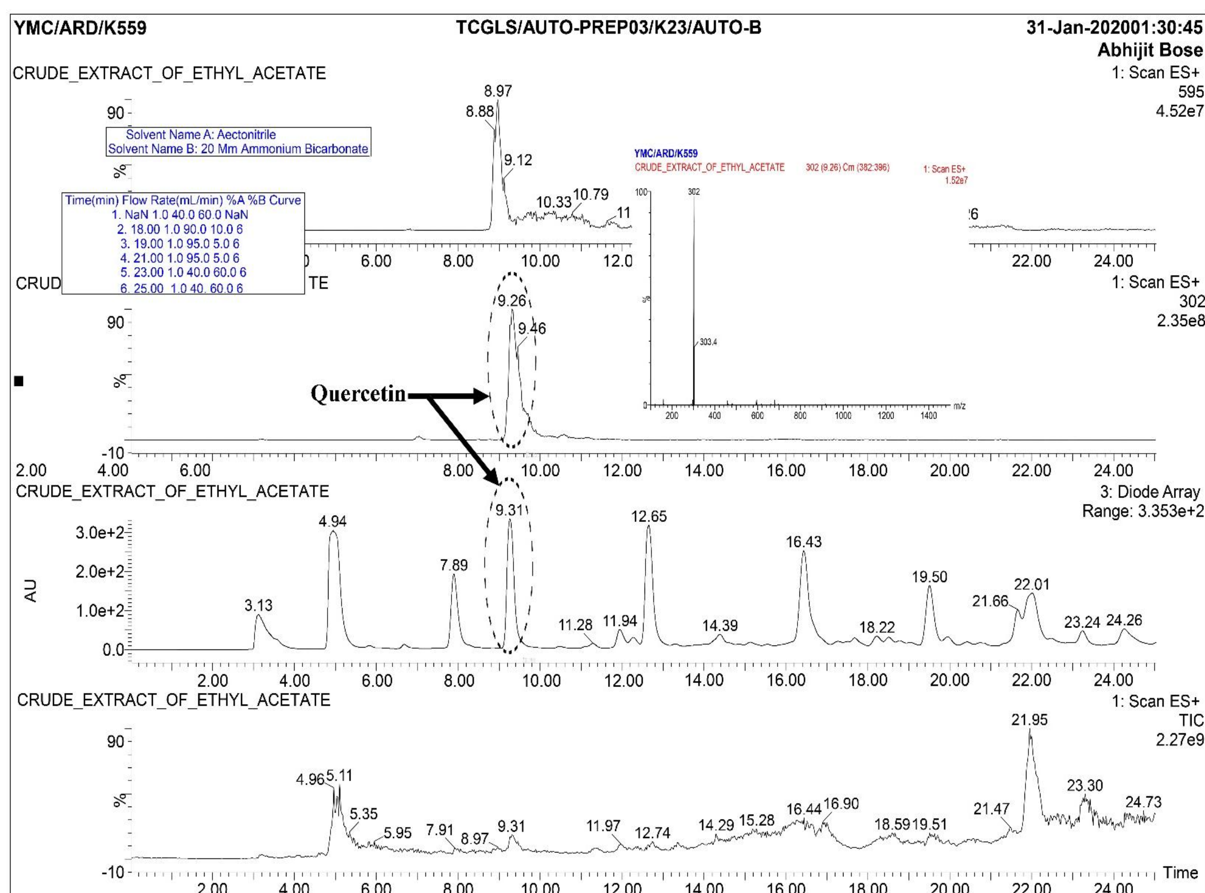
Table 4: Extract'sPercent yield and total flavonoid content

Extract	Yield(%w/w)	TFC (µg of QE/mg of extract)
Ethyl acetate extract of B. alba	8	9.48

Preparative-HPLC: The separation of all peaks of the extract was achieved through elution, which depends on their respective polarity, molecular mass, eluotropic strength, and structures. Quercetin, being a polar compound, exhibited a chromatographic behavior wherein it eluted in a polar region at 9.31 minutes (Retention time or RT) of retention time in PDA. Subsequently, it eluted other undesired peaks based on their respective polarities in the mid-polar and non-polar regions. The efficient pre-preparatory advancement of quercetin isolation necessitated subsequent purification on a large scale through preparative isolation procedures utilizing a PDA detector. This detector is non-invasive and minimizes sample loss during the purification process.

Following a fruitful pre-purification analysis, the quercetin peak of interest was obtained through mass triggerat a RT of 9.26 with no co-elution of extraneous compound peaks. Subsequently, the quercetin peak was eluted at approximately 9.31 minutes RT via UV/PDA trigger.The target product, quercetin, was identified by a single mass of M+H= 302, with no presence of impurities or extraneous masses (Figure **6**). According to the pilot study conducted during the analytical developmental stage, the target compound quercetin was successfully isolated and has been subsequentlyperformed in large-scale preparative isolation (Figure **7**).

Figure 6.Determination of quercetin peak before proceeding for Preparative-HPLC



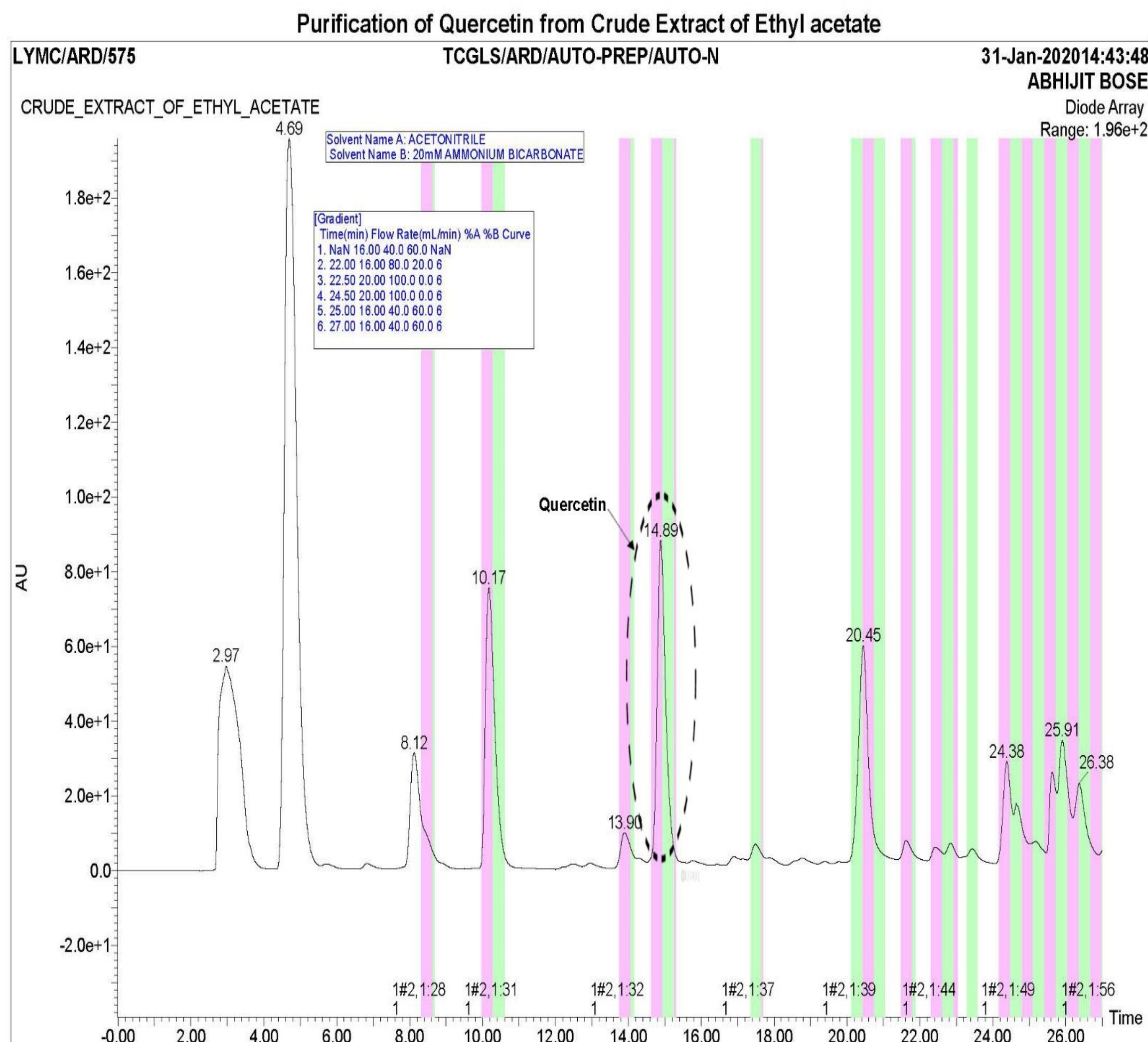


Figure 7.Isolation of quercetin by Preparative-HPLC

Fourier transforms infrared (FTIR): The FTIR spectra from the isolated substances are shown in Figure **8**, while Table **5** lists the distinctive or characteristic peak values. Phenol's highest peak of absorption was recognised as the —OH stretching vibration that occurred at approximately 3279 cm^{-1}. At 1665 cm^{-1}, C=O aryl ketonic stretching vibrations have been seen. The absorption maxima at 1520 cm^{-1}, 1608 cm-1, and 1456 cm^{-1} have been correlated to the C=O, C—C, and C=C aromatic stretching vibrations, respectively. We noticed the OH bending vibrations of phenol at 1380 cm^{-1}. The greatest absorption peak at 1317 cm^{-1} as well as the more modest or smaller absorption peaks between 950 cm^{-1} and 600 cm^{-1} were attributed to the C—H bending vibrations associated with aromatic hydrocarbons. TheC-O stretching vibrations of phenols and aryl ether are 1203 $cm^{-1}$and 1260 cm^{-1}, respectively-CO-C stretching and bending vibrations of ketones were detected at 1167 cm^{-1}, confirming that the isolated substance is the quercetin.The above finding is consistent with previous research on quercetin's molecular structure.

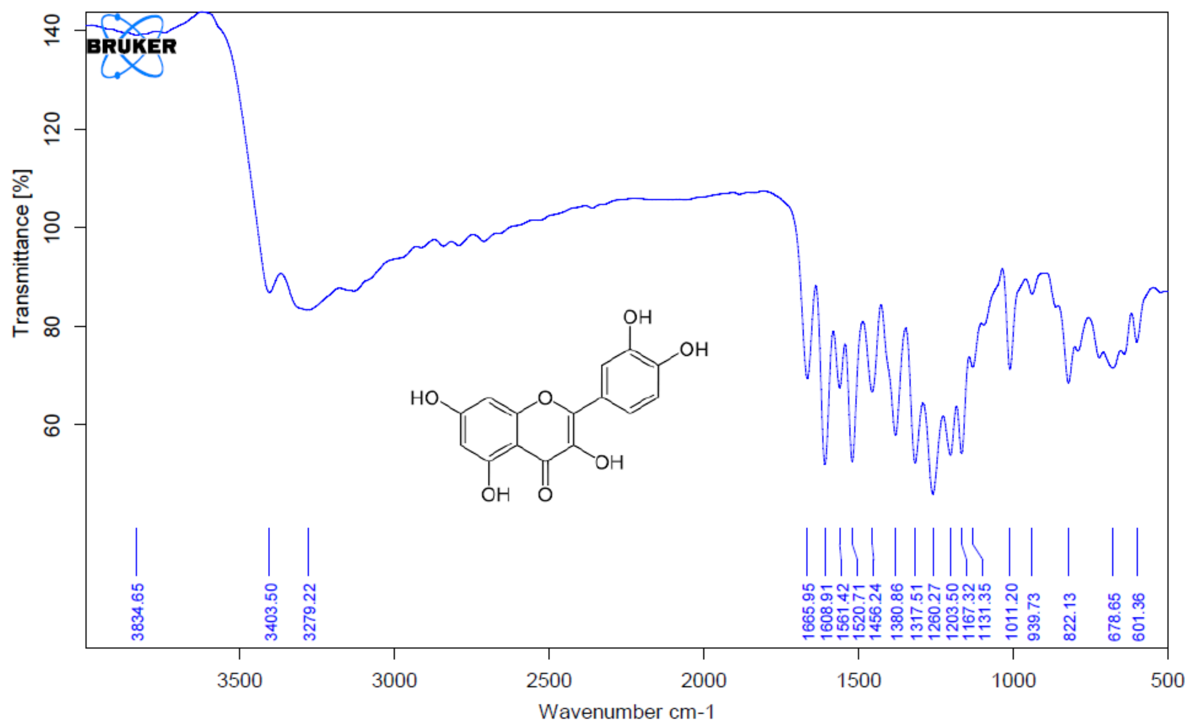


Figure8.Isolated compound's FTIR spectra

Table 5.FT-IR Peak values and probable bonds of isolated quercetin

Peak value	Bond type	Bond
939.73, 822.13, 678.65, 601.36	bending of aromatic hydrocarbons	C-H
1167.32	stretching and bending in ketone	C-CO-C
1203.50	stretching of phenol	C-O
1260.27	stretching of Aryl ether	C-O
1317.51	bond in Aromatic hydrocarbon	C-H
1380.86	bending of phenols	O-H
1456.24	aromatic stretching	C=C
1520.21	aromatic stretching	C=O
1608.91	Aromatic ring stretching	C---C
1665.95	Aryl ketonic stretching	C=O
3279.22	stretching vibration of phenol	O-H

NMR spectra: NMR spectroscopy was used to verify the binding site positions of carbons as well as protons. The isolated component's ^{1}H-NMR spectra had been more well resolved. Aromatic hydrogen and phenolic-OH groups seemed to be within the range from 6.1858 to 7.6714 ppm and 9.3423 to 12.4865 ppm respectively in the isolated compound's ^{1}H-NMR spectra (Figure**8**). Figure**9**shows the ^{13}C-NMR spectrum, which exhibits the carbonyl and the aromatic carbon group at 175.8579 ppm and 93.3952-163.9145 ppm respectively. Table **6** shows the respective^{1}H NMR and ^{13}C NMR spectral peak values of the isolated component. The peaks in the NMR spectra were found similar to pure quercetin, which was also verified by previous literature. As a consequence, this might be concluded that the isolated component is quercetin.

Table 6.^{1}H and ^{13}C NMR spectral peak values of isolated quercetin

^{1}H NMR	^{13}C NMR
12.4865(s, 1H, Ar-OH)	175.8579, (Ar-C=O)
10.7681(s, 1H, Ar-OH)	163.9145, (Ar-C)

9.5641(s, 1H, Ar-OH)	160.7485, (Ar-C)
9.3423 (s, 2H, Ar-OH)	156.1716, (Ar-C)
7.6714(d, 1H, J = 7.4 Hz, Ar-H)	147.7131, (Ar-C)
7.5448(q, 1H, J = 6.9 Hz, Ar-H)	146.8127, (Ar-C)
6.8925(d, 1H, J = 5.1 Hz, Ar-H)	145.0745, (Ar-C)
6.4053(d, 1H, J = 6.9 Hz, Ar-H)	120.0285, 122.0133, 135.7633, (Ar-C)
6.1858 (d, 1H, J = 7.2 Hz, Ar-H)	93.3952, 98.2262, 103.0468, 115.1039,115.6437 (Ar-C)

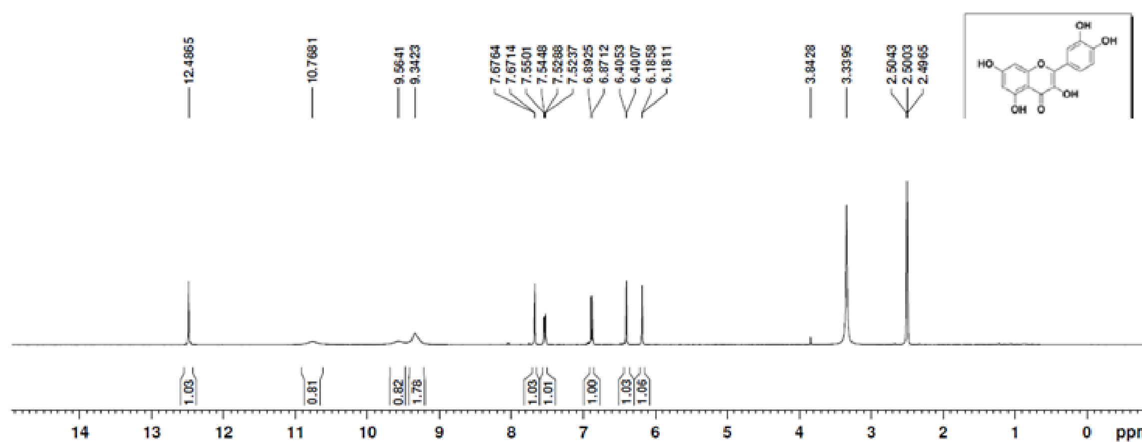


Figure9. Isolated compound's ^{1}H NMR Spectra

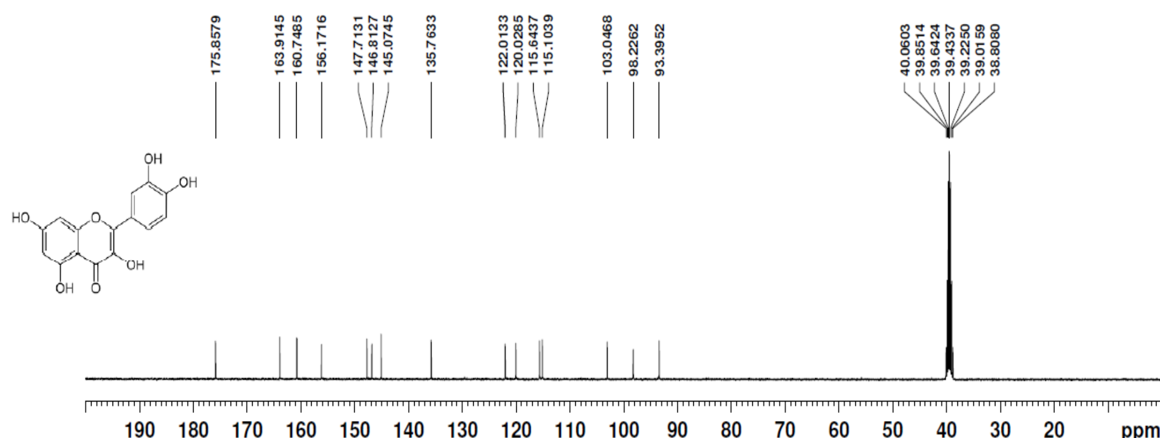


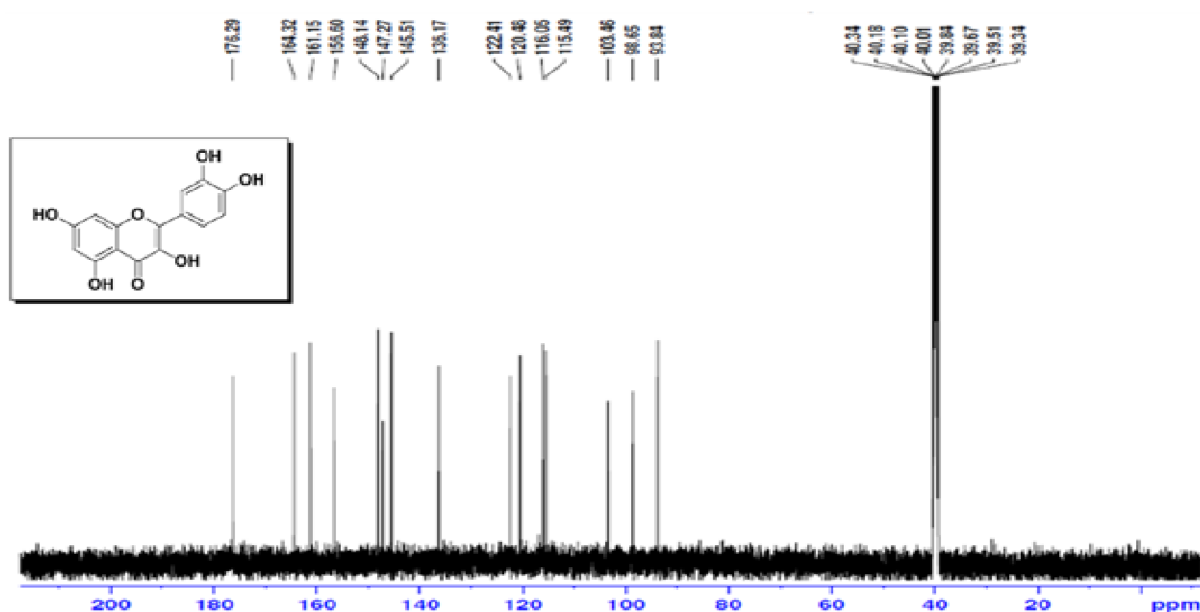
Figure10. Isolated compound's ^{13}C NMR Spectra

Liquid chromatography-tandem mass spectrometry (LC-MS-MS identification)

Liquid chromatography–mass spectrometryhad been used to investigate the isolated plant compound. It has been effectively used to separate and identify the isolated compound from B. alba (Indian spinach) in a shorter time period. The chromatogram of the isolated substance is displayed in Figure **10**. The fragmented pattern m/z 302 has been identified during its 1storder mass spectra, and it's hypothesized that it pertains to the quercetin's fragment pattern. The library system defined the substance that was isolated to be quercetin. Previous research has revealed an identical peak, confirming the isolated component is quercetin.

A molecular ion peak could indeed be seen at m/z 302 (M-1) that pertains to the compound's molecular weight of 301.

Figure11. Isolated compound's total ion chromatogram



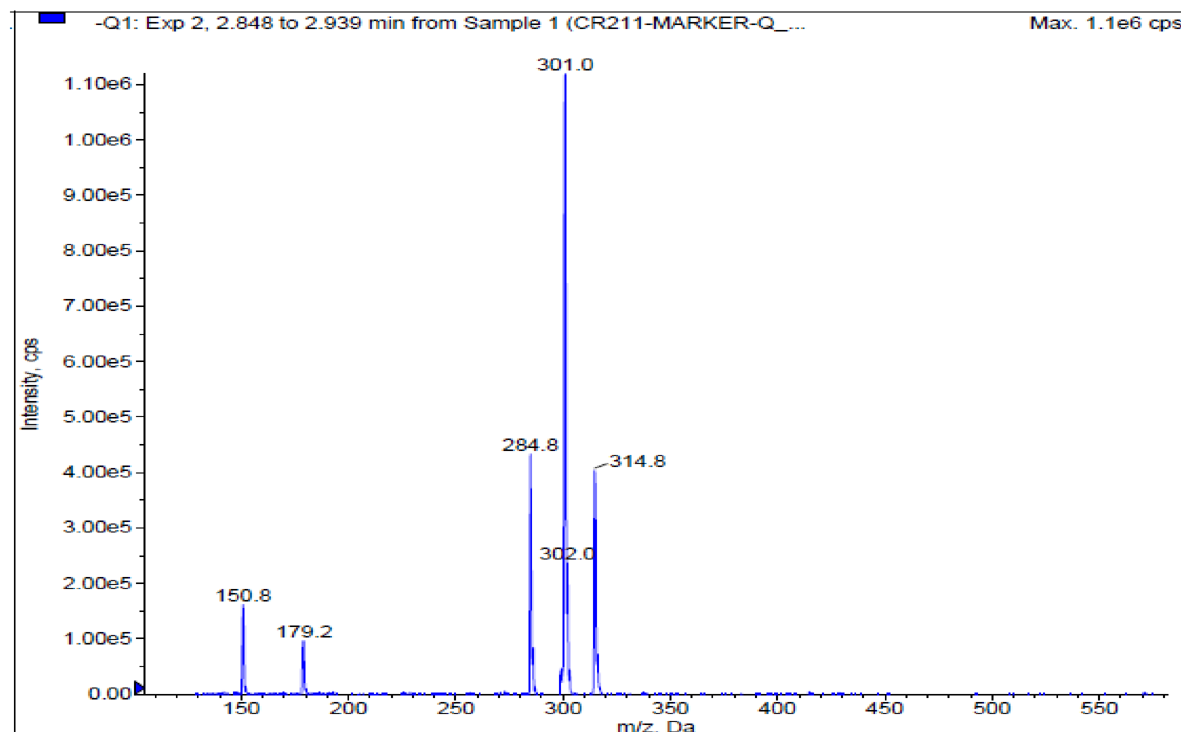


Figure12. Spectral analyses of isolated compound by Mass spectroscopy

UV Analysis

Determination of λ_{max}

Preparing standard stock solution

Standard stock solution has been made by solubilizing 100 mg of quercetin in a prepared solvent [n-butanol(C_4H_9OH): water: acetic acid] at 7:1:1 ratio. The final volume of a 100 ml volumetric flask was adjusted using the same solvent to obtain a quercetin solution (Stock A) of 1000 µg per millilitre.

Selecting the wavelength

One millilitre of standard stock "A" solution of quercetin was pipetted outinto a volumetric flask of 10 millilitre and diluted up to the mark

with the same developed solvent system mentioned above to obtain a concentration of 100 µg per millilitre (Stock B). The stock solution B was scanned between 200 and 400 nm respectively, and the maximum wavelength for absorption was determined to be 256.30 nm.

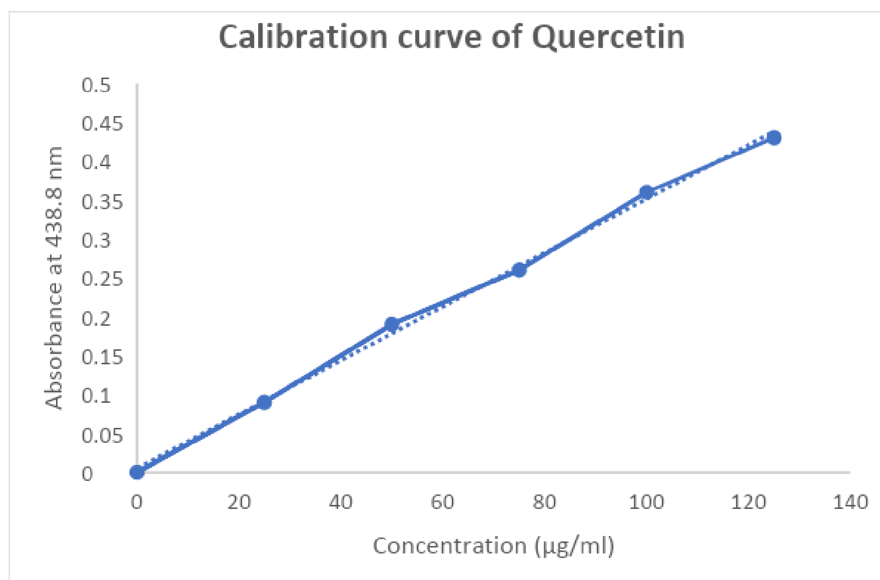


Figure 13. Calibration curve of standard quercetin

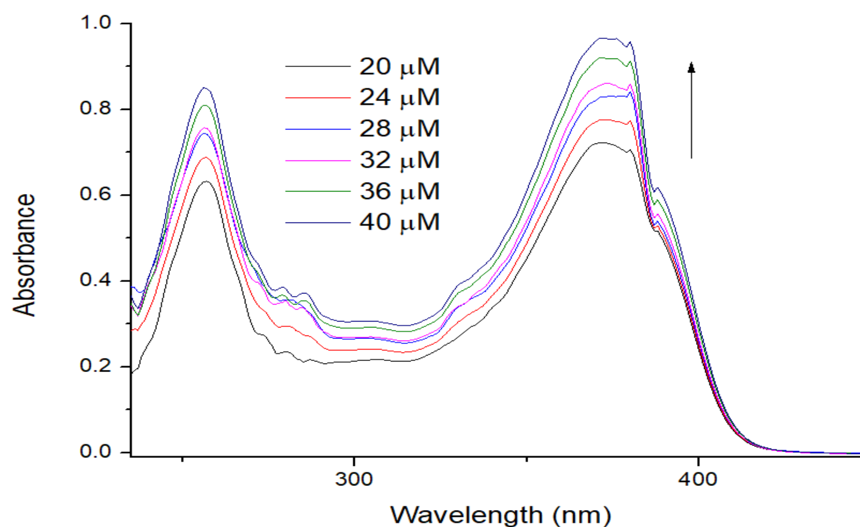


Figure 14. Linearity curve of standard quercetin

As a consequence, quercetin was effectively identified and quantified using UV spectrometry, which will aid in the standardization of various herbal formulations containing this active ingredient. The suggested UV technique is linear, accurate, and precise, and it could be utilized to measure the quercetin's concentration in a variety of samples from various herbs and formulations in a short period of time.

HPLC analysis of quercetin

Standard solution preparation

A 100 ml volumetric flask was precisely weighed with quercetin as the standard substance at a concentration of 100 parts per million (ppm). The flask was subsequently filled with methanol to reach the desired volume. A precise transfer of two millilitres was made into a volumetric flask with 10 millilitres capacity, and subsequent dilution with methanol was made to achieve the

intended volume. Therefore, a quercetin solution with a standard concentration of 20.6 µg/ml was made.

Calibration curve

2, 4, 8, 12, 16, and 20 µl of the standard stock solution were injected twice, correspondingly.then a calibration curve for quercetin was established. In order to assess linearity, the average peak area of quercetin based on different injection volumes were employed. The regression equation (y=74.805x) was then calculated, with a correlation coefficient (R^2) of 0.9999. An excellent linearity showed up when the quercetin content was between 41.2 and 412.0 µg/ml (Figure **15**).

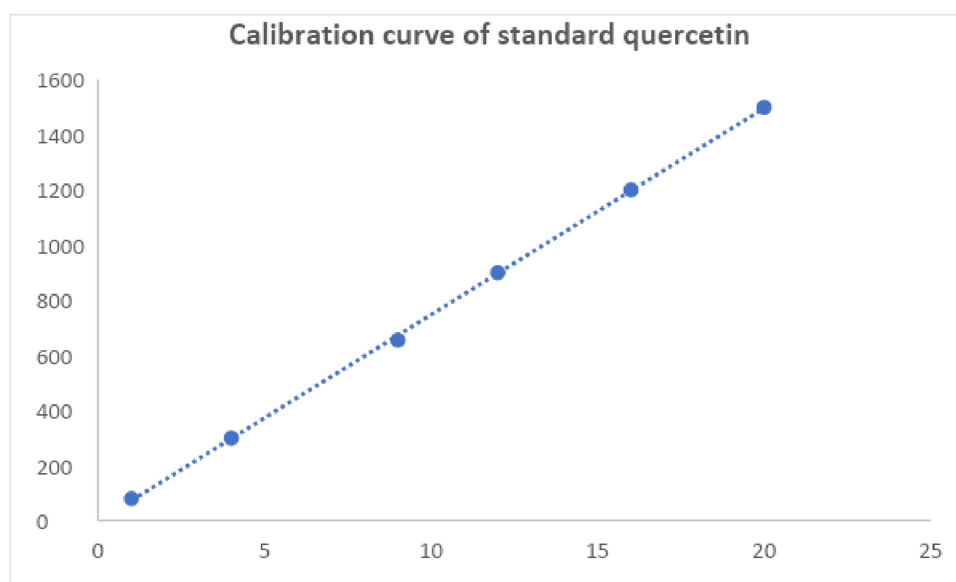


Figure 15. Calibration curve of standard quercetin

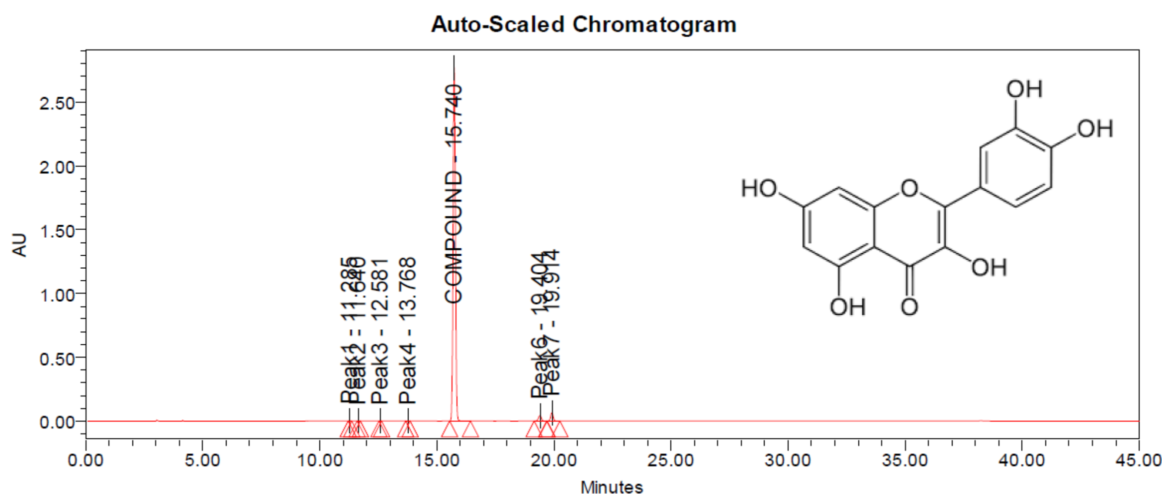


Figure 16.HPLC peak of standard quercetin

Acute Toxicity study of Quercetin

Observation period

Table 7. Observation-1 [Plain Eyes]

Changes in	Observation
skin	No change observed. Concerned parameter normal.

fur	No change observed. Concerned parameter normal.
eyes	No change observed. Concerned parameter normal.
mucous membranes	No change observed. Concerned parameter normal.
respiratory	No change observed. Concerned parameter normal.
circulatory	No change observed. Concerned parameter normal.
autonomic nervous system	No change observed. Concerned parameter normal.
central nervous system	No change observed. Concerned parameter normal.
somatomotor activity	No change observed. Concerned parameter normal.
behaviour pattern	No change observed. Concerned parameter normal.

Table 8. Observation-2 [plain eyes]

Observations of	**Comments**
tremors	Nil
convulsions	Nil
coma	Nil
diarrhoea	Nil
lethargy	Nil

Table 9. Dosing Protocol for introducing the test substances to female Swiss albino mice for an investigation on acute toxicity (300 milligram/kilogram)

Sl. No	**Group name**	**No. of animals**	**Group details**	**Dosing schedule**
1	Normal control (NC)	six	Acute toxicity study- “Normal Control Group”	Normal Saline
2	TG-A	six	Acute toxicity group- “A”	300milligram/kilogram

Table 10: Dosing protocol of Test substances to female Swiss albino mice for acute toxicity study (2000 milligram/kilogram)

Sl No	**Group name**	**No. of Animals**	**Group details**	**Dosing schedule**
1	Normal control (NC)	six	Acute toxicity study- “Normal Control Group”	Normal Saline
2	TG-B	six	Acute toxicity group- “B”	2000 milligram/kilogram

Table 11. Effect of test substances on physical and behavioural characteristics in mice during “14-day acute toxicity study”

Characteristics	**Normal control (NC) group observations**	**300 milligram/kilogram/day**	**2000 milligram/kilogram/day**
Body weight	22 g	22 g	22 g
Twenty-four-hour consumption of water	24 ml	23 ml	24 ml
Food consumption (24 h)	37g	40g	47g

Rate of respiration	No change observed. Concerned parameter normal.	No change observed. Concerned parameter normal.	No change observed. Concerned parameter normal.
Body temperature	No change observed. Concerned parameter normal.	No change observed. Concerned parameter normal.	No change observed. Concerned parameter normal.
Visible abnormalities	Nil(no visible anomalies)	Nil(no visible anomalies)	Nil(no visible anomalies)
Lethargy	No change observed. Concerned parameter normal.	No change observed. Concerned parameter normal.	No change observed. Concerned parameter normal.
Drowsiness	No change observed. Concerned parameter normal.	No change observed. Concerned parameter normal.	No change observed. Concerned parameter normal.
Diarrhoea	No change observed. Concerned parameter normal.	No change observed. Concerned parameter normal.	No change observed. Concerned parameter normal.
Urination	No change observed. Concerned parameter normal.	No change observed. Concerned parameter normal.	No change observed. Concerned parameter normal.
Stool colour	Deep Black	Deep Black	Deep Black
Eye colourPigmentation	No change observed. Concerned parameter normal.	No change observed. Concerned parameter normal.	No change observed. Concerned parameter normal.
Skin colour	Normal	No change observed. Concerned parameter normal.	No change observed. Concerned parameter normal.
Mucoid stool	Nil(no visible anomalies)	Nil(no visible anomalies)	Nil(no visible anomalies)
Mobility	34 min	35 min	34min
Rashes	Not noticed or monitored	Not noticed or monitored	Not noticed or monitored
Paw licking	Not noticed or monitored	Not noticed or monitored	Not noticed or monitored
Paw biting	Not noticed or monitored	Not noticed or monitored	Not noticed or monitored
Paw jumping	Not noticed or monitored	Not noticed or monitored	Not noticed or monitored
Mortality	Alive	Alive	Alive

Body weight and relative organ weight

Every other day across the entire duration of the acute toxicity investigation, the body weight was determined by means of a digital balance. After the 28-day period, the animals were put to death, and all of their organs were taken out. Each organ, including the heart, brain, liver, kidney, pancreas, gut, and lungs, had its blood evacuated before being weighed on a digital balance. The following approach was subsequently employed to calculate the relative organ weight (Pennock et al. 1973).

$$Relative\ organ\ weight = \frac{Organ\ weight}{Animal's\ weight\ on\ the\ day\ of\ the\ sacrifice} X\,100$$

Table 12. Effect of oral administration of Test substances on relative organ weight of mice

Organ	Distilled water(Control)	300 milligram/kilogram/day TS1(Test substance-1)	2000 milligram/kilogram/day TS2(Test substance-2)
Brain	1.763 ± 0.220	1.716 ± 0.208	1.844 ± 0.144
Heart	0.873 ± 0.038	0.81 ± 0.002	0.876 ± 0.066
Kidney (both)	1.247 ± 0.073	1.224 ± 0.046	1.25 ± 0.118
Liver	6.025 ± 0.432	5.94 ± 0.076	6.068 ± 0.450
Lung	1.293 ± 0.069	1.294 ± 0.044	1.303 ± 0.093

The outcomesvalues for every group of 5 mice were presented as means SD. For the study to figure out if there were statistically significant differences between the groups, a one-way ANOVA accompanied with a nonparametric paired t-test was employed. The values in the various treatment groups were determined to be non-significant ($p > 0.05$) when compared to the normal control group.

Haematological analysis

Under ether anesthesia, blood was taken from the posterior vena cava of every mouse through cardiac puncture (or wherever possible). Blood was put into simple bottles for clinical biochemistry analysis and into EDTA bottles for haematological assessment. Anautomatic haematological analyzermodel KX-21 (Manufacturer: Sysmex Corporation, Japan) was used to do an instantaneous analysis of the blood for the haematological examination. The parameters investigated were lymphocyte percentage, lymphocytes number, platelets, mean cell volume, mean corpuscular haemoglobin, white blood cells (WBC), haemoglobin (HGB), red blood cells (RBC), hematocrit (HCT), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC)(Gokarn et al. 2017, Wilkinson et al. 1969).

Table 13. Effect of Test substances on haematological parameter

Blood components	Distilled water(Control)	300 milligram/kilogram/day (TS1)	2000 milligram/kilogram/day (TS2)
Lymphocyte	78.258 ± 0.075	78.315 ± 0.266	78.35 ± 0.162
Neutrophils	17.598 ± 0.272	17.638 ± 0.631	18.334 ± 0.918
Monocyte	2.675 ± 0.246	2.68 ± 0.511	2.658 ± 0.458
Reticulocyte	1.6± 0.283	1.518 ± 0.179	1.538 ± 0.319
RBC (106/µL)	7.568 ± 0.255	7.585 ± 0.107	7.608 ± 0.242
WBC (103/µL)	8.83 ± 0.819	8.824 ± 1.044	8.87 ± 1.224
Platelet (103/µL)	1058.677 ± 25.339	1056.677 ± 54.078	1058.668 ± 54.118
MCV [L/L]	58.314 ± 0.333	58.363 ± 0.529	58.368 ± 0.389
PCV [L/L]	43.5 ± 0.249	43.348 ± 0.412	43.38 ± 0.245
MCH (pg)	20.047 ± 0.174	20.263 ± 0.289	20.277 ± 0.460
MCHC(gram/microlitre)	32.454 ± 0.333	32.494 ± 0.544	32.508 ± 0.591
Eosinophil's	1.615 ± 0.322	1.628 ± 0.378	1.66 ± 0.198
Basophils	0.515 ± 0.026	0.555 ± 0.102	0.578 ± 0.065
Hemoglobin	14.477 ± 0.353	14.564 ± 0.448	14.588 ± 0.294
Hematocrit	42.39 ± 0.384	42.42 ± 0.173	42.45 ± 0.129

The outcomes values for every group of 5 mice were presented as means SD. For the study to figure out if there were statistically significant differences between the groups, a one-way ANOVA accompanied with a nonparametric paired t-test was employed. The values in the various treatment groups were determined to be non-significant ($p > 0.05$) when compared to the normal control group.

Biochemical parameters

The following serum examinations were performed on serum samples in order to look at the investigating factors: serum glutamic pyruvate transaminase (SGPT) or Alanine transaminase (Ashraf et al.), serum glutamic oxaloacetic transaminase (SGOT) or aspartate aminotransferase (AST), serum alkaline phosphatase (ALP), and serum gamma-glutamyl transferase (GGT)(Fossati et al. 1986, Morand et al. 2000).

Table 14. Effect of Test substances on biochemical characteristics

Organ (Biochemical parameter)	**Distilled water(Control)**	**300 milligram/kilogram/day (TS1)**	**2000 milligram/kilogram/day (TS2)**
SGOT or AST (International unit per litre or IU per litre)	74.65 ± 3.822	75.208 ± 3.491	74.84 ± 3.812
SGPT or ALT (IU per litre)	43.989 ± 3.187	44.39 ± 2.711	43.708 ± 1.263
GGT (IU per litre)	0.418 ± 0.036	0.424 ± 0.040	0.418 ± 0.039
ALP(IU per litre)	564.25 ± 24.782	565.33 ± 24.717	571.454 ± 28.154

The outcomes (values) for every group of 5 mice were presented as means SD. For the study to figure out if there were statistically significant differences between the groups, a one-way ANOVA accompanied with a nonparametric paired t-test was employed. The values in the various treatment groups were determined to be non-significant ($p > 0.05$) when compared to the normal control group.

Protein estimation

The estimated amount of serum protein was carried out in order to look for changes in protein levels. The next characteristics were assessed. Albumin (Alb)(Mohamed et al. 1992), total protein (TP)(Ryan and Chopra 1976), and globulin (Glb). Serum globulin was produced by deducting serum albumin from serum total proteins and figuring out the albumin:globulin ratio (A/G ratio).

Table 15: Effect of Test substances on Protein content

Organ (Protein content)	**Distilled water(Control)**	**300 milligram/kilogram/day (TS1)**	**2000 milligram/kilogram/day (TS2)**
Total Protein (gram/decilitre)	6.335 ± 0.355	6.43 ± 0.234	6.27 ± 0.385
Globulin (gram/decilitre)	2.814 ± 0.116	2.824 ± 0.137	2.8 ± 0.096
Albumin (gram/decilitre)	2.574 ± 0.178	2.594 ± 0.091	2.594 ± 0.145
A/G (ratio)	0.918 ± 0.089	0.919 ± 0.035	0.928 ± 0.077

The outcomes (values) for every group of 5 mice were presented as means SD. For the study to figure out if there were statistically significant differences between the groups, a one-way ANOVA accompanied with a nonparametric paired t-test was employed. The

values in the various treatment groups were determined to be non-significant ($p > 0.05$) when compared to the normal control group.

Kidney function test

To monitor any changes in renal markers and electrolyte levels, kidney function tests were carried out. The following characteristics were calculated. The Jendrassik-Grof technique(Doumas et al. 1973) was used to assess the serum total bilirubin level. The Berthelot-Searcy technique(Fawcett and Scott 1960) was used to figure out the blood urea nitrogen (BUN). whereas the modified Jaffe technique was used to assess serum creatinine(Scharer 1977). While blood glucose, urea, and uric acid were all measured with commercially available diagnostic kits (Agappe Diagnostics Ltd., India), (Reckon Diagnostics Pvt. Ltd) respectively, sodium was investigated using an automated electrolyte analyser (Elyte-i5, Tulip Diagnostics (P) Ltd., India).

Table 16. Effect of Test substances on Renal function investigation

Organ (Kidney function test)	**Distilled water(Control)**	**300 milligram/kilogram/day (TS1)**	**2000 milligram/kilogram/day (TS2)**
Total Bilirubin content (milligram/decilitre)	0.514 ± 0.032	0.514 ± 0.032	0.514 ± 0.039
BUN(milligram/decilitre)	16.54 ± 0.408	16.554 ± 0.324	16.32 ± 0.376
Glucose level (milligram/decilitre)	133.668 ± 6.808	136 ± 5.242	134.32 ± 3.823
Sodium (milimole/litre)	134.52 ± 1.44	133.64 ± 0.89	136.48 ± 1.84
Total Creatinine level (milligram/decilitre)	0.45 ± 0.112	0.464 ± 0.106	0.438 ± 0.128
Uric acid level (milligram/decilitre)	2.05 ± 0.42	2.34 ± 0.51	2.84 ± 0.85
Urea (milligram/decilitre)	47.85 ± 2.45	46.92 ± 1.68	48.54 ± 2.58

The outcomes (values) for every group of 5 mice were presented as means SD. For the study to figure out if there were statistically significant differences between the groups, a one-way ANOVA accompanied with a nonparametric paired t-test was employed. The values in the various treatment groups were determined to be non-significant ($p > 0.05$) when compared to the normal control group.

Lipid profile attributes

Analyses were conducted on triglycerides (Gokarn et al.), low density lipoproteins (LDL), high density lipoproteins (HDL), and very low-density lipoproteins (VLDL). along with total cholesterol (TChol). The assessment was completed utilising semi-automatic analyzers and widely accessible diagnostic kits (BC300, Contec, China).

Table 17. Effect of Test substances on lipid profile

Organ(Lipid profile)	**Distilled water(Control)**	**300 milligram/kilogram/day (TS1)**	**2000 milligram/kilogram/day (TS2)**

LDL (milligram/decilitre)	35.95 ± 2.447	36.588 ± 2.115	35.809 ± 1.708
HDL (milligram/decilitre)	50.136± 3.254	51.378 ± 1.557	51.092 ± 8.835
VLDL(milligram/decilitre)	54.587 ± 2.976	53.556 ± 2.865	52.282 ± 3.853
Triglyceride level(milligram/decilitre)	95.728± 3.423	95.204 ± 3.486	94.822 ± 1.256
Total Cholesterol level(milligram/decilitre)	87.95 ± 6.158	86.696 ± 5.334	86.766 ± 9.942

The outcomes (values) for every group of 5 mice were presented as means SD. For the study to figure out if there were statistically significant differences between the groups, a one-way ANOVA accompanied with a nonparametric paired t-test was employed. The values in the various treatment groups were determined to be non-significant ($p > 0.05$) when compared to the normal control group.

Antioxidant enzyme estimation

Erythrocytes and their membranes weren't included in the experimental and control groups, almost altering the Dodge et al. methodology(DePierre and Karnovsky 1973). The hemolysate was used to assess the activity of the antioxidant enzymes glutathione peroxidase (GPx) (Moron et al. 1979), superoxide dismutase (SOD) (Beers and Sizer 1952), GSH (Fukatsu et al. 1999), and catalase (CAT) (Rotruck et al. 1973).

Table 18. Effect of Test substances on antioxidant enzymes

Antioxidant enzyme	**Distilled water(Control)**	**300 milligram/kilogram/ day (TS1)**	**2000 milligram/kilogram/ day (TS2)**
GSH mg/dl(glutathione)(milligram/decilitre)	4.18 ± 0.03	4.32 ± 0.04	4.23 ± 0.03
GPx (units per gram of haemoglobin) (U/gHb)	33.72 ± 1.09	35.42 ± 1.63	36.72 ± 1.25
SOD(units per gram of haemoglobin) (U/gHb)	348.09 ± 21.34	354.53 ± 27.96	359.39 ± 29.11
CATALASE (units per gram of haemoglobin)(U/gHb)	64.52 ± 2.89	65.43 ± 2.82	71.37 ± 3.18

The outcomes (values) for every group of 5 mice were presented as means SD. For the study to figure out if there were statistically significant differences between the groups, a one-way ANOVA accompanied with a nonparametric paired t-test was employed. The values in the various treatment groups were determined to be non-significant ($p > 0.05$) when compared to the normal control group.

Statistical analysis

Mean and standard deviation (SD) were used to represent the results. The statistical analysis programme Graph Pad Prism6.0 was used in order to carry out the quantitative statistical analysis. To figure out the level of significance, an analysis of variance (one-way an ANOVA) was implemented to compare a few variables followed by the Dunnet test to determine the differences between the test group and normal control groups(using distilled water or purified water as normal control substance). $P<0.05$ was selected as the significance criteria. A "p" value of 0.05 or above was regarded as insignificant.

4. Discussion

Numerous studies provide support for the claim that frequent consumption of fruits and vegetables can mitigate the possibility of developing chronic ailments such as cancer and diabetes. Vegetables and fruits contain a multitude of phytochemicals, such as phenols and flavonoids, which have been demonstrated to possess diverse biological activities and confer a range of health advantages. According to literature, certain phyto-substances have demonstrated potential anti-inflammatory and anti-diabetes mellitus properties (Yamada et al. 2011). The botanical species B. alba, which is a type of leafy green vegetable, possesses remarkable antioxidant properties due to the presence of flavonoids, its active constituents. Therefore, it is recommended that this plant be encouraged for cultivation as it exhibits various pharmacological actions. This suggestion is supported by existing literature (Namrata et al. 2011).

Flavonoids or polyphenols, which are the most abundant and widely distributed class of plant phenolic compounds, can be distinguished by their benzo-pyrone chemical structure (Kifayatullah and Waheed 2014). These compounds possess antioxidant properties. Both fruits and vegetables are a source of it. Flavonoids, a subclass of polyphenolic chemical compounds, are widely present in plants and are referred to as natural biological modifiers (Vaghasiya et al. 2011). Flavonoids, being biological regulators, have been observed to mitigate the detrimental impacts of viruses, allergies, and carcinogens (Mythilypriya et al. 2007). Flavonoids, a subclass of polyphenolic chemicals, are widely distributed in plants and are referred to as natural biological modifiers. Flavonoids function as biological regulators by mitigating the deleterious impact of viruses, allergies, and carcinogens (Sushruta et al. 2006). The substance exhibits properties that include anti-inflammatory, anticancer, anti-allergic, antioxidant, and antibacterial effects. As per the findings of published research, a higher intake of flavonoids has been associated with a reduced risk of developing cardiovascular disease, carcinoma, and neoplastic diseases (Alsarhan et al. 2014).

The whole Basella alba plant was extracted employing the solvent extraction technique to produce crude extracts. Various solvents, such as hexane, ethanol, and ethyl acetate, were utilized to conduct a qualitative analysis of flavonoids. The best approach for isolating quercetin from the chosen plant was found to be ethyl acetate. The findings are summarized in Tables **1**, **2**, **4**, and Figure **4**. Ethyl acetate has been identified as a highly effective solvent for the extraction of flavonoids due to its efficient chemical extraction capabilities and safe properties for human consumption. Kiassoset al. (2009) reported that an increase in ethyl acetate concentration resulted in enhanced extraction of flavonoids from different sections of Basella alba(Anandarajagopal et al. 2011, Shantha et al. 2016, Siddique et al. 2010).

The ethyl acetate extract derived from the entire B. alba plant exhibits a total flavonoid content of 9.49 µg/g quercetin, whereas the ethanol and hexane extracts obtained from the entire plant exhibit lesser flavonoid content

(Figure **4**). The investigation referred to above successfully detected the presence of flavonoids in the ethyl acetate fraction, isolated quercetin flavonoid, and conducted purification via preparative-HPLC using an appropriate solvent system to establish the final confirmation of the quercetin flavonoid (Rahmatullah et al. 2011, Reddy et al. 2014, Selvakumaran et al. 2003).

Upon undergoing ethyl acetate extraction, the crude ethyl acetate extract exhibited a notable increase in quercetin content from 1.52% to 9.63%. Subsequently, preparative HPLC purification and lyophilisation were carried out, resulting in a quercetin recovery rate of 85.5%. The isolation and purification of Quercetin were carried out through reverse phase preparative HPLC chromatography using a gradient elution technique with a YMC-Actus C18 column (250 x 20 mm, 5 µm particle size). The mobile phase consisted of acetonitrile as the solvent and 20mm ammonium bicarbonate as the modifier, with a flow rate of 16 mL/min. The isolation and purification of the target compound quercetin using conventional column chromatography proved to be challenging due to its laborious and time-consuming nature, low precision, and limited ability to yield a pure compound with high recovery rates. The objective of this study was to devise a straightforward and efficient approach for the extensive extraction and refinement of quercetin, while ensuring the attainment of an ultra-pure compound with a substantial recovery rate. The method employed involved the collection of pure fractions, specifically the desired peak of quercetin, through the utilization of a PDA trigger. The utilization of preparative chromatography is imperative for the rapid and efficient separation of bioactive compound quercetin from a large quantity of crude impurities or undesired products. This advanced technique boasts high throughput and accuracy, while minimizing the time required to achieve optimal purity and recovery. The utilization of modern preparative HPLC separation technique proved to be highly advantageous in our study. This technique enabled us to directly isolate pure quercetin from the crude profile by assessing LCMS purity.

The compound exhibiting an R_f value of 0.46 underwent phytochemical screening, physical property analysis, and spectral characterization through various techniques such as HPLC, FTIR, LC-MS, ^{13}C NMR, and ^{1}HNMR to determine its structural elucidation. The preparative isolate compound was verified as quercetin (Iwamoto et al. 1985, Siddique et al. 2010), as per the findings of the study.
Quercetin, a phenolic super antioxidant, has been found to possess therapeutic potential in the treatment of various ailments such as diabetes, cancer, HIV, influenza, among others. This adds to the existing abundance of therapeutic plants in the herbal domain. In the study on acute toxicity of quercetin, Swiss albino mice were orally administered doses of 300 milligram/kilogram body mass and 2000 milligram/kilogram body mass. The mice were observed for a total of 72 hours, with continuous monitoring for the first 4 hours, to assess any potential toxic or adverse effects throughout the treatment period. No statistically significant alterations in behaviour or mortality were observed across all treatment cohorts(Adeneye and Olagunju 2009, Ukwuani et al. 2012, Wang et al. 2017).

In comparison to the control group, the administration of quercetin at varying doses ranging from 300 milligram/kilogram to 2000 milligram/kilogram did not elicit any significant abnormalities in behaviour, body weight, food and fluid consumption, or mortality. Thus, it can be deduced that the oral ingestion of quercetin during the acute toxicity testing phase didn't have any unfavourable effects on the biological or physiological mechanisms and growth of mice. In comparison to the control group, our study demonstrates that the treated groups didn't exhibit a significant increase in organ weight or a notable decrease in relative organ weight. In addition, it can be inferred that the administration of quercetin at two different levels does not result in any adverse effects on vital organs, as per the findings of a study (Adeneye and Olagunju 2009). Medications that possess an oral LD_{50} (lethal dose) exceeding 1000 milligram/kilogram may be considered safe (Kunimatsu et al. 2004). The findings indicate that the administration of

quercetin at a dose of 2000 milligram/kilogram body weight, commonly employed for the management of chronic ailments, does not pose any risk to the relative weight of organs, haematological parameters, lipid profile, or biochemical attributes (Donkor et al. 2014). Differences in organ weight between animals subjected to treatment and those in the control group may exhibit significant variations, even in the absence of any observable morphological abnormalities. As such, these variations represent the most sensitive indicators of potential drug toxicity. The utilization of relative organ weight as an assessment tool for determining the toxicity level of chemicals or medications is considered dependable. This is due to its ability to provide information on the individual impact of the test component on each organ, regardless of body mass. This has been documented in various studies (Arsad et al. 2013, Piao et al. 2013). There exist several possible mechanisms by which deleterious effects could lead to a reduction in the weight of one organ concomitant with an increase in the weight of another organ, while the net weight of the organism remains unchanged. The haematological analysis is a highly vulnerable area for deleterious drugs and substances, and serves as a pivotal measure for the pathological and physiological state in both human and animal subjects (Kunimatsu et al. 2004). In order to differentiate the potential adverse effects of quercetin, it may be advantageous to conduct a haematological evaluation on the blood of experimental animals. Such an assessment could shed light on the crucial role of quercetin in regulating blood flow. The haematological profile observed after administering quercetin was not significantly different from the normal control substance. The parameters remained within the expected range for the research animals used in the study. The findings indicate that the administration of quercetin did not result in any notable adverse effects on the haematological profile of mice(Mukinda and Syce 2007, Yakubu et al. 2007).

The evaluation of the hazardous nature of a given experimental material necessitates the consideration of its biochemical properties as an essential aspect. The biochemical characteristics of the kidneys and liver, two of the most significant and vital organs, can provide a better understanding of their functioning. Transaminase, along with ALP and GGT, is considered to be the most accurate and dependable marker of inflammation in visceral organs such as the liver, heart, and kidney (Ramaiah 2011). Elevated levels of marker enzymes are associated with hepatotoxicity or liver disorders. The present study indicates the absence of significant variations in the biochemical markers (Mukinda and Syce 2007).

Proteins serve as fundamental constituents of every organism, and their elevated levels within an organism's system signify a successful recovery or restoration of damaged organs or tissues. Fluctuations in the total water content in plasma and the concentration of one or more specific proteins in the plasma exerted an influence on the total serum protein quantity. The increase in albumin (A), globulin (G), and A/G ratio, along with total serum proteins observed in the treated mice, may suggest the absence of liver lesions that could have impeded certain hepatic functions. In situations where dehydration is caused by either excessive water loss or insufficient water intake, a reduction in the quantity of plasma water (hyperproteinemia) has been observed. The quercetin-treated groups exhibited significantly lower plasma lipid levels compared to the untreated control group (i.e., normal control). According to the study, the concentration of high-density lipids (HDL) was significantly higher, while the levels of low-density lipids (LDL) were lower (Féres et al. 2006). The findings indicate that quercetin possesses favourable attributes that could mitigate the reduction of cardiovascular risk factors, thereby serving as an inhibitor or prophylactic measure against the onset or advancement of atherosclerosis and related coronary artery ailments. The results suggest that quercetin possesses hypolipidemic properties, leading to a reduction in lipid profile levels. This effect may be attributed to the antioxidant properties of quercetin or the presence of a hypolipidemic component within it (Sundaram et al. 2018).

Alterations in kidney function assessment are indicative of notable renal dysfunction. In the present study, upon comparison of animals treated with quercetin to control mice, no significant variations in the renal parameters were observed. There was no significant alteration observed in the levels of glucose and creatinine in TS1 and TS2. Consequently, the body weights of both TS1 (treated with 300 mg/kg) and TS2 (treated with 2000 mg/kg) did not exhibit any deleterious impacts. The study findings indicate that the administration of quercetin does not have any adverse impact on renal function, as evidenced by the comparison of treated mice with control groups (Ibrahim et al. 2010).
The enzyme known as superoxide dismutase (SOD) catalyses the conversion of two superoxide anions into hydrogen peroxide and molecular oxygen. The lower level of SOD activity might be attributed to the fact that SOD (superoxide dimutase) is constantly being employed to shield or protecting cells from the damaging consequences of superoxide radicals. Catalase (CAT) is recognized as a secondary antioxidant enzyme that plays a crucial role in safeguarding peroxidative compounds. The feedback effect of hydrogen peroxide on the expression of mRNA may be responsible for the increased level of CAT. The principal antioxidant, GPx (glutathione peroxidise), exploits reduced glutathione to get rid of the hydrogen peroxide generated when superoxide radicals are dismutated. The consumption of reduced glutathione is observed to increase as GPx effectively eliminates a greater number of hydrogen peroxide molecules. The concentration of GPX in plasma decreases as a result of a deficiency or absence of glutathione (Karincaoglu et al. 2005).

The antioxidant enzyme status of the experimental animals was examined subsequent to the administration of quercetin. With the exception of the glutathione (GSH) level observed in the TS2 group, minimal alterations were detected in the blood levels of SOD, GSH, GPx, and CAT. The increase in GSH levels was previously believed to be non-toxic due to its inconsistency and potential influence from animal physiological variability. Additionally, histological analyses did not provide evidence to support its impact. The results of this study provide evidence in favour of the proposal that quercetin did not elicit any deleterious outcomes and upheld the typical enzymatic antioxidant mechanisms in the liver and kidneys (Scharer 1977).
The acute toxicity profile of Quercetin is expected to be advantageous for future in-vivo and clinical investigations. The results of oral acute toxicity studies conducted on mice indicate that quercetin exhibits no toxicity and is non-harmful. The findings of this investigation suggest that quercetin exhibits a favourable safety profile, thereby allowing further clinical investigations to determine its potential clinical toxicity.

5. Conclusions

The present study reports the successful extraction of quercetin, a desired plant compound, from the whole plant of Basella alba. The study highlights the significance of solvent in the extraction process of plant constituents. Notably, the ethyl acetate extract exhibited a higher flavonoid content compared to hexane and ethanol extracts. The identification of quercetin was attempted by direct comparison with its retardation factor, and the isolated constituent was further characterised using HPLC, FT-IR, NMR, and mass spectroscopy.Preparative HPLC separation and purification of quercetin resulted in a purity of 100%, high yield, and high throughput. This advanced and efficient analytical chromatography process is technically significant, with zero error and good accuracy in terms of purity and recovery. It also saves time, particularly when dealing with a complex crude profile such as the crude ethyl acetate extract, where undesired peaks are merged with the target product quercetin, making separation difficult using conventional techniques. Upon conducting preparative isolation and subsequent analysis of the physical characteristics, HPLC, and spectral data, it was verified that the compound in question is quercetin.

The results of the present study have validated the presence of bioactive phytochemicals throughout the entire Basella alba plant. The data indicates that the entire Basella alba plant

extract contains a noteworthy amount of quercetin. Further investigation into the pharmacological properties of Basella alba may reveal its potential as a potent plant with therapeutic properties. Upon oral administration of quercetin to Swiss albino mice at doses of up to 2000 milligram/kilogram body weight, no significant toxicological effects were observed in terms of behaviour, physical health, or hypersensitivity. The absence of any mortalities within the range of 300 milligram/kilogram to 2000 milligram/kilogram indicates that the quercetin LD_{50} is deemed safe for administration at dosages exceeding 2000 milligram/kilogram body weight, as per the findings of the toxicity analysis. When formulating new nutraceuticals, it is important to consider the potential for providing therapeutic benefits to the body while also offering dietary supplements at a reduced level. The utilization of alternative approaches may prove efficacious in addressing issues pertaining to dose-dependent toxicity of phytocompounds.

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