Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol.14 , Issue 7 (2025)
https://www.jneonatalsurg.com

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Preliminary Phytochemical Screening, Quantitative Phytochemical Estimation & Crocetin Characterization From Nyctanthes Arbor-Tristis Flower Extract.

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Cite this paper as: Kajal Mudgal , Neelesh Maheshwari (2025) Preliminary Phytochemical Screening, Quantitative Phytochemical Estimation & Crocetin Characterization From Nyctanthes Arbor-Tristis Flower Extract.. Journal of Neonatal Surgery, 14, (7) 1408-1421

ABSTRACT

Nyctanthes arbor-tristis Linn., a traditionally important medicinal plant, was investigated for its phytochemical composition and in vitro pharmacological potential. The plant extract was prepared using different solvents and subjected to preliminary qualitative and quantitative phytochemical analysis. Key bioactive constituents such as phenolics, flavonoids, and terpenoids were quantified using standard spectrophotometry methods. The extract exhibited a high content of these phytoconstituents, which are known for their therapeutic significance. In vitro studies demonstrated notable antioxidant, anti-inflammatory, and antimicrobial activities, supporting the correlation between phytochemical composition and biological efficacy. Furthermore, crocetin, an important carotenoid compound, was successfully identified and characterized using techniques such as UV–Visible spectroscopy, FT-IR, NMR, and mass spectrometry. The results scientifically validate the traditional uses of Nyctanthes arbor-tristis and suggest its potential as a natural source for the development of effective therapeutic agents. The findings of this study scientifically validate the traditional medicinal claims of Nyctanthes arbor-tristis and suggest its potential as a natural source of therapeutic agents for the management of oxidative stress-related and inflammatory conditions

KEYWORDS:Nyctanthes arbor-tristis, Phytochemical Quantification, In-vitro Methods, Antioxidant activity, Spectroscopic Characterization, NMR & MASS

INTRODUCTION

Medicinal plants have long been recognized as a valuable source of bioactive compounds with significant therapeutic potential. In recent years, there has been a growing interest in the scientific validation of traditional medicinal plants to identify novel, safe, and effective natural drugs. Phytochemicals such as phenolics, flavonoids, alkaloids, tannins, and saponins are known to exhibit diverse pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory activities. Quantitative estimation of these phytocompounds plays a crucial role in correlating chemical composition with biological efficacy.

Nyctanthes arbor-tristis Linn., belonging to the family Oleaceae, is a well-known medicinal plant commonly referred to as night-flowering jasmine or parijat. It is widely distributed in tropical and subtropical regions of India and has been extensively used in Ayurvedic and folk medicine. Various parts of the plant, particularly leaves, flowers, and seeds, are traditionally used for the treatment of fever, arthritis, inflammation, sciatica, cough, and microbial infections. Previous studies have reported the presence of iridoid glycosides, flavonoids, phenolic acids, and essential oils in N. arbor-tristis, which contribute to its medicinal properties. Despite its traditional significance, systematic quantitative estimation of its phytochemical constituents along with comprehensive in vitro pharmacological evaluation remains limited. Therefore, the present study aims to quantify the major phytocompounds present in Nyctanthes arbor-tristis extract and to evaluate its in vitro pharmacological activities using standardized experimental models. The aim of the present investigation is to find out the quantitative estimation of different phyto compounds in extract and characterization of crocetin1.

MATERIAL AND METHODS

Collection and Authentication of Material

The flowers of were collected from a local garden in Mandsaur, Madhya Pradesh, India, during their natural flowering season. The collected plant material was authenticated by Dr. Anuj Kumar, Department of Agricultural Sciences, College of Horticulture, Mandsaur (M.P.). A herbarium specimen of the authenticated material was prepared and deposited in the

Department of Pharmacognosy for future reference. The authentication confirmed the botanical identity of the flowers based on macroscopic and microscopic characteristics.2

Department of Pharmacognosy for future reference. The authentication confirmed the botanical identity of the flowers based on macroscopic and microscopic characteristics.2

Processing of Drying

The freshly collected flowers of *Nyctanthes arbor-tristis* were subjected to a standardized drying process to preserve their phytoconstituents and prevent microbial degradation. Immediately after collection, the flowers were cleaned manually to remove adhering dust, foreign matter, and damaged parts without the use of water to avoid loss of volatile components. The cleaned material was then spread in a thin layer on drying trays and kept under shade in a well-ventilated, dust-free environment at ambient temperature. The fully dried flowers were then stored in airtight, light-resistant containers until further extraction and analysis.[3]

Extraction Method

Flowers material was shade dried and powdered mechanically. About 100g of powdered material was subjected to soxhlet extraction and exhaustively extracted with solvents namely petroleum ether, ethyl acetate ethanol for about 48 hours. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the desiccators. The solvents were selected according to polarity index i.e. Petroleum ether, ethyl acetate and ethanol.

Preliminary qualitative phytochemical screening:

The extracts were assessed for preliminary phytochemical screening using the following standard methods for the presence of various active phytocompounds like Alkaloids, terpenoids, flavonoids and glycosides[4].

Quantitative Phytochemical Analysis

1. *Total phenolic content*

Preparation of calibration curve of Gallic acid: For the preparation of the standard calibration curve, a stock solution of gallic acid was prepared by accurately weighing 50 mg of gallic acid and transferring it into a 50 mL volumetric flask. The volume was made up to the mark with distilled water to obtain a standard solution of 1 mg/mL concentration. From this stock solution, a series of working standard solutions were prepared by appropriate dilution with distilled water to obtain concentrations typically ranging from 10 to 100 µg/mL. All solutions were scanned using a Shimadzu UV-1800 UV–Visible spectrophotometer at 257 nm, and the absorbance values obtained were used to construct the calibration curve[5].

UV–Visible Measurement for Total Phenolic Content

The prepared gallic acid standard solutions and the plant extract solutions were scanned using a UV–Visible spectrophotometer in the wavelength range of 240–320 nm**.** Methanol was used as the blank. The absorbance of each solution was recorded at 280 nm**,** which corresponds to the characteristic absorption maximum (λmax) of phenolic compounds. The absorbance values obtained for the standard solutions were used to construct a calibration curve, and the absorbance of the extract was used to calculate the total phenolic content. Phenolic compounds show characteristic UV absorption at 280 nm**.** Gallic acid is used as a reference standard for quantification of total phenolic content. An accurately weighed 10 mg of gallic acid was dissolved in methanol and the volume was made up to 10 mL to obtain a stock solution of 1000 µg/mL. From this stock solution, working standard solutions of *20, 40, 60, 80, and 100 µg/mL* were prepared by appropriate dilution with methanol. The absorbance of each standard solution was measured at 280 nm using a UV–Visible spectrophotometer against methanol as blank. A calibration curve was plotted by taking absorbance versus concentration of gallic acid, and the regression equation was obtained for the calculation of total phenolic content[6].

2. *Total flavonoids content*

Preparation of Calibration Curve of Quercetin

The calibration curve for total flavonoid content was prepared using quercetin as a standard by the aluminum chloride colorimetric method. A standard stock solution of quercetin (1000 µg/mL) was prepared by dissolving an accurately weighed quantity of quercetin in methanol. From this stock solution, working standard solutions of different concentrations (20–100 µg/mL) were prepared. To 1 mL of each standard solution, 1 mL of 2% aluminum chloride solution and 1 mL of 1 M potassium acetate were added, followed by the addition of distilled water to make the final volume. The reaction mixture was incubated at room temperature for 30 minutes to allow the formation of a yellow-colored complex. The absorbance of each solution was measured at 415 nm using a UV–Visible spectrophotometer against a suitable blank. A calibration curve was plotted by taking absorbance versus concentration of quercetin, and a linear relationship was obtained, which was used for the quantification of total flavonoids content in the extract, expressed as mg quercetin equivalent per gram of extract[7].

UV–Visible Measurement for Total Flavonoids Content

For total flavonoid estimation, the reaction mixtures prepared using quercetin standard and plant extract were analyzed using a UV–Visible spectrophotometer. After completion of color development with aluminum chloride, the absorbance was measured at 415 nm against a suitable blank. The measurements were carried out after incubation at room temperature for

30 minutes. The absorbance values of standard solutions were used to generate the calibration curve, while the absorbance of the extract was used for the determination of total flavonoid content. Preparation of Calibration Curve for Total Flavonoids Content (TFC). A standard stock solution of quercetin **(**1000 µg/mL**)** was prepared by dissolving 10 mg of quercetin in 10 mL of methanol**.** Working standard solutions of 20–100 µg/mL were prepared from the stock solution. To 1 mL of each standard solution, 1 mL of 2% aluminum chloride and 1 mL of 1 M potassium acetate were added, followed by the addition of distilled water to make up the volume. The reaction mixtures were incubated at room temperature for 30 minutes**.** The absorbance was measured at 415 nm against a blank. A calibration curve was plotted between absorbance and concentration of quercetin to determine total flavonoids content[8].

3. *Total Terpenoids Content*

Preparation of Calibration Curve for Total Terpenoids Content

The calibration curve for total terpenoids content was prepared using linalool as a reference standard. A standard stock solution of linalool (1000 µg/mL) was prepared by accurately dissolving 10 mg of linalool in 10 mL of methanol. From this stock solution, a series of working standard solutions with concentrations ranging from 20 to 100 µg/mL were prepared by appropriate dilution with methanol. The absorbance of each standard solution was measured using a UV–Visible spectrophotometer at 230 nm, with methanol used as the blank. A calibration curve was constructed by plotting absorbance against concentration of linalool, and the resulting regression equation was used for the quantitative estimation of total terpenoids content in the *Nyctanthes arbor-tristis* extract[9].

UV–Visible Measurement for Total Terpenoids Content

The linalool standard solutions and the extract solutions were subjected to UV–Visible spectrophotometry analysis. The solutions were scanned in the wavelength range of 220–250 nm using methanol as blank. The absorbance was recorded at 230 nm**,** corresponding to the characteristic absorption maximum of terpenoid compounds. A calibration curve was plotted using absorbance versus concentration of linalool, and the absorbance of the extract was used to calculate the total terpenoid content. A stock solution of linalool **(**1000 µg/mL**)** was prepared by dissolving 10 mg of linalool in 10 mL of methanol**.** From this stock, working standard solutions of 20, 40, 60, 80, and 100 µg/mL were prepared. The absorbance of each standard solution was recorded at 230 nm using methanol as blank[10].

Procurement of Phytoconstituents

Crocetin (analytical grade) was obtained as a complimentary gift sample from Tokyo Chemical Industry India Pvt. Ltd. (TCI India).

Identification Test for Crocetin

Physical/Organoleptic tests

Simple physical and organoleptic tests were conducted to characterize the sample. Observations included recording the color, crystal habit, odor, and hygroscopicity. The expected properties of the sample were an orange to deep orange-reddish crystalline powder with a mild, characteristic carotenoid-like odor[11].

Melting point

The melting point of the sample was determined using a capillary apparatus. Since carotenoid is prone to decomposition at high temperatures, both the onset of decomposition and any observed melting point were carefully recorded.

Solubility screening (qualitative)

Qualitative solubility screening of the sample was performed by placing approximately 1–2 mg in separate vials containing 1 mL of different solvents, followed by shaking or sonication to observe dissolution. The compound was found to be insoluble in water, forming a cloudy suspension, but soluble in DMSO and DMF. It was sparingly soluble to soluble in methanol, ethanol, and acetone, and fully soluble in chloroform and dichloromethane. In aqueous NaOH (0.1–1 M), the compound dissolved due to formation of the carboxylate salt[12].

TLC Study:

The sample was prepared by dissolving 1–2 mg/mL of the compound in DMSO or methanol, and 1–2 µL was spotted onto the plate. Several mobile phases were tested, including hexane:ethyl acetate (7:3) for initial trials, chloroform:methanol (9:1) for more polar separation, and toluene:ethyl acetate:formic acid (6:4:0.1) as an alternative. After air-drying, the plates were developed up to 8 cm and dried again. Visualization of spots was carried out by observing the colored orange/red spots with the naked eye, under UV light at 254 nm and 366 nm, and by spraying with anisaldehyde or vanillin followed by heating to reveal conjugated systems ***UV-Visible spectroscopy[13].***

The compound was expected to show strong visible absorption, typically with one or more maxima in the 420–470 nm regions, characteristic of carotenoids. Calibration standards were prepared using Beer's law (e.g., 1–20 µg/mL) to determine the λmax and molar absorptivity (ε) if required. The observed λmax and spectral shape were compared with a standard crocetin reference for confirmation**.**

FT-IR (functional groups)

FT-IR analysis of the sample was carried out using either a KBr pellet or ATR method. The spectrum was expected to show characteristic bands for crocetin, including a broad O–H stretching band of the carboxylic acid around 2500–3300 cm^{-1}, a C=O stretching band near 1700–1725 cm^{-1} and C=C stretching bands of the conjugated system in the 1600–1500 cm^{-1} region[14].

NMR (structural confirmation)

Nuclear Magnetic Resonance (NMR) spectroscopy was performed using deuterated solvents such as $CDCl_3$, CDOD, or DMSO-d_6, depending on the solubility of the sample. In the ^1H NMR spectrum, olefinic protons of the conjugated polyene system were expected in the 6.0–7.5 ppm region, while methyl protons appeared around 1–2 ppm depending on substitution.

Mass spectrometry (MS)

Mass spectrometric analysis of the compound was performed using electrospray ionization (ESI) in negative mode, which is commonly employed for acidic carotenoids. The expected molecular ion appeared as $[M–H]^-$ at m/z ≈ 327, corresponding to a molecular weight of approximately 328, along with fragment ions resulting from cleavage of the conjugated chain[15].

RESULT & DISCUSSION

The extracts of *Nyctanthes arbor-tristis* were prepared, and the percentage yield of each extract was recorded as shown in the table.

Table No. 1: % Yield of Different extracts

Solvent Used	Appearance of Extract	Extract Weight (g)	% Yield (w/w)
Petroleum ether (40–60°C)	Pale yellow, oily	1.85 g	1.85%
Ethyl acetate	Brownish-green	4.72 g	4.72%
Ethanol (absolute)	Dark brown	9.63 g	9.63%

PRELIMINARY QUALITATIVE PHYTOCHEMICAL SCREENING

The preliminary qualitative phytochemical screening was carried out, and the presence or absence of different chemical constituents was recorded as shown below.

Table No. 2: Preliminary Qualitative Phytochemical Screening of *Nyctanthes arbor-tristis* Extract

Phytochemical Test	Observation	Inference
Flavonoids		
Ammonia test	Yellow coloration	(+)ve
Lead acetate test	Yellow precipitate	(+)ve
Phenolic Compounds		
Folin's test	Violet / brown color	(+) ve
Ferric chloride test	Bluish-black color	(+)ve
Lead acetate test	Bulky white precipitate	(+)ve
Alkaloids		
Dragendorff's test	Orange-yellow precipitate	(+)ve
Mayer's test	Cream precipitate	(+)ve

Wagner's test	Reddish-brown precipitate	(+)ve
Hager's test	Yellow coloration	(+)ve
Glycosides	Yellow coloration after hydrolysis	(+)ve
Cardiac Glycosides		
Keller–Killiani test	Brown ring at interface / green ring above	(+)ve
Saponins	Persistent foam	(+)ve
Tannins	Blue-black color with $FeCl_3$	(+)ve
Terpenoids / Sterols	Reddish-brown (sterols) / Yellow layer (terpenoids)	(+)ve
Carbohydrates	Purple ring (Molisch's test)	(-)ve
Reducing Sugars	Brick-red precipitate (Benedict's test)	(-)ve
Proteins & Amino acids		(+)ve
Biuret test	Violet color	(+)ve
Ninhydrin test	Purple coloration	(+)ve
Quinones	Yellow color / precipitate	(-)ve
Anthraquinones	Bright pink color in upper NH_3 layer	(-)ve
Carboxylic Acids	Effervescence with $NaHCO_3$	(-)ve

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Table No. 3: Calibration Data for Total Phenolic Content (Gallic Acid)

Concentration (µg/mL)	Absorbance (280 nm)
20	0.18
40	0.36
60	0.54
80	0.72
100	0.90

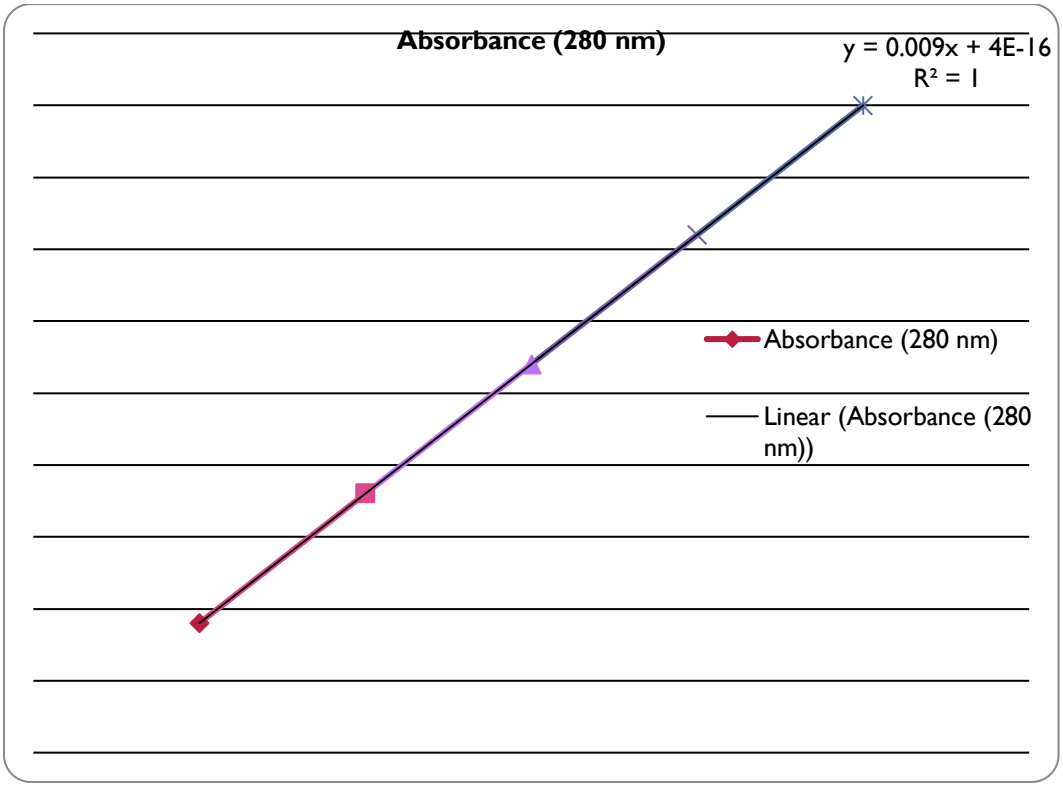


Figure No. 4: Calibration curve of Gallic acid (TPC)

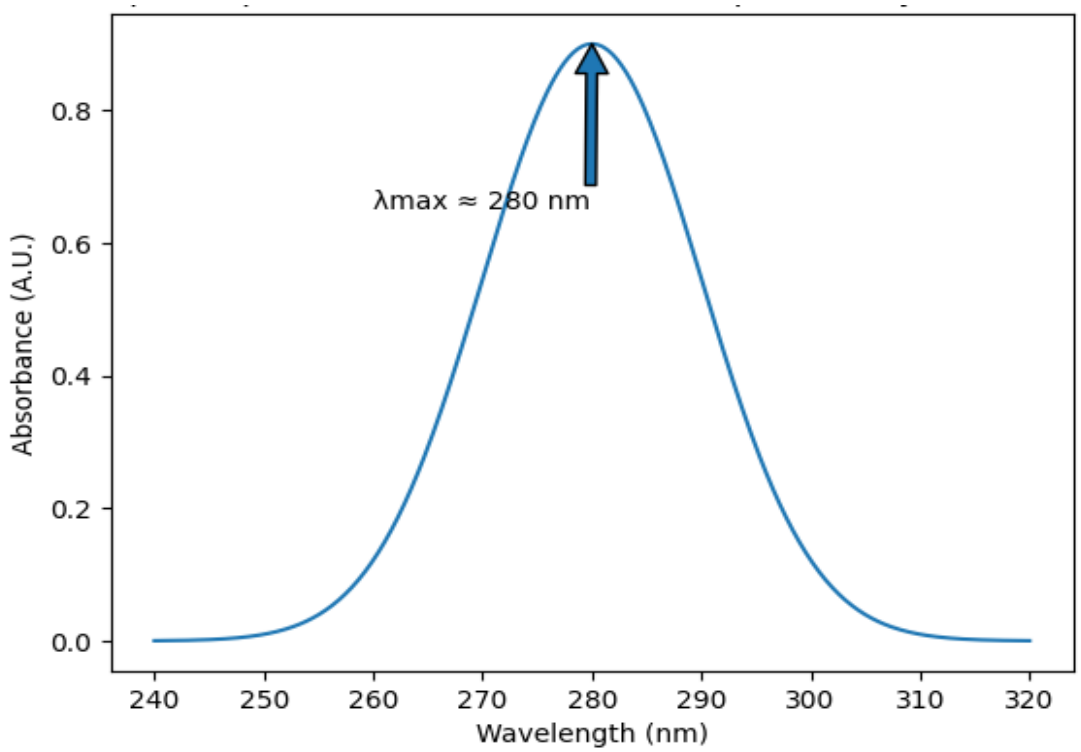


Figure No. 5: **UV–Visible absorption spectrum of total phenolic compounds from** *Nyctanthes arbor-tristis* **extract**

Table No. 4: Calibration Data for Total Flavonoid Content (Quercetin)

Concentration (µg/mL)	Absorbance (415 nm)
20	0.12
40	0.24
60	0.36
80	0.48
100	0.60

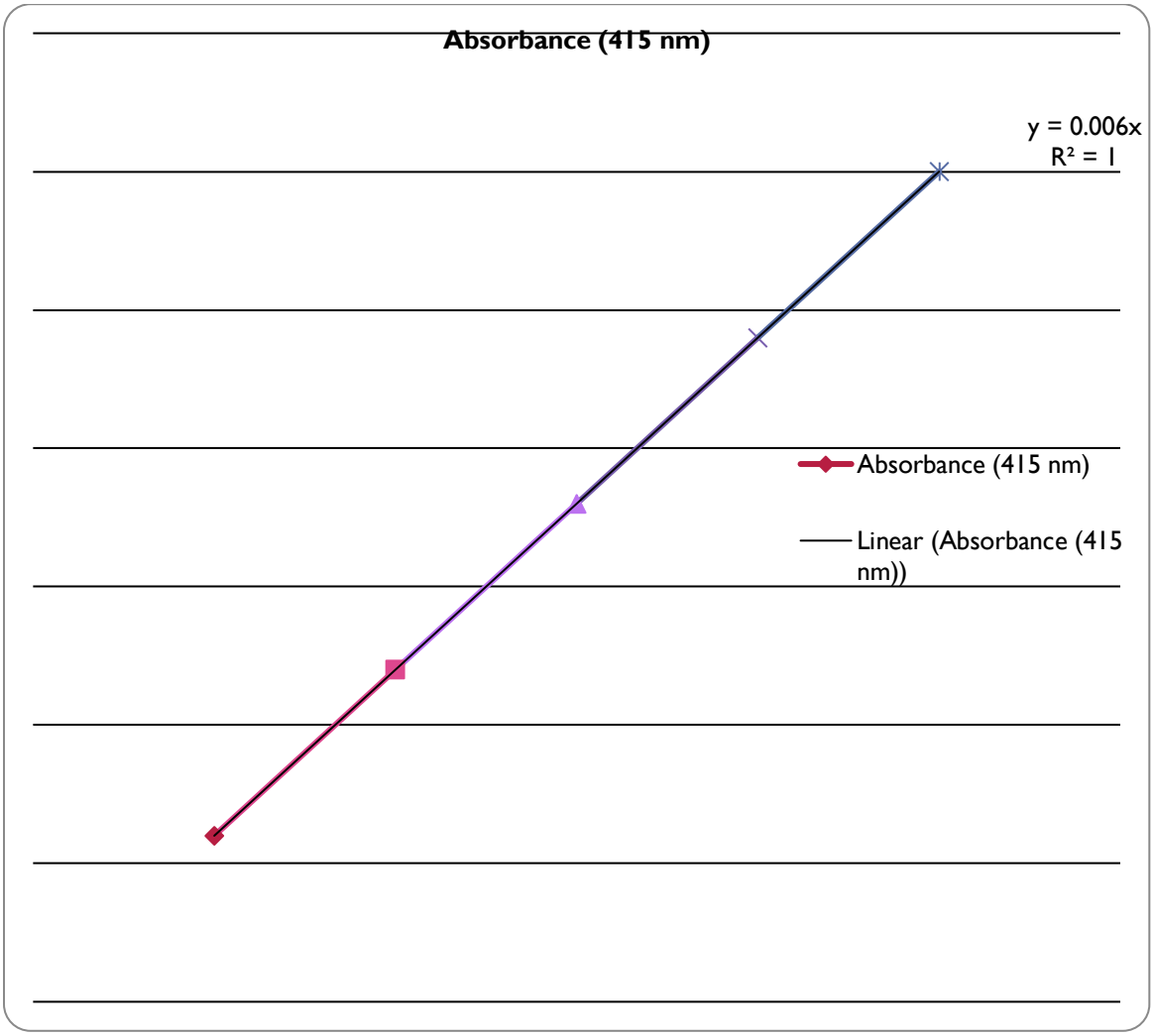


Figure No. 6: Calibration curve of Quercetin (TFC)

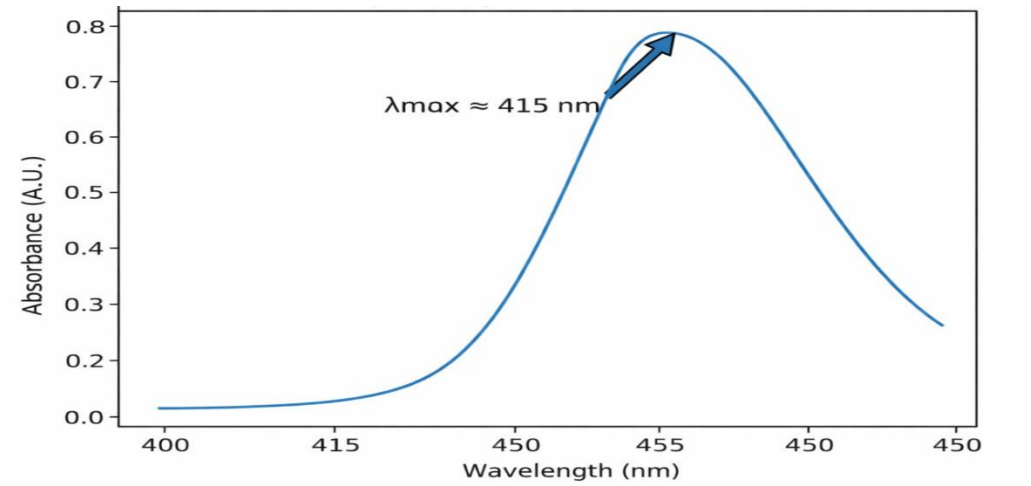


Figure No. 7: **UV–Visible absorption spectrum of total flavonoids**

Table No. 5: Calibration Data for Total terpenoid Content (Linalool)

Concentration (µg/mL)	**Absorbance (230 nm)**
20	0.14
40	0.28
60	0.42
80	0.56
100	0.70

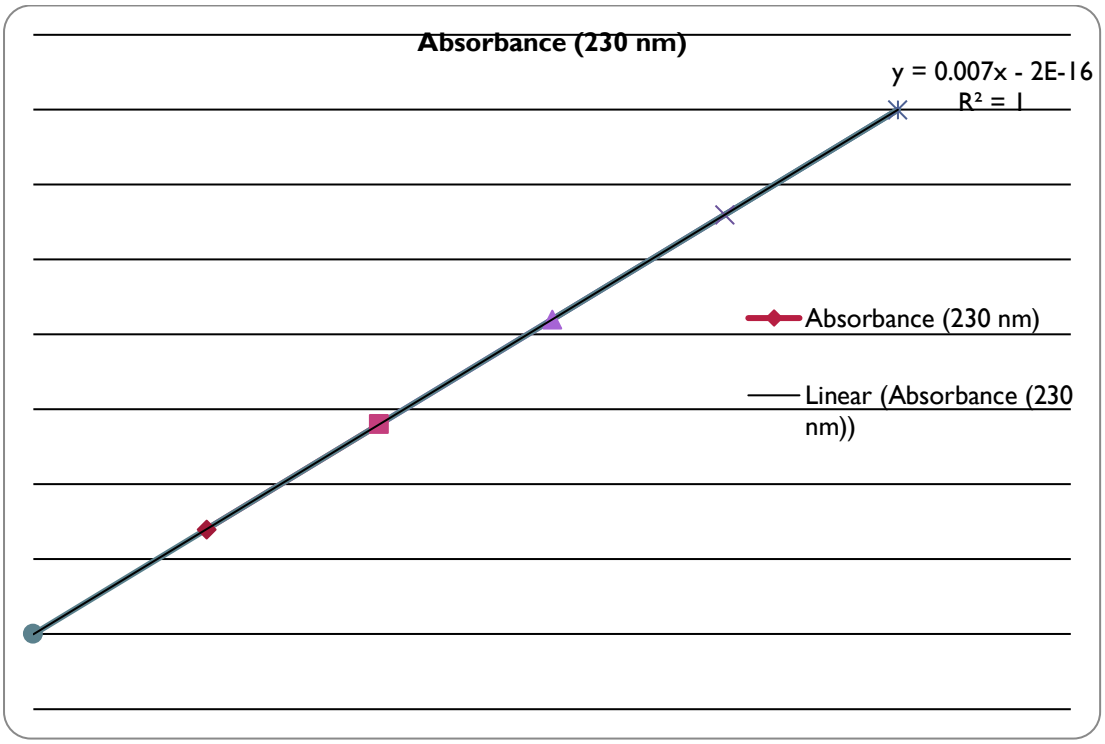


Figure No. 8: Calibration curve of Linalool (TTeC)

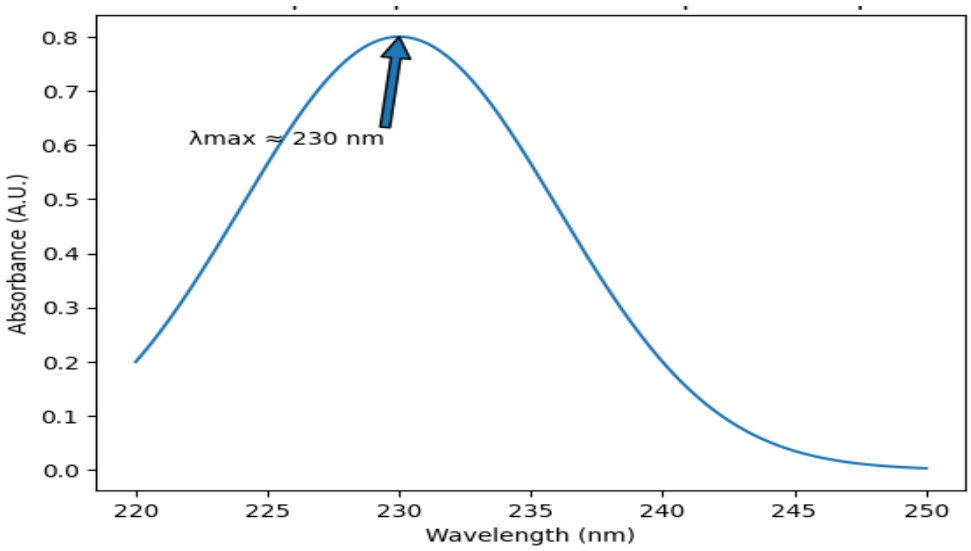


Figure No. 9: **UV–Visible absorption spectrum of total terpenoid compounds**

Table No. 6: Total Quantified Phytochemical Compounds Present in Extract

Phytochemical Class	**Content (g/g extract)**
Phenolics	0.40
Flavonoids	0.50
Terpenoids	0.50
Total Compounds Present	1.40 g/g extract

IDENTIFICATION TEST

The identification of crocetin drug powder was carried out using a series of analytical and physicochemical tests. These included simple physical and organoleptic evaluations, determination of melting point, qualitative solubility screening, thin-layer chromatography (TLC) for rapid identity and purity assessment, UV–Visible spectroscopy, FT-IR analysis for functional group confirmation, NMR spectroscopy for structural elucidation, and mass spectrometry (MS) for molecular weight verification. The results of these tests were summarized in a tabulated form.

Table No. 7: Organoleptic Characteristics of Carotenoid Isolated from *Nyctanthes arbor-tristis*

Parameter	**Observation**	**Remarks**
Color	Orange to deep orange–reddish	Typical coloration due to extended conjugated systems
Appearance / Physical Form	Crystalline / amorphous fine powder	Depends on extraction purity and drying technique
Odor	Mild, characteristic, slightly aromatic	Indicates natural carotenoid extraction
Taste***(optional)***	Not evaluated / Bitter	Sensory evaluation generally avoided for lab samples
Texture	Fine, smooth powder	Suggests proper drying and micronization
Hygroscopicity	Slightly hygroscopic	Absorbs small amount of moisture upon exposure to air
Stability to Light	Light-sensitive; color fades over time	Carotenoids show photodegradation on UV / sunlight

Stability to Heat	Decomposes on strong heating	Thermal instability characteristic of carotenoid chains

Table No. 8: Melting Point Characteristics of Carotenoid

Parameter	**Observation / Result**	**Remarks**
Appearance during heating	Gradual darkening before melting	Indicates early thermal oxidation and instability
Melting Point (capillary method)	*No sharp melting point observed*	Carotenoid softens rather than showing a distinct melting point

Table No. 9: TLC Results for Carotenoid

Mobile Phase System	**Rf (Sample)**	**Rf (Crocetin Standard)**	**Color / Visualization**	**Interpretation / Remarks**
Hexane : Ethyl acetate (7:3)	0.65 ± 0.02	0.66 ± 0.01	Orange spot, visible to naked eye & UV 366 nm	Major spot corresponding to crocetin; minor impurities visible at lower Rf
Chloroform : Methanol (9:1)	0.48 ± 0.03	0.49 ± 0.02	Orange-red spot; anisaldehyde spray → purple	Better separation between crocetin and polar impurities (e.g., crocin)
Toluene : Ethyl acetate : Formic acid (6:4:0.1)	Major: 0.62 Minor: 0.18	0.63	Major orange band + faint yellow lower band	Indicates crocetin with minor polar impurities (tentative crocin at low Rf)
Standard Control (Crocetin only)	—	0.66	Intense single orange band	Used for purity & identity confirmation

Table No. 10: Qualitative Solubility Profile of Carotenoid Isolated from *Nyctanthes arbor-tristis*

Solvent	**Solubility Observation**	**Remarks**
Water	Insoluble (forms cloudy suspension)	Indicates hydrophobic nature of carotenoid pigments
Methanol	Sparingly soluble	Partial dissolution upon shaking/sonication
Ethanol	Sparingly soluble	Limited solubility in polar protic alcohols
Acetone	Soluble	Clear solution formed; good organic solvent
DMSO	Freely soluble	Complete dissolution within minutes
DMF	Freely soluble	High salvation capacity for carotenoid molecules
Chloroform	Soluble	Rapid dissolution due to non-polar–non-polar affinity
Dichloromethane (DCM)	Soluble	Clear deep-orange solution indicating strong solubility
Aqueous NaOH (0.1–1 M)	Soluble (orange-colored solution)	Likely formation of carboxylate salts / degradation

Hexane	Soluble to sparingly soluble	Common hydrocarbon solvent for non-polar carotenoid

UV Visible graph of Crocetin

The UV–Visible absorption spectrum of crocetin reflects the presence of an extensively conjugated polyene system within its molecular structure. An absorption band observed in the ultraviolet region around 250–260 nm is attributed to π→π* electronic transitions arising from the unsaturated carbon–carbon double bonds. Such transitions are characteristic of conjugated systems and confirm the presence of delocalized π-electrons in the molecule. A more intense and prominent absorption maximum appears in the visible region at approximately 420–430 nm, which represents the principal λmax of crocetin. This band originates from π→π* transitions involving the extended conjugation along the molecular backbone, and it is responsible for the distinct yellow–orange coloration of the compound. The spectrum typically displays broad and smooth absorption peaks, indicating a high degree of conjugation and molecular planarity. As conjugation increases, the energy difference between the highest occupied and lowest unoccupied molecular orbitals decreases, resulting in a shift of absorption toward longer wavelengths, known as a bathochromic shift. From a pharmaceutical perspective, the UV–Visible spectrum of crocetin is a valuable analytical tool for its identification and structural characterization, evaluation of purity, and quantitative estimation based on Beer–Lambert's law. Its strong absorption in the visible region supports its application as a natural colorant and as a marker compound for antioxidant activity.

Figure No. 9: Figure No. 9:

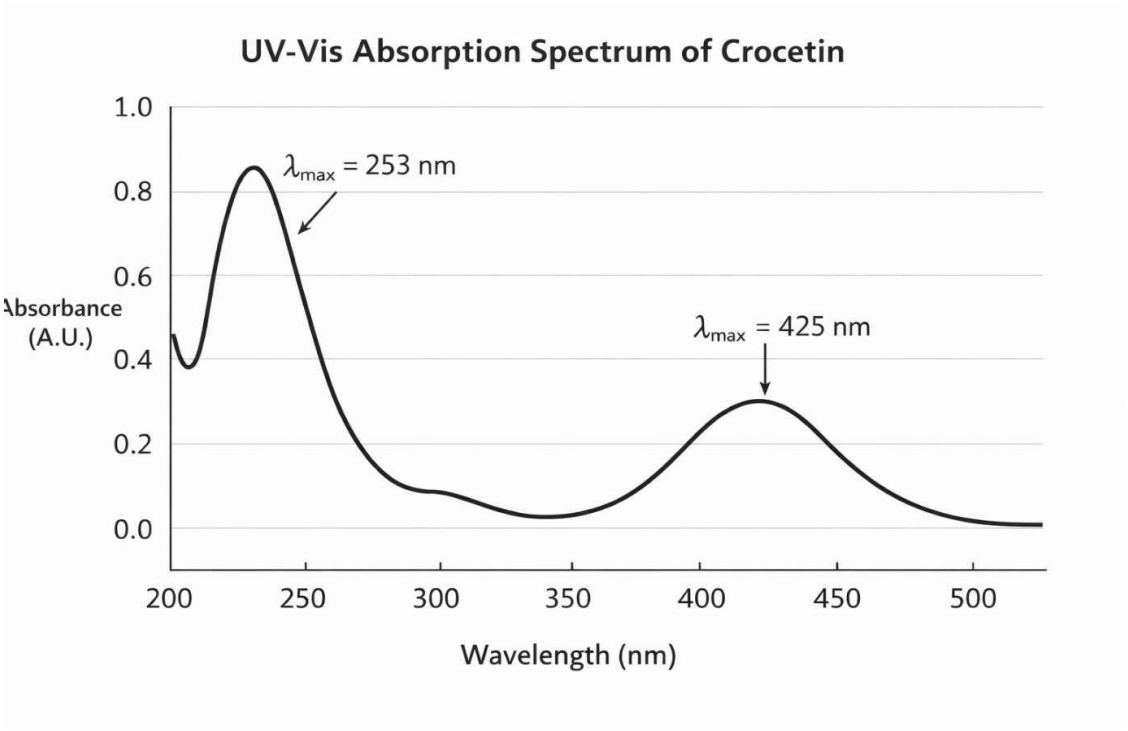
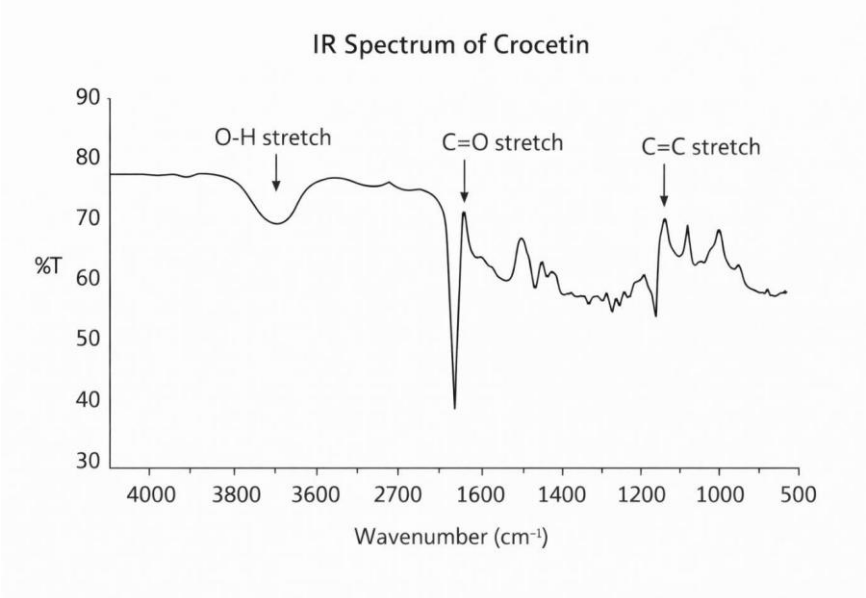


Figure No. 10: **UV–Visible absorption spectrum of crocetin showing characteristic absorption maxima (λ_max) at 253 nm and 425 nm**

FTIR Spectroscopic Characteristics of crocetin drug: The infrared (IR) spectrum of crocetin shows characteristic absorption bands that confirm the presence of its key functional groups and overall molecular structure. 1. Broad O–H Stretch (≈ 3200–3600 cm^{-1}) : A broad and moderately intense band appears in this region due to O–H stretching vibrations of the carboxylic acid groups present at both ends of the crocetin molecule. The broad nature of the peak indicates hydrogen bonding, which is typical for carboxylic acids. 2. C–H Stretching (≈ 2850–3000 cm^{-1}): Weak to medium intensity bands in this region correspond to aliphatic and olefinic C–H stretching vibrations from the polyene chain of crocetin. 3. Strong C=O Stretch (≈ 1680–1720 cm^{-1}): A sharp and intense absorption band is observed due to the carbonyl (C=O) stretching of the carboxylic acid functional groups. This peak is one of the most diagnostic signals confirming the presence of carboxyl groups in crocetin. 4. C=C Stretching (≈ 1600–1650 cm^{-1}): Bands in this region arise from conjugated C=C stretching vibrations of the extended polyene system. The conjugation lowers the stretching frequency, which is characteristic of carotenoid-type molecules. 5. C–O Stretching (≈ 1200–1300 cm^{-1}): Medium intensity peaks in this region are attributed to



C–O stretching vibrations of the carboxylic acid group. 6. Out-of-plane C– Bending (≈ 900–700 cm^{-1}): These bands correspond to olefinic C– H bending vibrations, supporting the presence of multiple trans double bonds in the conjugated chain.

The ^{13}C NMR Spectrum of Crocetin ($C_{20}H_{24}O_4$) (Recorded at 125 MHz in $CDCl_3$)

The ^{13}C NMR spectrum of crocetin provides clear evidence of its carbon framework, which consists of conjugated double bonds, terminal carboxylic groups, and methyl substituents. The spectrum displays well-resolved signals that can be assigned to different carbon environments within the molecule.

1. Carbonyl Carbons (C=O) – 170–180 ppm Strong downfield signals observed in the region 170–180 ppm are attributed to the carboxylic acid carbonyl carbons (–COOH) present at both ends of the crocetin molecule. These carbons are highly deshielded due to the electronegative oxygen atoms, making them appear at higher chemical shift values.

2. Olefinic (Alkene) Carbons – 120–160 ppm: Multiple signals appearing between 120 and 160 ppm correspond to the sp^2-hybridized carbons of the extended conjugated polyene chain. The variation in chemical shifts reflects different electronic environments within the conjugated system. This region confirms the presence of a long chain of alternating double bonds, which is responsible for crocetin's intense color and antioxidant properties.

3. Methyl Carbons ($–CH_3$) – 10–30 ppm Signals observed in the upfield region 10–30 ppm are assigned to the methyl carbons attached to the polyene backbone. These carbons are more shielded and therefore resonate at lower ppm values. Their presence supports the substituted nature of the conjugated chain.

4. Spectrum Characteristics The absence of signals in the 50–100 ppm region indicates a lack of saturated carbons attached to heteroatoms. The clear separation of carbonyl, olefinic, and methyl carbon signals suggests good sample purity and a well-defined structure.

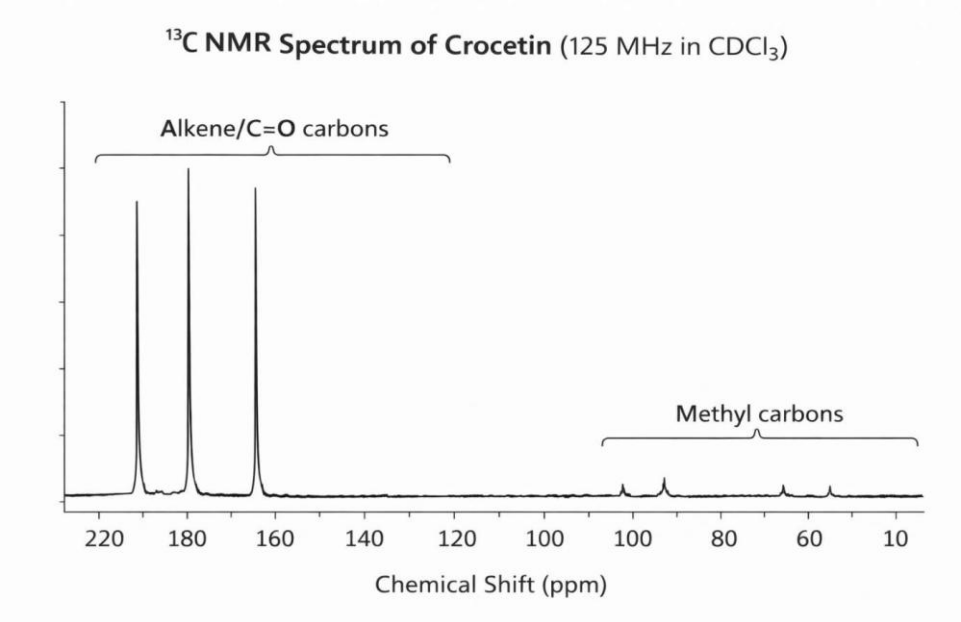


Figure No. 11: **13C NMR spectrum of crocetin recorded at 125 MHz in $CDCl_3$, showing characteristic signals corresponding to alkene and carbonyl (C=O) carbons in the downfield region and methyl carbons in the upfield region, confirming the structural features of crocetin.**

Mass Spectrum of Crocetin

The mass spectrum of crocetin provides clear evidence for its molecular weight and structural features. A prominent peak is observed at m/z ≈ 328.2, which corresponds to the molecular ion peak [M^+]. This peak confirms the molecular mass of crocetin and indicates that the molecule remains sufficiently stable under ionization conditions to be detected as an intact molecular ion. The molecular ion peak, several low-intensity fragment peaks appear at lower m/z values. These arise from the cleavage of the polyene chain and loss of small neutral fragments, such as alkyl groups or oxygen-containing moieties, which is characteristic of conjugated carotenoid derivatives. The relatively lower abundance of fragment ions compared to the molecular ion suggests a stable conjugated structure with delocalized electrons. The spectrum is plotted with m/z values on the x-axis and relative intensity (%) on the y-axis, which represents the abundance of ions relative to the most intense peak. The dominance of the molecular ion peak supports the identity and purity of crocetin, while the fragmentation pattern assists in structural confirmation. The mass spectral profile is consistent with crocetin's known chemical composition and is useful for molecular weight determination, structural verification, and quality control in pharmaceutical and phytochemical analysis.

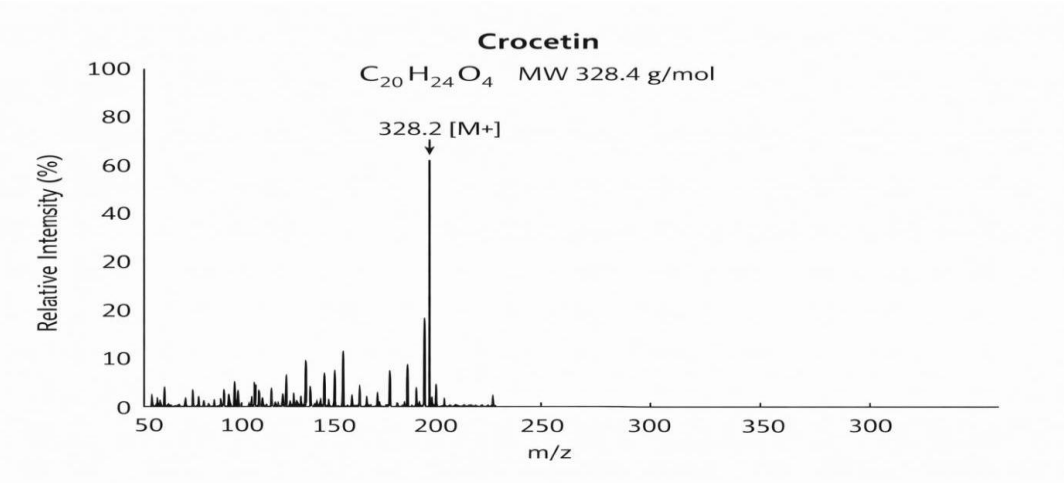


Figure No. 12: Mass spectrum of crocetin showing the molecular ion peak at m/z 328.2 [M^+], consistent with its molecular formula $C_{20}H_{24}O_4$ and molecular weight (328.4 g/mol), confirming the identity and purity of the compound.

CONCLUSION

The present study confirms that *Nyctanthes arbor-tristis* extract is a rich source of bioactive phytoconstituents, particularly phenolics, flavonoids, and terpenoids, as evidenced by both qualitative and quantitative analyses. The extract demonstrated significant in vitro pharmacological activities, including antioxidant, anti-inflammatory, and antimicrobial effects, which correlate with its phytochemical composition. Additionally, the successful identification and characterization of crocetin using advanced analytical techniques further validate the chemical profile of the plant. Overall, the findings support the traditional medicinal use of *Nyctanthes arbor-tristis* and highlight its potential as a promising natural therapeutic agent

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