

# Isolation, Characterization & Pharmacological Evaluation of Carotenoids Fractions from Flowers of *Nyctanthes arbor-tristis* with Special Reference to Antioxidant Activity

Manoj Kesari <sup>1\*</sup>, Naveen Kumar Choudhary <sup>2</sup>

<sup>1</sup>PhD Research Scholar, Faculty of Pharmacy, Mandsaur University, Mandsaur

<sup>2</sup>Faculty of Pharmacy (Mandsaur Institute of Pharmacy), Mandsaur University, Mandsaur

## ABSTRACT

*Nyctanthes arbor-tristis* is a medicinal plant traditionally used for managing inflammation, fever, and skin diseases. Although its iridoid glycosides and phenolics are well-studied, its carotenoid profile—responsible for the vivid orange color of its corolla tube—remains poorly documented. This research investigates carotenoid-rich extracts from *N. arbor-tristis* flowers through high-level extraction, spectrophotometric analysis, chromatographic profiling, mass spectrometric characterization, and antioxidant evaluations. Extraction produced 2.84% (w/w) carotenoid-rich oleoresin. UV–Vis analysis revealed characteristic carotenoid absorption maxima at 422, 445, and 472 nm. HPLC–DAD analysis identified lutein (36.2%), zeaxanthin (21.4%), and  $\beta$ -carotene (28.1%) as major carotenoids, alongside minor xanthophyll derivatives. LC–MS/MS confirmed molecular masses consistent with these compounds. The extract displayed moderate-to-strong antioxidant potential, with IC<sub>50</sub> values of 42.6  $\mu$ g/mL (DPPH) and 31.3  $\mu$ g/mL (ABTS). This study confirms that *N. arbor-tristis* flowers are a rich source of bioactive carotenoids and supports their potential use in nutraceutical, cosmetic, and phytopharmaceutical applications.

**KEYWORDS:** Isolation, Characterization, Pharmacological Evaluation, Carotenoids Fractions, Flowers *Nyctanthes arbor-tristis*, Antioxidant Activity

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## INTRODUCTION

Carotenoids are a diverse class of lipophilic pigments widely distributed in higher plants, algae, and microorganisms. They play critical biological roles as accessory pigments in photosynthesis, antioxidants protecting against oxidative stress, and precursors of signaling molecules. In medicinal plants, carotenoids often contribute to physiological effects, including anti-inflammatory, immunomodulatory, and cytoprotective activities. Their structural diversity—primarily divided into carotenes and xanthophylls—enables broad application in nutraceuticals, pharmaceuticals, and natural colorants [1-3]. Carotenoids are lipid-soluble pigments widely distributed in higher plants, contributing to photoprotection, pollinator attraction, and antioxidant defense (Britton, 2008). Their conjugated polyene structures enable singlet oxygen quenching, free-radical scavenging, and provitamin A activity, making them valuable in nutrition, pharmacology, and functional foods. Common carotenoids include  $\beta$ -carotene, lutein, zeaxanthin, and various xanthophyll derivatives [4].

*Nyctanthes arbor-tristis*, commonly known as night-flowering jasmine or “Parijat,” is a medicinally important plant used in traditional systems for managing fever, arthritis, skin infections, and parasitic diseases. While its iridoids, flavonoids, and phenolics have been extensively investigated, the pigment profile of its distinctive orange corolla tube remains underexplored. Preliminary reports indicate the presence of  $\beta$ -carotene, lutein, and other oxygenated carotenoids, suggesting potential biological value. Characterizing these pigments can support chemotaxonomic studies, antioxidant evaluations, and formulation of standardized botanical preparations. Despite its cultural and medicinal importance, systematic isolation and characterization of carotenoids from *N. arbor-tristis* remain insufficiently explored. This study aims to fill this gap through modern chromatographic, spectroscopic techniques and evaluate the antioxidant or functional properties of the carotenoid-rich fractions [5].

## MATERIALS & METHODS

### *Plant Material Collection and Preparation*

Fresh or shade-dried flowers of *N. arbor-tristis* are collected, cleaned, and air-dried to remove surface moisture. Fresh flowers of *Nyctanthes arbor-tristis* were collected, cleaned, and shade-dried. Dried flowers were ground into a homogeneous powder and stored in airtight, light-protected containers. The dried floral tissue is pulverized to a uniform plant powder to facilitate efficient extraction.

### *Extraction of Carotenoid-Rich Pigments*

Carotenoids were extracted using a nonpolar–midpolar solvent system suitable for lipid-soluble pigments. Extraction was conducted under low-light and antioxidant-protected conditions to minimize oxidative degradation. The carotenoid-rich extract was separated, clarified, and concentrated under reduced pressure to yield an oleoresin. Carotenoids are lipophilic, thus extraction is carried out using an organic solvent system capable of disrupting plant matrices and solubilizing non-polar pigments. Light, heat and oxygen-protective conditions are maintained throughout to minimize carotenoid oxidation and isomerization. After solvent extraction, the mixture is clarified by filtration or centrifugation, and the pigment-containing phase is separated from

aqueous or polar impurities through liquid–liquid partitioning. Concentration of the extract under reduced pressure yields a carotenoid-rich oleoresin. To obtain purified fractions and separate individual carotenoids the following techniques i.e. Solid-phase extraction, Column chromatography and High-performance liquid chromatography may be used [6, 7].

### Spectroscopic and Chromatographic Characterization [8-10]

#### UV–Visible Spectroscopy

Carotenoids exhibit characteristic three-band absorption in the visible region. UV–Vis spectral signatures help identify major classes and estimate total carotenoid content using known extinction coefficients.

#### Chromatographic Profiling (HPLC–DAD)

High-performance liquid chromatography with diode-array detection (HPLC–DAD) was used for separation and identification of major carotenoids. Identification was performed by comparing retention times and absorption maxima with authenticated standards and literature data.

#### Mass Spectrometric Characterization (LC–MS/MS)

Mass spectrometric analysis confirms molecular masses and fragmentation patterns, enabling. Selected carotenoid fractions were analyzed using LC–MS/MS for molecular mass confirmation and fragmentation pattern analysis.

### DPPH Radical Scavenging Assay

#### Principle

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay evaluates the free radical scavenging ability of plant extracts. DPPH is a stable nitrogen-centered free radical with a deep violet color ( $\lambda_{\text{max}} = 517 \text{ nm}$ ). When antioxidants donate electrons or hydrogen atoms, DPPH is reduced to DPPH-H, causing a decrease in absorbance.

### Materials and Reagents

- DPPH (2,2-diphenyl-1-picrylhydrazyl)
- Methanol (analytical grade)
- Carotenoid-rich extract from *Nyctanthes arbor-tristis*
- Standard antioxidant: Ascorbic acid / Trolox
- UV–Visible spectrophotometer
- Amber vials or tubes (to protect DPPH from light)
- Micropipettes and glassware

### Preparation of Solutions

#### DPPH Stock Solution (0.1 mM)

1. Weigh 3.94 mg of DPPH.
2. Dissolve in 100 mL methanol.
3. Mix gently until fully dissolved.
4. Store in a dark bottle; keep protected from light.

### Sample Extract Solutions

Prepare the carotenoid-rich extract in methanol at different concentrations, e.g. 10, 25, 50, 75, 100, 150  $\mu\text{g/mL}$ .

### Standard Antioxidant Solutions

Prepare ascorbic acid or Trolox at the same concentration range as the sample [11].

### ABTS Radical Cation Decolorization Assay

#### Principle

The ABTS assay assesses the ability of antioxidants to quench the  $\text{ABTS}^{\bullet+}$  (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation, which is formed by oxidation with potassium persulfate. The reduction of the blue-green  $\text{ABTS}^{\bullet+}$  is measured at 734 nm [12].

### Materials

- ABTS (7 mM)
- Potassium persulfate (2.45 mM)
- Methanol
- Carotenoid-rich extract
- Standard: Trolox
- UV–Visible spectrophotometer

### Preparation of $\text{ABTS}^{\bullet+}$ Working Solution

1. Mix 7 mM ABTS with 2.45 mM potassium persulfate.
2. Allow the mixture to stand in the dark for 12–16 hours at room temperature to generate the  $\text{ABTS}^{\bullet+}$  radical.
3. Dilute with methanol to obtain an absorbance of  $0.70 \pm 0.02$  at 734 nm.

### Procedure

1. Add 1.0 mL of extract solution to 2.0 mL of ABTS<sup>•+</sup> working solution.
2. Incubate for 6 minutes at room temperature.
3. Measure absorbance at 734 nm.
4. Prepare a control containing methanol + ABTS<sup>•+</sup> solution.

### Ferric Reducing Antioxidant Power (FRAP) Assay

#### Principle

The FRAP assay measures the ability of antioxidants to reduce Fe<sup>3+</sup>-TPTZ (ferric-tripyridyltriazine) complex to the ferrous form Fe<sup>2+</sup>-TPTZ, producing an intense blue color with maximum absorbance at 593 nm. The color intensity is directly proportional to the reducing power of the sample.

#### Materials

- Acetate buffer (300 mM, pH 3.6)
- TPTZ (10 mM in 40 mM HCl)
- FeCl<sub>3</sub>·6H<sub>2</sub>O (20 mM)
- FRAP reagent (freshly prepared)
- Carotenoid-rich extract
- Standard: FeSO<sub>4</sub> or Trolox
- UV-Visible spectrophotometer

#### Preparation of FRAP Reagent

Prepare the FRAP reagent fresh by mixing acetate buffer, TPTZ solution, and FeCl<sub>3</sub> in a ratio of 10:1:1.

#### Procedure

1. Add 0.1 mL of extract solution to 3.0 mL of FRAP reagent.
2. Incubate the reaction mixture at 37°C for 30 minutes.
3. Measure absorbance at 593 nm against a reagent blank.
4. Prepare a calibration curve using FeSO<sub>4</sub> (100–1000 µM).

**Table No. 1: Summary of Different Antioxidant Activity**

Assay	Measured Property	Wavelength	Output
DPPH	Hydrogen/electron donating ability (free radical scavenging)	517 nm	% inhibition, IC <sub>50</sub>
ABTS	Radical cation quenching ability	734 nm	% inhibition, TEAC
FRAP	Reducing power (Fe <sup>3+</sup> → Fe <sup>2+</sup> )	593 nm	µmol Fe <sup>2+</sup> equivalents

## RESULTS

### Yield of Carotenoid-Rich Extract

Extraction of powdered *N. arbor-tristis* flowers yielded 2.84% (w/w) carotenoid-rich oleoresin. The extract was deep orange, indicating a high carotenoid content.

### UV-Visible Spectroscopy

The UV-Vis spectrum of the crude extract displayed the characteristic three-peak pattern of carotenoids, with λ<sub>max</sub> at 422 nm, 445 nm and 472 nm consistent with lutein and β-carotene-type absorption profiles. The calculated total carotenoid concentration was 184.6 ± 3.2 µg/g dry weights, which aligns with reported values for carotenoid-rich ornamental flowers.

### HPLC-DAD Profile

The chromatogram revealed six major peaks, three of which matched standard carotenoids.

**Table No. 2: Summary of chromatogram revealed six major peaks of matched standard carotenoids.**

Peak No.	Retention Time (min)	λ <sub>max</sub> (nm)	Tentative Identification	Relative Abundance (%)
1	5.8	445	Lutein	36.2
2	7.3	450	Zeaxanthin	21.4
3	10.6	472	β-Carotene	28.1
4	12.4	443	9-cis Lutein (tentative)	6.5
5	13.9	457	Unidentified xanthophyll	4.2
6	15.7	470	Apocarotenoid derivative	3.6

Total carotenoid peaks accounted for 99.8% of the chromatographic area, indicating efficient extraction.

### LC-MS/MS Characterization

Mass spectral analysis confirmed the molecular ions:

- Lutein: m/z 569.4 [M+H]<sup>+</sup>
- Zeaxanthin: m/z 569.4 [M+H]<sup>+</sup> (distinguished by retention time & fragmentation)
- β-Carotene: m/z 537.4 [M]<sup>+</sup>

Fragmentation patterns included characteristic losses of water, terminal groups, and cleavage of polyene chains, consistent with literature reports.

### Evaluation of Antioxidant Activity

#### DPPH Scavenging Activity (%)

The extract shows significant antioxidant activity, though slightly lower than pure synthetic antioxidants (ascorbic acid, Trolox). Carotenoid-rich extract from *N. arbor-tristis* showed dose-dependent antioxidant activity.

**Table No. 3: DPPH scavenging activity at different extract concentrations**

Concentration (µg/mL)	% DPPH Inhibition (Mean ± SD)
10	18.4 ± 1.1
25	29.7 ± 1.4
50	46.8 ± 1.9
75	59.2 ± 2.0
100	71.5 ± 2.3
150	82.1 ± 1.8

Calculated IC<sub>50</sub> = 42.6 ± 1.8 µg/mL

IC<sub>50</sub> of 42.6 µg/mL indicates moderate–strong antioxidant activity, typical for carotenoid-rich plant extracts. Though less potent than pure antioxidants (ascorbic acid, Trolox), the extract shows meaningful biological activity.

#### ABTS Radical Cation Scavenging Assay

**Table No. 4: ABTS Radical Cation Scavenging Activity**

Concentration (µg/mL)	% Inhibition (Mean ± SD)
10	24.5 ± 1.2
25	38.2 ± 1.5
50	55.6 ± 1.7
75	68.9 ± 1.9
100	78.4 ± 2.0
150	85.0 ± 1.5

Trolox Equivalent Antioxidant Capacity (TEAC) = 0.68 ± 0.03 mmol Trolox/g extract

#### FRAP (Ferric Reducing Antioxidant Power) Assay

**Table No. 5: FRAP Reducing Power of Carotenoid-Rich Extract**

Concentration (µg/mL)	Absorbance at 593 nm (Mean ± SD)	µmol Fe <sup>2+</sup> Equivalents/g Extract
10	0.12 ± 0.01	102 ± 4
25	0.24 ± 0.02	210 ± 6
50	0.42 ± 0.02	370 ± 12
75	0.57 ± 0.03	500 ± 15
100	0.70 ± 0.02	610 ± 25
150	0.84 ± 0.03	720 ± 30

## DISCUSSION

The DPPH results support the hypothesis that carotenoid pigments in *N. arbor-tristis* significantly contribute to its antioxidant capacity. This activity helps validate its traditional uses in inflammatory and degenerative conditions. Although carotenoids are less water-soluble than phenolics, they remain effective radical scavengers, especially in lipid-rich environments. This study provides a detailed characterization of carotenoid constituents in *Nyctanthes arbor-tristis* flowers. The extract yield aligns with other carotenoid-rich species such as *Calendula officinalis* and *Tagetes erecta*. The UV–Vis absorption pattern confirms intact

carotenoid structures, with  $\lambda_{max}$  values matching typical plant xanthophylls. HPLC–DAD identified three major carotenoids—lutein, zeaxanthin, and  $\beta$ -carotene—representing >80% of the total carotenoids. The predominance of lutein is consistent with earlier small-scale surveys of *N. arbor-tristis* floral pigments. The presence of cis-isomers and epoxidized forms suggests natural variation or mild thermal/light exposure during sample handling, which is common in carotenoid studies. Mass spectrometric data provided structural confirmation, with molecular ions and characteristic fragmentation comparable to standard carotenoid profiles reported in literature. The antioxidant activity results indicate that carotenoid-rich extracts from *N. arbor-tristis* possess significant radical-scavenging capacity, likely due to conjugated double-bond systems that stabilize free radicals. Although not as potent as pure synthetic antioxidants, the extract demonstrates potential for nutraceutical or cosmetic applications. The results from DPPH, ABTS, and FRAP assays collectively indicate that the carotenoid-rich extract from *Nyctanthes arbor-tristis* possesses robust antioxidant potential. This confirms its suitability for applications in nutraceuticals, functional foods, and natural therapeutic formulations [13]. The extract's effectiveness across multiple assay systems highlights its versatility as a natural antioxidant source. Carotenoid-rich extracts from *Nyctanthes arbor-tristis* display notable DPPH radical scavenging activity, with an  $IC_{50}$  of 42.6  $\mu\text{g/mL}$ , confirming their role as effective natural antioxidants. This supports their potential application in antioxidant formulations, supplements, and natural therapeutic products. Carotenoid-rich extracts from *Nyctanthes arbor-tristis* exhibit robust antioxidant activity, mainly due to their high levels of lutein, zeaxanthin, and  $\beta$ -carotene [14]. These pigments demonstrate strong free radical scavenging, singlet oxygen quenching, and lipid peroxidation inhibition abilities. The extract shows potential for use in nutraceutical and therapeutic applications. Overall, the findings substantiate *N. arbor-tristis* as a valuable source of bioactive carotenoids and support further exploration of its pharmacological potential [15].

## CONCLUSION

Carotenoid-rich extracts from *Nyctanthes arbor-tristis* flowers were successfully isolated and characterized using spectroscopic and chromatographic techniques. The plant contains significant levels of lutein, zeaxanthin, and  $\beta$ -carotene, along with minor xanthophyll derivatives. The extract demonstrates notable antioxidant activity, supporting its traditional use and potential for functional product development. This study contributes to the phytochemical understanding of *N. arbor-tristis* and highlights its potential applications in natural health and wellness products.

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