Exploring The Therapeutic Potential of *Carissa Carandas*: In Vitro Antioxidant and Anti-Parkinson Activity Evaluation

Priyanshu Srivastava[1], Anubhav Dubey[1*], Vikram Kumar Sahu[1], Sribatsa Lanchhana Dash[1], Amit Mishra[2]

[1] Maharana Pratap College of Pharmacy Kothi, Mandhana, Kanpur -209217, Uttar Pradesh, INDIA.
[2] Maharana Pratap College of Pharmaceutical Sciences, Kothi, Mandhana, Kanpur -209217, Uttar Pradesh, INDIA.
Corresponding Author:
anubhavdwivedi803@gmail.com

Abstract:

Background:

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by dopaminergic neuronal loss, oxidative stress, mitochondrial dysfunction, and neuroinflammation. Natural products rich in bioactive phytochemicals have emerged as promising candidates for neuroprotection. Carissa carandas (Karonda) contains flavonoids, phenolics, triterpenoids, and other secondary metabolites with reported antioxidant and anti-inflammatory properties. This study evaluated the antioxidant and anti-Parkinson potential of the n-hexane extract of Carissa carandas fruits through phytochemical characterization and in vitro biological assays.

Methods:

Fruits of Carissa carandas were collected, authenticated, and extracted using n-hexane. The extract was subjected to CHNS elemental analysis, qualitative phytochemical screening, estimation of total phenolic and flavonoid contents, and GC–MS analysis for phytochemical profiling. Antioxidant activity was assessed using the DPPH free radical scavenging assay. Anti-Parkinson potential was evaluated by monoamine oxidase (MAO-A/B) inhibition assay and neuroprotective assessment using the SH-SY5Y human neuroblastoma cell line through the MTT assay.

Results:

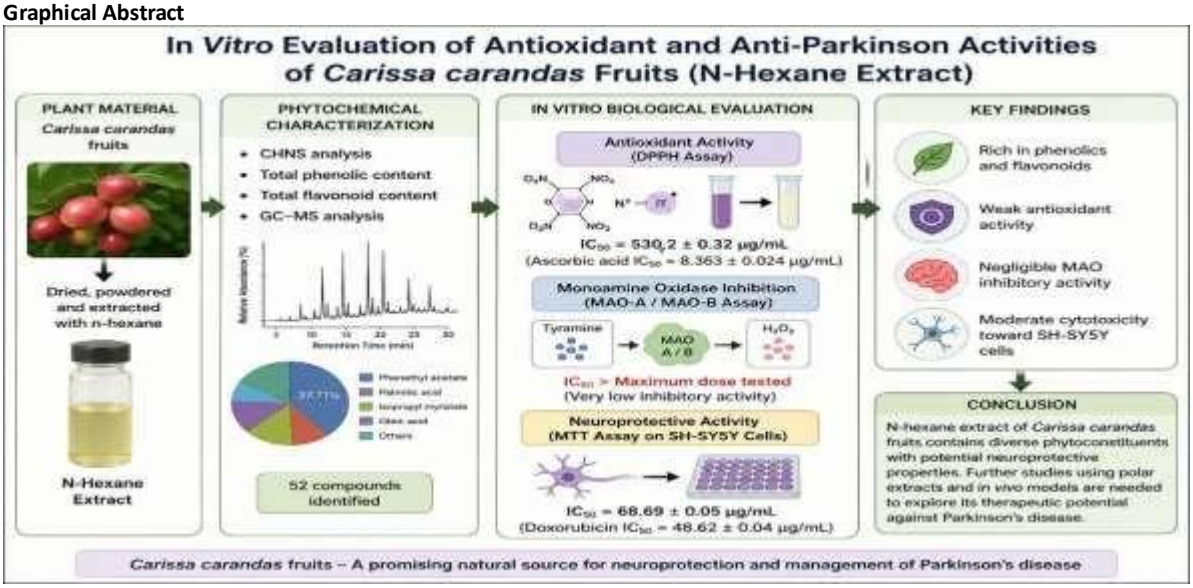
The n-hexane extract showed a recovery yield of 3.34%. Total phenolic and flavonoid contents were 5.82 ± 0.38 mg GAE/100 g extract and 0.49 ± 0.02 mg QE/100 g extract, respectively. The DPPH assay demonstrated comparatively weak antioxidant activity with an IC_{50} of 530.2 ± 0.32 µg/mL compared with ascorbic acid (8.363 ± 0.024 µg/mL). GC–MS analysis identified 52 phytochemical constituents, including phenethyl acetate, palmitic acid, oleic acid, isopropyl myristate, globulol, and long-chain hydrocarbons. The extract exhibited negligible MAO inhibitory activity (IC_{50} above the maximum tested dose). In the MTT assay, the extract showed moderate cytotoxicity toward SH-SY5Y cells with an IC_{50} of 68.69 ± 0.05 µg/mL compared with doxorubicin (48.62 ± 0.04 µg/mL).

Conclusion:

Although the n-hexane extract of Carissa carandas fruits demonstrated limited antioxidant and MAO inhibitory activities, GC–MS analysis confirmed the presence of several biologically relevant phytoconstituents with potential neuroprotective significance. Further investigations using polar extracts, mechanistic cellular models, and in vivo Parkinson's disease models are warranted to validate its therapeutic potential and identify the bioactive constituents responsible for neuroprotection.

Keywords: Carissa carandas, Parkinson's disease, Neuroprotection, GC–MS analysis, Antioxidant activity, Monoamine oxidase inhibition.

Graphical Abstract



Introduction

Parkinson disease (PD) is a progressive neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra and the accumulation of alpha-synuclein within Lewy bodies. The condition affects approximately 1% of individuals older than 60 years and represents the fastest-growing neurodegenerative disorder worldwide. Clinical presentation most commonly begins with asymmetric motor features, including bradykinesia, resting tremor, rigidity, and postural instability. Nonmotor symptoms such as constipation, sleep disturbance, depression, cognitive decline, and autonomic dysfunction often precede or accompany motor findings, contributing significantly to morbidity. By the time of diagnosis, more than half of dopaminergic neurons are already lost, and evidence suggests that pathological changes may begin a decade or more before motor manifestations [1-2]. Carissa carandas L. (family: Apocynaceae), commonly known as Karonda, is a medicinal plant widely recognized for its rich phytochemical composition and diverse pharmacological activities. The plant contains numerous bioactive compounds, including flavonoids (quercetin, kaempferol), phenolic acids, triterpenoids (ursolic acid, oleanolic acid), saponins, tannins, alkaloids, and vitamin C, which collectively exhibit potent antioxidant, anti-inflammatory, and neuroprotective properties. Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra, resulting in motor dysfunction, oxidative stress, mitochondrial impairment, and chronic neuroinflammation. The phytoconstituents of Carissa carandas have the potential to mitigate these pathological mechanisms. Flavonoids and phenolic compounds effectively scavenge reactive oxygen species (ROS), reducing oxidative damage to neuronal cells. Triterpenoids such as ursolic acid inhibit inflammatory mediators, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and nuclear factor-kappa B (NF-κB), thereby attenuating neuroinflammation. Furthermore, these compounds may enhance endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), helping maintain neuronal integrity. Emerging experimental evidence suggests that these phytochemicals may protect dopaminergic neurons from apoptosis and improve mitochondrial function. Therefore, Carissa carandas represents a promising natural source of neuroprotective phytochemicals that could serve as complementary therapeutic agents for the prevention and management of Parkinson's disease, although further preclinical and clinical studies are required to validate its efficacy and safety [3-4].

Methodology

Plant material collection and identification

Carissa Carandas fruits were employed in this investigation. The authors gathered the samples at Maharana Pratap College of Pharmacy's and approved the plant Central Institute of Medicinal & Aromatic Plants in Lucknow. They placed a voucher specimen in the Plant Systematics Laboratory. (Ang./2025-26/85 25 Feb.2026).

Extraction

5 gm of sample was taken and mixed with 50 ml of solvent (Hexane). Sample mixture was then incubated on a rocker shaker for 24 hours. Then extract was then filtered through Whatman filter paper 1, and the extract was then completely dried in the oven at 40°C. Extract were collected in micro centrifuge tube and stored at 4°C [5-6]. Using the calculation, we determined the yield of the soluble ingredients by weighing the drained extracts.

$$Yield\ \% = \text{Weight of dry extract} / \text{Weight of dry fruits powder} \times 100$$

Chemicals: All the chemical were of AR grade and were purchased from Vikas Sales Corporation, India.

CHNS analysis: On Unicube (Germany, Elementar), an elemental analysis of Carissa Carandas fruits powder was done. CHNS was quantified and analysed at the Energetic Materials Lab of IIT. Kanpur [7].

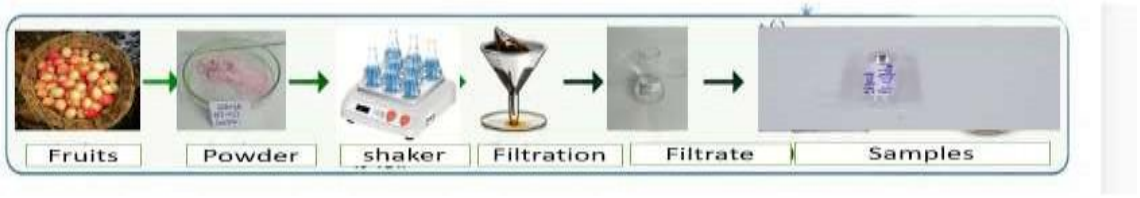


Figure-1 N-hexane extractions of Carissa Carandas fruits

Qualitative phytochemical screening:

To confirm the existence of different bioactive components, a standard qualitative investigation of the N-hexane extractions extract was performed.

Determination of total flavonoid contents:

Aluminum chloride colorimetric techniques were used to determine flavonoid concentrations. Carissa Carandas fruits extract was sonicated for 15 minutes at maximum power in a volume of 10 milliliters of 80% methanol before being filtered using Whatmann filter paper No.42 (125 millimeters). Then, in a test tube, they combined 0.3 mL of filtrate, 3.4 mL of 30% methanol, 0.15 mL of 0.5 M $NaNO_2$ and 0.15 mL of 0.3 M $AlCl_3$-$6H_2O$. The absorbance was taken at 510 nm after adding 1

mL of 1M NaOH and letting it sit for 5 minutes. Using quercetin quantities that were previously determined, a standard calibration curve was generated. Absorbance was used to measure total flavonoid concentration which was then expressed as mg quercetin equivalent per gram of extract (mg QE/g powder) [7-8].

Determination of total phenolic compound:

The extracts' total soluble phenolic content was calculated using a modified version of the Folin-Ciocalteu technique15. The sample solution (1.0 mg/mL) and the 10% Folin-Ciocalteu solution (400 µll) were combined in a volumetric flask and incubated for 10 minutes at room temperature before being combined with the 7% Na2CO3 solution (0.2 mL). Finally, 10 mL of the solution was prepared by diluting the original solution with deionized distilled water in a volumetric flask. After 2 hours at room temperature, the absorbance of the combination was measured at 725 nm to determine its stability. Using the equation y = 0.2177x+0.0005, r2=0.999, we were able to convert the total phenolic components in the extract into grams of gallic acid equivalent (GAE). If x represented concentration and y represented absorbance, then gallic acid equivalents per gram of extract (mg GAE/g extract) were used to determine total phenolics [9-10].

In vitro antioxidant activity

DPPH Scavenging Assay:

To evaluate antioxidant activity, 10 µL of different concentrations of test samples or standard (Ascorbic Acid, SRL Cat. No. 23006) were mixed with 200 µL of 0.1 mM DPPH solution (SRL Chem, Cat. No. SR-29128) in a 96-well plate. Reactions were prepared in quadruplicate; duplicate blanks (sample + methanol without DPPH), red blanks (without DPPH), and untreated wells served as controls. Plates were incubated in the dark for 30 min, and absorbance was read at 517 nm using a microplate reader (iMark, Bio-Rad). A combination of test solution with 20 µL deionized waters served as control. Scavenging activity (%) was calculated relative to control. IC_{50} values were derived using GraphPad Prism 6 by plotting inhibition (%) vs. sample concentration [11].

Calculations:

% RSA= (ABS control-Abs Sample)/Abs control) x100

GC-MS Analysis GC-MS analysis of N-hexane extracts was performed using a PerkinElmer Clarus 600 system equipped with an Rtx-5MS capillary column. Helium (99.99%) was used as the carrier gas at a constant flow rate of 1.0 mL/min. Injection volume: 1 µL, split ratio: 10:1, ionization energy: 70 eV, scan range: 40–650 m/z, scan time: 0.2 sec. Injector temperature: 260°C. Oven temp held at 50°C for 3 min, then ramped to 300°C at 10°C/min. Compounds were identified by comparing retention times and mass spectra with those in the NIST and Wiley-8 libraries [12-13].

In Vitro Models of Anti-Parkinson Activity

MTT assay: Cytotoxicity of the provided samples on SHSY5Y (Passage No. 23, Procured from NCCS Pune, India, RRID-CVCL_0019) cell line was determined by MTT Assay. The cells (10000 cells/well) were cultured in 96 well plate for 24 h in DMEM medium (Dulbecco's Modified Eagle Medium-AT149-1L) supplemented with 10% FBS (Fetal Bovine Serum - HIMEDIA-RM 10432) and 1% antibiotic solution (Penicillin-Streptomycin-Sigma-Aldrich P0781) at 37°C with 5% CO2. Next day cells were treated from different concentrations of the samples (concentration as per mentioned in excel sheet). Stock solution of samples was prepared in DMSO and further diluted to get different concentrations in incomplete Cell culture Medium (Without FBS). After incubation for 24 hours, MTT Solution (5 mg/ml) was added to cell culture and further incubated (Air-jacketed CO2 incubator-Heal force-HF90) for 2 h. Cells without treatment were considered as Control and cells without MTT were considered as Blank. At the end of the experiment, culture supernatant was removed, and cell layer matrix was dissolved in 100 µl Dimethyl Sulfoxide (DMSO –SRL- Cat no.- 67685) and read in an Elisa plate reader (iMark, Biorad, USA) at 540 nm. IC50 was calculated by using Graph Pad Prism -6. Images were captured under inverted microscope (Olympus ek2) using Camera (AmScope digital camera 10 MP Aptima CMOS). 50% inhibitory concentration (IC50) was presented as Mean ± SEM (Standard Error of Mean) [13-14].

Calculation

% Viable cells = (Atest / AControl) *100

(Atest = Absorbance of test sample)

Enzyme Inhibition Assay -Monoamine Oxidase A/B: 5 µl of different stock dilutions of the test sample (0 to 1000 µg/ml) or inhibitor (0 to 50 µM) were added to the defined wells of the 96-well plate. 5 µl of enzyme were added to the wells of all the sets. After that, the plate was incubated (Basil Scientific Corp. India- Incubator) for 10 minutes at 37°C. 5 µl of tyramine (Cat No: T90344) 40 mM were added to the defined wells. 15 µl of the reaction mixture (containing 5 µl of hydrogen peroxidase (Cat No: HP6520) (10 U/ml), 5 µl of amino antipyrine (1 mM), and 5 µl of 5-dichloro-2-hydroxybenzenesulfonic acid (DCHBS)) were added to all wells of the plate. The plate was incubated for 30 minutes at 37°C. The absorbance was measured at 515 nm using ELISA micro plate reader. (iMark, BioRad). Inhibitor, Acarbose (SRL- Cat no-65457) (50 µg/ml final Concentration) was used as a positive control. IC-50 was calculated using Software Graph Pad Prism 6[15-16].

Statistical investigation:

All data were investigated by means of the analysis of variance (ANOVA) followed by the Post Hoc Tukey's test (Graph Pad Prism Software-6). All data represent three independent values and results are presented as mean ± S.E. Statistical significance was considered as P value ≤ 0.05.

Result and Discussion

The Hexane leaver extracts of PS-01had yields in percentages of 3.34% respectively in table-1.

Table-1 Represents recovery rate of Hexane fruits extracts

S. No.	Sample Code	Weight of Powder (mg)	Weight of Extract (mg)	% Recovery
1	PS-01Carissa Carandas fruits	5000	167	3.34

CHNS analysis:

A compound's elemental makeup may be determined using elemental analysis. Carbon, hydrogen, nitrogen and sulphur were identified in the study. Both nitrogen and sulphur are essential macronutrients for plant development. Plants benefit from the presence of certain elements, while humans benefit from obtaining those elements via ingestion. Carbohydrates and hydrocarbons may be extracted from plants with a high carbon and hydrogen content. Because of its high nitrogen and sulphur content, this plant is likely a good source of proteins and vitamins, both of which are crucial to good health. Table 2 demonstrates that the required percentage of components may be found in Carissa Carandas fruits powder.

Table-2 Elements Percentage composition of Carissa Carandas fruits powder

S.N.	Elements	Results %
1.	Carbon	66.26%
2.	Hydrogen	9.923 %
3.	Nitrogen	2.81%
4.	Sulphur	0.359%

Determination of total phenolic and flavonoid compounds:

Table 3 displays the phenolic and flavonoid content of Carissa Carandas fruits powder. There were more total phenolics in the hexane extract

Table-3 Phenolic and flavonoid contents of Carissa Carandas fruits powder

Herbal extracts	Total phenolic contents (mg gallic acid/100g extract)	Total flavonoids (mg Quercetin/100g extract)
Hexane extract	5.82±0.38*	0.49±0.02*

Oxidative stress has been linked to age-related neurodegenerative diseases in a number of studies. Numerous studies have also examined the protective effects of antioxidants in preventing or reducing neuronal death that occurs in the pathophysiology of this disorder. Indicators of a compound's antioxidant potential also include its total antioxidant activity and ability to scavenge radicals. To determine the plant extracts' ability to act as antioxidants, activities—DPPH evaluated. The 1, 2-diphenyl-2-picryl hydroxyl radical (DPPH) was used to examine the antioxidant activity of many extracts. Table 4presents the results.

Table-4DPPH Scavenging Assay

Sample code	IC_{50} value (µg/ml) (Mean ± SEM)
Ascorbic Acid	8.363±0.024
PS-01	530.2±0.32

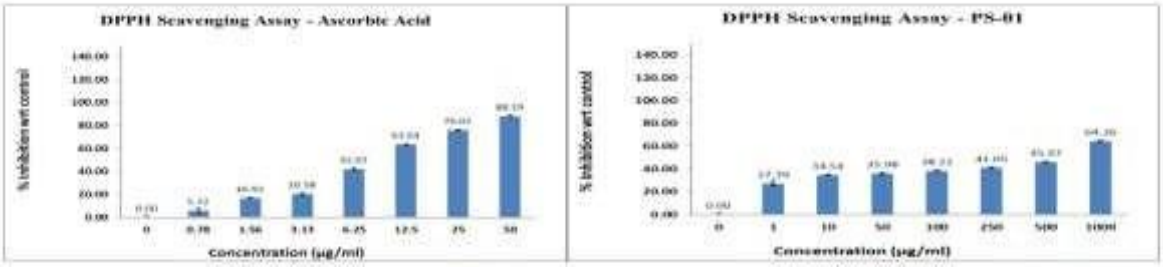


Figure-2 Graph represents the DPPH Scavenging Assay of Carissa Carandas fruits powder Extract

Based on the results obtained from the experimental work, Antioxidant activity (DPPH Assay) was estimated in sample, and 50% inhibitory concentration (IC_{50}) was mentioned in table 1. Sample – PS-01 was found to be less active. 530.2µg of sample-PS-01 was found equivalent to 8.363µg of standard Ascorbic acid. The standard compound, being a purified compound and highly active substance, was assessed using a lower concentration range (0 to 50 µg/ml). In contrast, the sample's potency remains unbound; therefore, a broader concentration range (0 to 1000 µg/ml) was employed for the sample analysis.

Identification of components: Identification was done using the calculated fragments, molecular mass, and molecular structure. The NIST database and the Willy8-library, which has more than 62,000 pattern entries, were used to interpret the GC-MS spectra. Together with their molecular weights and chemical structures, the components of the test materials were determined. The percentage amounts discovered in the sample were contrasted with component spectra from the Willy8 collection and the NIST library. This is done in order to determine whether this plant species has any chemicals or a combination of them that could support its traditional and commercial therapeutic purposes. It also helps determine the most effective methods for removing harmful compounds. The test materials' constituent parts were determined (Table 5, and Figure 3).

Table 5- Compounds of Carissa Carandas fruits powder Extract

Peak Report TIC-Sample- PS (01) n-hexane					
Peak	**R. Time**	**Area**	**Area %**	**Name**	**Common Names**
1	10.438	335644	1.82	DECANE	Decane
2	11.196	297754	1.62	1,1-Dibromo-2-(2,2-dimethylpropyl) cyclopropane	Brominated cyclopropane derivative
3	11.345	175400	0.95	Hexane, 2,4-dimethyl-	2,4-Dimethylhexane
4	11.532	473347	2.57	UNDECANE, 4,7-DIMETHYL-	4,7-Dimethylundecane
5	12.188	99272	0.54	TETRADECANE	Tetradecane
6	13.156	122844	0.67	1-Dodecene	1-Dodecene
7	13.267	620623	3.37	TETRADECANE	Tetradecane
8	13.469	110331	0.60	Octane, 3,4,5,6-tetramethyl-	3,4,5,6-Tetramethyloctane
9	13.911	61953	0.34	DODECANE	Dodecane
10	13.990	83941	0.46	Octane, 2,4,6-trimethyl-	2,4,6-Trimethyloctane
11	14.079	347065	1.88	TETRADECANE	Tetradecane
12	14.350	174445	0.95	Decane, 2,4,6-trimethyl-	2,4,6-Trimethyldecane
13	14.407	615220	3.34	HEPTADECANE	Heptadecane
14	14.551	117656	0.64	DODECANE	Dodecane
15	14.682	87235	0.47	Phenol, 3,5-bis(1,1-dimethylethyl)-	3,5-Di-tert-butylphenol
16	14.966	30490	0.17	Decane, 3,7-dimethyl-	3,7-Dimethyldecane
17	15.329	66210	0.36	DECANE, 2,9-DIMETHYL-	2,9-Dimethyldecane
18	15.417	64131	0.35	UNDECANE, 3-METHYL-	3-Methylundecane
19	15.685	230764	1.25	1-Tetradecene	1-Tetradecene
20	15.778	555591	3.02	TETRADECANE	Tetradecane
21	16.083	108000	0.59	3,4,5,6-TETRAMETHYLOCTANE	Tetramethyloctane
22	16.376	103370	0.56	METHYL DIHYDROJASMONATE	Methyl dihydrojasmonate (Hedione)
23	16.630	89416	0.49	(-)-GLOBULOL	Globulol
24	16.872	162839	0.88	Undecane, 3,7-dimethyl-	3,7-Dimethylundecane
25	16.932	726966	3.95	OCTADECANE	Octadecane
26	17.412	78473	0.43	UNDECANE, 4,7-DIMETHYL-	4,7-Dimethylundecane
27	17.953	191473	1.04	1-HEXADECANOL	Cetyl alcohol
28	18.029	265159	1.44	HEPTADECANE	Heptadecane
29	18.258	745973	4.05	ISOPROPYL TETRADECANOATE	Isopropyl myristate
30	18.410	195886	1.06	Octane, 3,4,5,6-tetramethyl-	Tetramethyloctane
31	18.533	196720	1.07	Oleic Acid	Oleic acid
32	19.116	145152	0.79	TETRADECANE	Tetradecane
33	19.204	512099	2.78	NONADECANE	Nonadecane
34	19.395	6947867	37.71	Benzeneacetic acid, 2-phenylethyl ester	Phenethyl acetate/phenethyl ester
35	19.781	1051591	5.71	HEXADECANOIC ACID	Palmitic acid
36	19.990	340438	1.85	ETHYL HEXADECANOATE	Ethyl palmitate
37	20.070	125297	0.68	Tridecane	Tridecane
38	20.509	40395	0.22	Sulfurous acid, 2-ethylhexyl isohexyl ester	Sulfite ester
39	20.881	59321	0.32	1-Octanol, 2-butyl-	2-Butyl-1-octanol
40	21.219	357901	1.94	NONADECANE	Nonadecane
41	21.650	477579	2.59	HEXADECANOIC ACID	Palmitic acid
42	21.794	127803	0.69	BUTYL STEARATE	Butyl stearate
43	21.927	33226	0.18	Tridecane	Tridecane
44	22.414	67731	0.37	Hexadecane	Hexadecane
45	22.689	66410	0.36	Methyl 2-hydroxydodecanoate	Hydroxy dodecanoate ester

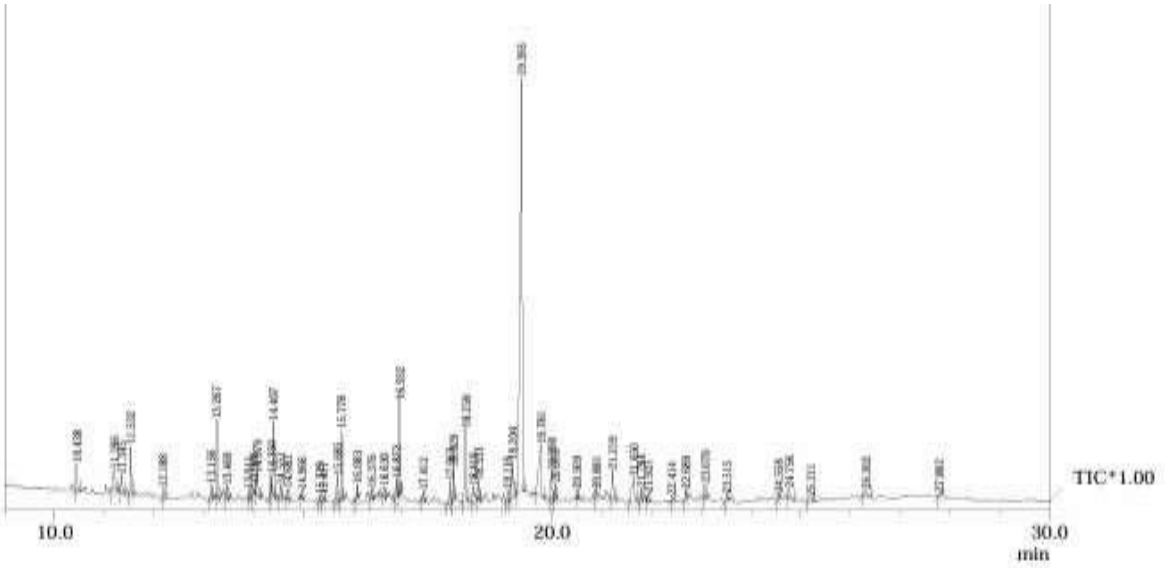


Figure-3 Carissa Carandas fruits powder Extract chromatogram

The GCMS analysis of sample- PS (01) n-hexane revealed the presence of many compounds. In sample- PS (01) n-hexane a total of 52 plant compounds were identified, which are already mentioned in above table. Based on the results obtained from the study, it was found that Enzyme Inhibition Activity Monoamine Oxygenase was observed in sample PS01. Sample-PS01 showed very low inhibitory activity

Table 6: Represents the Enzyme Inhibition Activity

Sample code	IC_{50} value (Mean ± SEM)
PS01	Above maximum dose limit
Chlorgyline (MAO-A inhibitor)	30.04 ± 0.004
Pargyline (MAO-B inhibitor)	41.69 ± 0.02

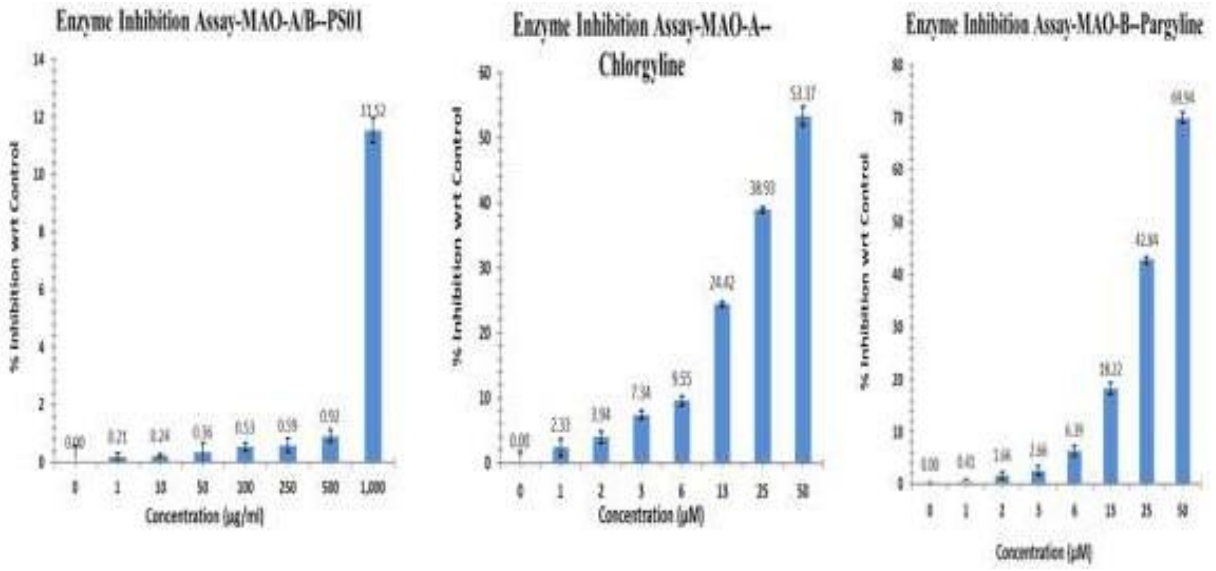


Figure-4 Represents the Enzyme Inhibition Activity of Chlorgyline and Pargyline

Table-6 Neuroprotective activity of Hexane extract and Percentage Viability of cells by MTT assay

Sample code	IC_{50} value (µg/ml) Mean ± SEM
Doxorubicin	48.62±0.04
PS01	68.69±0.05

Based on the results obtained from the MTT assay, it was observed that when the SHSY5Y cell line was exposed to different concentrations of the sample, cytotoxic activity was estimated for the sample and 50% inhibitory concentration as mentioned in table 6. Sample- PS01 was found to be moderately cytotoxic. IC_{50} is the concentration of an inhibitor/sample/ formulation at which the viable cells are reduced by half.

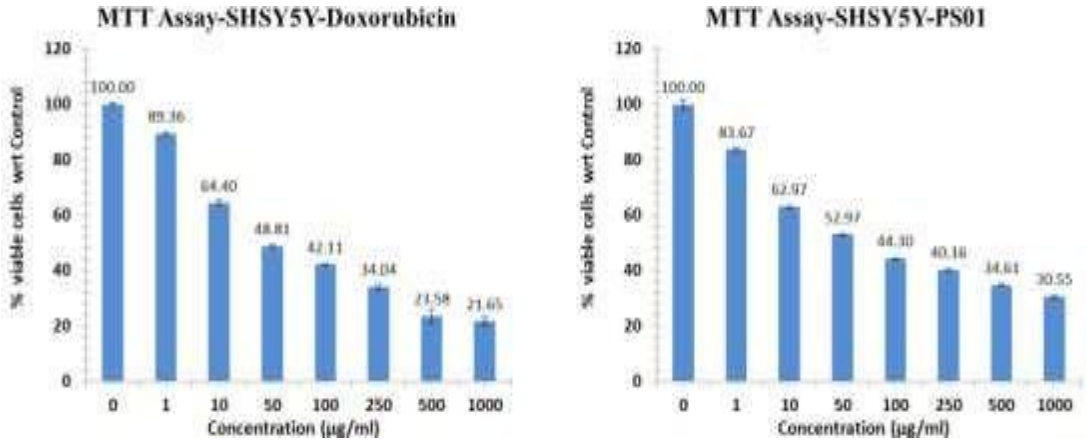


Figure-5 Represents the Neuroprotective activity of N-Hexane extract of Carissa Carandas fruits powder and Percentage Viability of cells by MTT assay

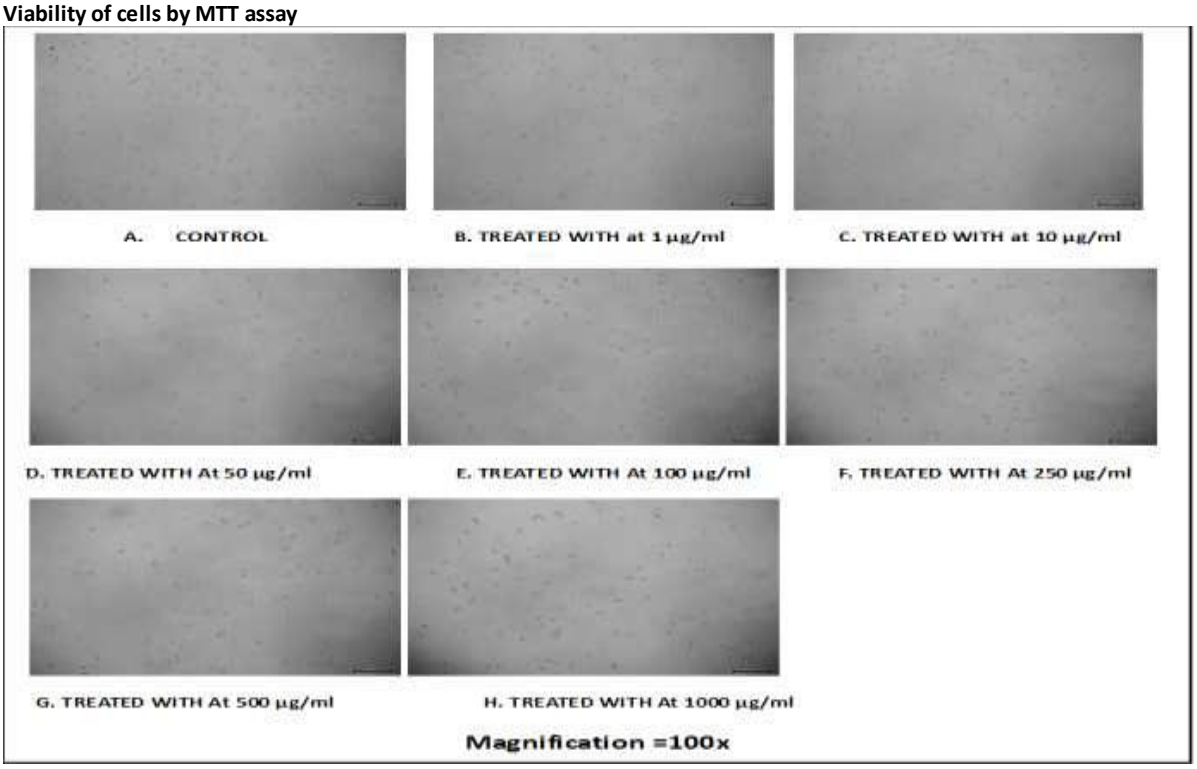


Figure-6 Cytopathic effects on SH-SY5Y treated by Doxorubicin

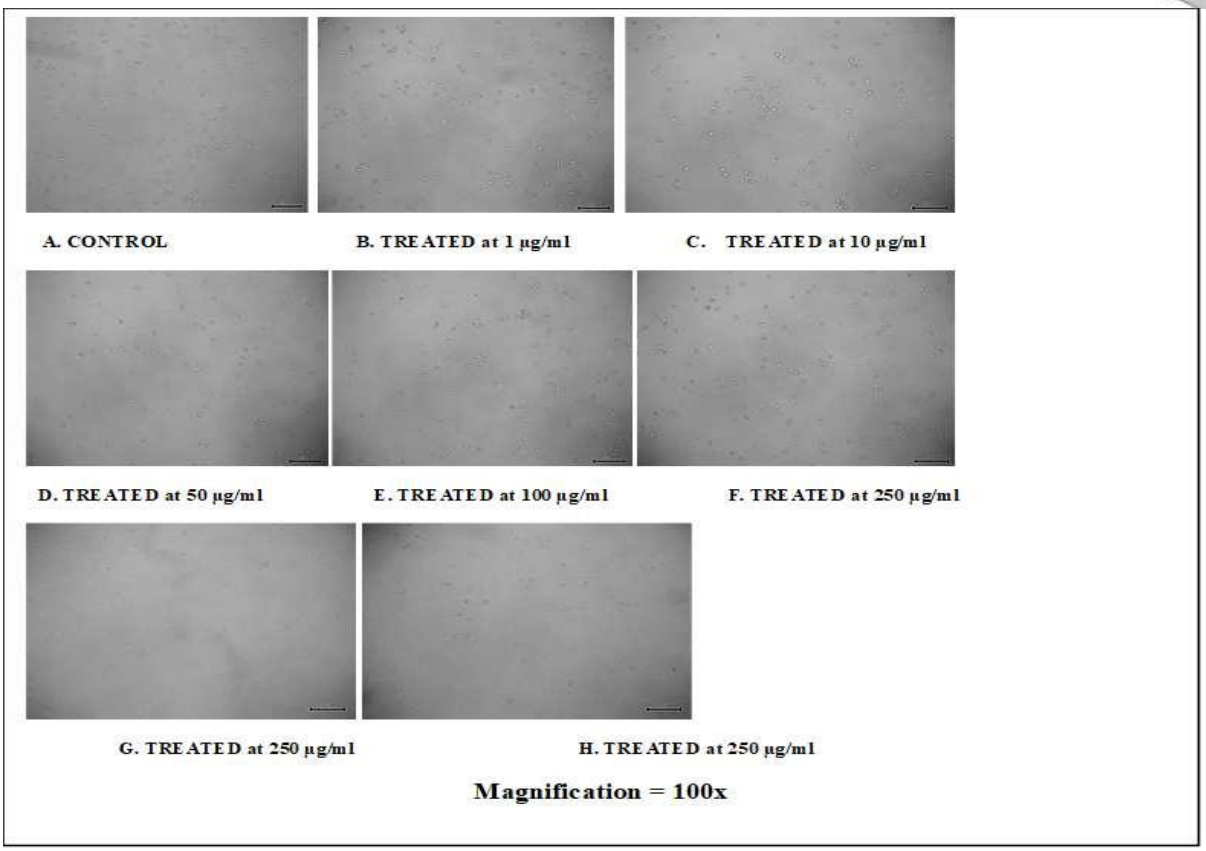


Figure-7 Cytopathic effects on SH-SY5Y treated by N-Hexane Carandas fruits extract of Carissa powder

Discussions:

The present study investigated the antioxidant and anti-Parkinson potential of the n-hexane extract of Carissa carandas fruits using phytochemical characterization and in vitro biological assays. Although the extract exhibited relatively low total phenolic and flavonoid contents and weak DPPH radical scavenging activity (IC_{50} = 530.2 µg/mL), these findings are consistent with the non-polar nature of n-hexane, which preferentially extracts lipophilic constituents rather than phenolic antioxidants. Previous studies have demonstrated that polar extracts of C. carandas, particularly methanolic and ethanolic fractions, possess significantly greater antioxidant activity owing to their higher phenolic and flavonoid content. GC–MS analysis identified several biologically relevant compounds, including phenethyl acetate, oleic acid, palmitic acid, globulol, and isopropyl myristate, which have been reported to exhibit antioxidant, anti-inflammatory, and membrane-protective activities. Despite the absence of significant monoamine oxidase (MAO) inhibition, these phytoconstituents may exert neuroprotective effects through alternative mechanisms such as attenuation of oxidative stress, preservation of mitochondrial function, and modulation of neuroinflammatory signaling pathways. The moderate cytotoxicity observed in SH-SY5Y cells suggests that dose optimization is essential before therapeutic application. A major limitation of the present study is the exclusive use of a non-polar extract and in vitro screening assays without mechanistic or in vivo validation. Future investigations should evaluate phenolic-rich polar extracts, isolate active constituents, and examine their effects in established Parkinson's disease models targeting oxidative stress, mitochondrial dysfunction, α-synuclein aggregation, and neuroinflammation. Such studies will provide stronger evidence for the therapeutic potential of Carissa carandas as a natural neuroprotective agent.

Conclusion:

The present study demonstrates that the n-hexane extract of Carissa carandas fruits contains diverse phytoconstituents with potential biological significance, although it exhibited limited antioxidant activity and negligible monoamine oxidase inhibitory effects. GC–MS analysis confirmed the presence of several lipophilic compounds that may contribute to neuroprotection through antioxidant and anti-inflammatory mechanisms rather than direct MAO inhibition. The moderate cytotoxicity observed in SH-SY5Y cells highlights the importance of dose optimization for future investigations. Overall, Carissa

carandas represents a promising source of neuroactive phytochemicals, warranting further studies using phenolic-rich extracts, mechanistic cellular assays, and in vivo Parkinson's disease models to validate its therapeutic potential.

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