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Development and Evaluation of Antiparkinsonian Potential of *Ficus carica* Phytosomes

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Abstract

The symptoms of Parkinson disease (PD) are manifested in old life i.e., bradykinesia (slow movements), and stiffness or tremor. The study was focused on the phytosomal formulation of *Ficus carica* and its targeted evaluation for anti-parkinsonian efficacy. The fig is the edible fruit of the tiny shrub Ficus carica, which belongs to the Moraceae family of flowering plants. The fresh barks of *Ficus carica* was collected from Kukrail picnic spot, Lucknow, Uttar Pradesh, India. The bark of *Ficus carica* plant was authenticated by the Prof. N. K. Pandey, Centre of Advanced Study in Botany, Institute of Sciences, Banaras Hindu University, Varanasi- 221005, UP, India (plant voucher no. mora. 2023/1). The fresh barks of *Ficus carica* were cleaned, washed, and dried under shade. It was extracted through Soxhlet apparatus using hydroalcoholic (ethanol 10%: distilled water 1%) solution for 48 hours. Total phenolic and flavonoid contents, HPTLC finger printing were determined. After pre-formulation study, the phytosomes of hydroalcoholic bark extract of *Ficus carica* were developed through Thin-layer hydration method. The phytosomes were characterized for Physical appearance, Particle size, PDI & Zeta potential, entrapment efficiency, In vitro drug release, SEM analysis, FTIR & NMR Spectroscopy. In group design, rats were kept separately into 3 groups (n = 6 each) and treated for 14 days, daily i.e., group 1: vehicle (distilled water), group 2: F1 (100 mg/kg, p. o.) and group 3: F2 (100 mg/kg, p. o.). The Antiparkinsonian activity was evaluated by estimation of antioxidant enzyme parameters i.e., SOD, CAT and GSH. In results, the evaluation of bark extract of *F. carica* showed increasing level of antioxidant enzymes which may function as a natural source of anti-parkinsonian role. A dose-dependent increase was seen in facilitating the release of antioxidant enzymes i.e., SOD, CAT, and Reduced Glutathione by the hydroalcoholic bark extract of *F. carica*. In conclusion, it suggests to assess the safety and efficacy of *F. carica's* hydroalcoholic bark extract as a potential natural neuromodulator and anti-Parkinson treatment. In order to improve bioavailability and pharmacological response, its physiologically active component would be added in an appropriate dosage form.

Keywords: Anti-parkinsonian activity, *Ficus carica,* hydroalcoholic bark extract, SOD, GSH.

Introduction

The symptoms of Parkinson disease (PD) are manifested in old life i.e., bradykinesia (slow movements), and stiffness or tremor (1). Additional symptoms include constipation, excessive salivation, mood abnormalities, sleep disturbances, loss of smell, and excessive periodic limb movements during sleep (2) (3). At any given time, PD affects 1 to 2 out of every 1000 persons; as people age, the frequency rises to 1% of those over 60. A

hereditary tendency is present in 5–10% of cases. Parkinson disease is more common in males than in women, and its incidence and prevalence do rise with age (4). Our knowledge of the etiology of Parkinson's disease has greatly advanced during the last century. The loss of pigmentation in the midbrain's substantia nigra was initially identified as a characteristic of post-mortem brain examinations of Parkinson's disease (PD) patients in 1919. It became clearer in the 1950s that the pigmented neurons lost in the substantia nigra are dopaminergic, and that the cause of the movement dysfunction in PD is related to dopamine loss in subcortical motor circuitry (5). The basal ganglia, which are made up of numerous additional nuclei, are the site of Parkinson's disease. Numerous areas of the cortex provide excitatory and inhibitory input to the striatum. The loss of dopaminergic neurons, which causes the symptoms, is the main pathology (6).

Plant extract and phospholipids interact to form lipid-compatible molecular complexes known as phytosomes. Studies have shown that, in comparison to traditional plant extracts, phytosomes can boost chemical absorption by up to 20 times (7). When water-soluble polyphenolic compounds interact with the water-containing portion of phospholipid and when the polyphenolic group of phytochemicals establishes hydrogen bonds with the phosphate group of phospholipid, phytosomes are formed in non-polar solvents (8)(9). Phytosomes shield the constituents of herbal extracts from intestinal bacteria and digestive juices. They improve bioavailability and pharmacokinetics, which enables them to cross lipid-rich biomembranes and enter the bloodstream. Phytosomes play a key role in extending the drug's circulation, reducing toxicity, and postponing the removal of drugs that are quickly metabolized. Because the medication is coupled with lipids to form persistent vesicles that efficiently encapsulate the active chemicals, phytosomes have a high entrapment capacity. By enhancing the therapeutic efficacy and safety profile of plant extracts, phytosomes provide clinical benefits. Better clinical results are a direct result of their improved absorption and administration (10). Compared to standardized extracts, they have higher bioavailability and activity, which makes them beneficial for a range of conditions (11).

Cell membranes include phospholipids, which give them flexibility and can be employed as a vehicle for medication delivery. Phospholipids are biocompatible and offer a number of benefits, such as flexibility in formulation and the option to choose from a variety of innovative drug delivery methods according to the intended use. Phospholipids are lipids with polar and non-polar structures that contain phosphorus (12). The pharmaceutical business frequently uses phospholipids, both synthetic and natural. In addition to having a minimal toxicity profile, they can be applied topically, orally, or parenterally. Synthetic polymers are not suitable for all routes; however phospholipids are superior excipients. The minimal quality of phospholipid content, which varies depending on the mode of administration, must be taken into account while choosing natural phospholipids. Oral and topical delivery of natural phospholipids containing at least 45% phosphatidylcholine (PC) is possible; however, parenteral treatment usually necessitates 70% PC. (13). Phospholipids such phosphatidylethanolamine, PC, phosphatidylinositol, phosphatidylserine, and phosphatidic acid are found in human biological cell membranes. The phytochemical is soluble in both the lipid and aqueous phases because the nitrogen atom in the phosphate group loses electrons while the oxygen atom can gain electrons (14).

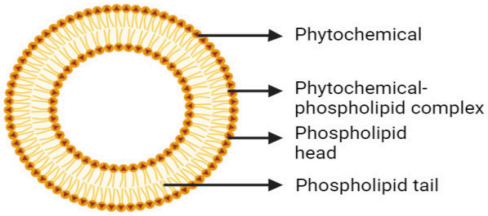

Fig. 1 Structure of Phytosome

Plant profile: Ficus carica

The fig is the edible fruit of the tiny shrub Ficus carica, which belongs to the Moraceae family of flowering plants. It is found all over the world in subtropical climates, including South Africa, Europe, America, and Australia. The minimum requirements for growing conditions are 200 c, 40–45% soil moisture, and 500–550 mm of yearly precipitation. The fig tree can be single or multi-stemmed, and it has a sparsely branched, spare crown that can reach a height of 8 to 10 metres. It sheds its leaves in the winter. Fresh sprouts are olive green and young branches are ashen. It has a gray-brown body. The lower side of the hand-shaped leaves are light green and hairy, while the top face is rough and has three to seven lobes. Leaf width is 6–18 cm and length is 8–20 cm. In the autumn, its leaves turn yellow (15).

Taxonomy

Kingdom: Plantae
Order: Rosales
Family: Moraceae
Genus: *Ficus*
Species: *carica*

It has numerous bioactive constituents i.e., mucilages, flavonoids, vitamins, enzymes, nicotinic acid, and tyrosine are present in F. carica. Bergaptine, stigmasterol, ficusin. The leaf contains the compounds psoralen, taraxasterol, beta-sitosterol, rutin, sapogenin, calotropenyl acetate, lepeolacetate, and oleanolic acid sistosterol. The plant also includes xanthotoxol, arabinose, amyrins, carotines, glycosides, and sitosterol, campestrol, fucosterol, umbelliferone, and fally acids, 6-(2-methoxy Z- vinyl)-7- methyl-pyranocoumarin, 9,19- cycloarlane triterpenoid, 6-O-acyl- D- glycosyl- sitosterol, calotropenyl acetate, and lupeol acetate (16).

Materials and methods

Chemicals

Ethanol, hexane, Mayer's, Dragendorff's reagents, nitric acid, sod. Hydroxide, and sodium hydrogen carbonate were bought from Renkem by avatar performance materials India. Gallic acid, quercetin hydrate, are standards for phenolic acids and flavonoids, respectively. (DPPH) from Sigma Aldrich. The aluminium chloride (AlCl3), and phenol reagent used by Folin-Ciocalteu were all obtained from Sigma Aldrich. Systronics Double beam spectroscopy type 2203.

Collection and authentication of the plant

The fresh barks of *Ficus carica* were collected from Kukrail picnic spot, Lucknow, Uttar Pradesh, India. The bark of *Ficus carica* plant was authenticated by the Prof. N. K. Pandey, Centre of Advanced Study in Botany, Institute of Sciences, Banaras Hindu University, Varanasi- 221005, UP, India (plant voucher no. mora. 2023/1).

Extraction process

The fresh barks of *Ficus carica* were cleaned, washed, and dried under shade. These were grinded into a fine powder for optimum extraction. It was extracted through Soxhlet apparatus using hydroalcoholic (ethanol 10%: distilled water 1%) solution for 48 hours. After the extraction process, it was filtered with filter paper and finally with Whatman filter paper. The filtrate (slurry) was made concentrated using the rotatory evaporator. Thus, the herbal extract obtained in dried powdered form and stored in to desiccator to prevent the *Ficus carica* extract from the moisture.

Determination of total phenolic contents

0.5ml Folin-Ciocalteu reagent was mixed with 0.5ml of the ethanolic and aqueous extracts, each individually. After 5-8 min (25°C), 2ml sodium carbonate solution (7.5%) was added, and the volume was made up to 8ml. The absorbance at 725nm was measured after two hours. Gallic acid was utilized as standard. Total phenolic content was expressed per gram of sample (mg/g) (17).

Determination of total flavonoids contents

4ml distilled water were mixed with 100µl ethanolic and aqueous extract. A 5% sodium nitrite solution was added in 0.3 milliliters. After five minutes, 0.3 cc of 10% aluminum chloride was added. After six minutes, the liquid was mixed with two milliliters of 1M sodium hydroxide. The mixture was thoroughly mixed after 3.3 cc of distilled water was added to dilute it. Quercetin was used as reference (510nm). The extract's TFC was measured in milligrams of Quercetin (18).

HPTLC finger printing (at 254nm)

Pre-coated silica gel 60 F_{254} aluminium plates were used in stationary phase. Mobile phase includes Toluene: Ethyl acetate: Formic Acid (6: 4: 0.2). The chamber saturation time was kept for 20 mins. In test solution, 1g of dried formulation dissolved in methanol and then filtered the liquid extract. Volume was made up to 10ml using methanol. Visualization and detection was performed at wavelengths i.e., 254 nm, 366 nm and 510 nm.

Procedure

Take a TLC plate that has been previously cleaned with methanol and dried, establish the dimensions at the X position, and use a pencil to mark the base at 10 and 90 mm, leaving 15 mm on both sides of the plate. Using the Linomats applicator's programming, apply the test sample solutions 10, 15, and 20 µl in bands. After letting the solvent evaporate, place the plate in the saturated tank, preferably vertically, so that any bands or spots are higher than the mobile phase level. After closing the tank, let it stand at room temperature until the mobile phase reaches the designated line. After removing the plate, dry it out and examine it under UV-Vis light at 254 and 366 nm. Create two scanning programs: one for a fully dried plate at 254 nm and another for a 366 nm wavelength. Once the plate has developed, let it air dry. After spraying the plate with 5% sulfuric acid in methanol reagent, it is heated for approximately ten minutes at 105 degrees Celsius. Scan at 510 nm once more. Obtain the report file that includes the scanning chromatograms' graphical data and photos.

Pre-formulation studies
Organoleptic properties

Chemicals and herbal extracts were examined for the organoleptic characteristics prior using in the formulation development. The organoleptic characteristics include colour, odor, and physical appearances.

Melting point

Thiel's melting point tube was used to determine the melting point of an organic compound (capillary tube method). Thus, melting point of the herbal extract was determined.

Preparation of standard calibration curve of F. carica extract

F. carica extract stock solution was prepared by dissolving 10mg of the dried extract, 10ml of methanol to give a 1000µg/ml concentration. To develop stock II, which has a concentration of 100µg/ml, take 1ml of this stock solution and diluted it with methanol (solvent) up to 10ml. A 10ml volumetric flask was filled with 1ml of the stock solution (100µg/ml), and the line was then filled with ethanol to a volume equal to 10µg/ml. The sample was then scanned with a UV-Visible spectrophotometer in the 200-400nm wavelength range using methanol as a blank. Further dilutions of 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml, and 12µg/ml were made from the stock solution (100µg/ml). The absorbance of the dilutions was measured at absorption maxima. The calibration curve was then constructed (59).

Preparation of phytosomes (Thin-layer hydration method)

Thin-layer hydration method was used to create phytosomes loaded with *Ficua carica* bark. In short, ethanol was used to dissolve varying amounts of phosphatidylcholine and Ficua carica extract. At a predetermined temperature and time, the mixture was refluxed and swirled. A rotary vacuum evaporator was

used to evaporate the final mixture under vacuum at 34–45 °C until a thin layer formed. For six hours, the thin film was further hydrated using 90% v/v ethanol in water. After that, the mixture was kept overnight in a desiccator. For additional characterization, the obtained extract-phospholipid complex was subsequently transferred into a vial and kept in a freezer (19).

Characterization parameters (20)(21)(22)

Physical appearance

The physical characteristics of the prepared phytosomes were evaluated. The formulations were characterized using an optical microscope. A drop of each formulation was placed on a slide and covered with a cover slip after it had been individually suspended in phosphate buffer pH 7.4. The complex was viewed under a microscope at a 10×10 magnification.

Particle size, PDI & Zeta potential

Using a particle size analyzer (Zetasizer Nano ZS90, Malvern Instruments, UK), the dynamic light scattering approach determines the particle size, PDI, and zeta potential. The mean particle diameter and PDI range from 0 to 1 at a scattering angle of 90 degrees at 250 degrees Celsius. Five millilitres of the diluted sample were put into a cuvette, and the zeta potential was measured after the sample had been diluted to ten millilitres with water. As the electrostatic repulsion between the particles grows, so does the stability.

Entrapment efficiency

The centrifugation technique was used to calculate the Phytosomes' entrapment efficiency. Methanol was used to dilute the phytosome, which was subsequently centrifuged using a high-speed cooling centrifuge at 10,000 rpm for 30 minutes at -4oC. The supernatant was taken, and the amount of free extract for the extracts of C. equisetifolia and M. koenigii was measured using UV visible spectrophotometers at 328 nm and 366 nm, respectively.

In vitro drug release

The United States Pharmacopoeia's dissolution test technique II (paddle) was followed in order to achieve the dissolution test (USP 43). The phosphate buffer pH 6.8 was employed to simulate intestinal fluid. In order to ensure uniform dispersion of vasicine, the dissolution bowls were filled with 900ml of dissolution fluid and placed in a dissolution device to maintain a temperature of 37±0.5°C. The paddle speed was set at 150 rpm. After adding 5 mg of the extract and phytosome to the dissolving media, 5 mL of samples were taken out at various intervals (0, 1, 2, 4, 6, 8, 10, 12, 24 hours) and replaced with new dissolution media.

SEM Analysis

Five microliters of the phytosomes were converted into a cover slip, which was then put on a specimen tab. At room temperature, the samples were left to dry. After that, a scanning electron microscope (Sigma, Carl Zeiss) was used to inspect and take pictures of the formulation's particle size. The JEOL JSM-6701F Field Emission SEM was used to view and take pictures of the coated samples after the particles were coated with platinum using a vacuum evaporator.

FTIR Spectroscopy

Infrared spectroscopy can confirm the synthesis of the complex by comparing its spectra to that of its component parts and their mechanical mixing. FTIR spectroscopy is another useful method for controlling the stability of phytosomes when they are microdispersed in water. By contrasting the complex's solid-state spectrum with its water-soluble micro-dispersion spectrum at different points after lyophilization, the stability may be practically confirmed.

NMR Spectroscopy

This technique (1H NMR, 13C NMR) can be used to evaluate the complex formation between the phosphatidylcholine moiety and the active phytoconstituents.

Preparation of animals

Wistar rats (130-150g) were obtained from the Institutional Animal Ethics Committee, Era's Lucknow Medical College & Hospital, Lucknow with reference no. IAEC/ELMCH/1/23/-3. They were kept in polypropylene cages with a 12-hour light/dark cycle at temperature of 25±1°C and 45-55% RH. The animals had water ad libitum and normal feeding pellets. Rats were kept on fasting for 24 hours, prior experiment (23).

Experimental design

Wistar Rats were kept separately into 3 groups, each consisting 6 rats. Group 1 (control) was administered distilled water, Group 2 (Test 1) administered F1 (100 mg/kg, p. o.) and Group 3 (Test 2) administered F2 (100 mg/kg, p. o.). Treatments were performed once a day for 14 days.

Estimation ofanti-oxidant enzyme parameters

Superoxide Dismutase (SOD) test

To prepare a total volume of 1 ml, an equal amount (0.5 ml) of plasma and ice-cold water were completely combined. After adding around 2.5 ml of ethanol and 1.5 ml of cold reagent (chloroform), the mixture was agitated for 60 seconds before being centrifuged at 4˚C. The following is the measurement of the enzyme activity in the supernatant. 0.1 ml of the sample, 0.2 ml of NADH, 0.1 ml of 186µM phenazine methosulphate, 1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052M), and 0.3 ml of 300µM nitrobluetetrazolium were all included in the assay mixture. After 90 seconds of incubation at 30˚C, the mixture was stirred with 4 ml of n-butanol. Prior to centrifugation and the separation of the butanol layer, the mixture was allowed to stand for ten minutes. SOD concentration was expressed as units/mg protein, and the color intensity of the chromogen in the butanol layer was determined using spectrophotometry at 560 nm. The quantity of enzyme that produced 50% inhibition of NBT reduction in a minute was considered one unit of enzyme activity (24).

Catalase test

One milliliter of 0.01 M phosphate buffer (pH 7.0) was added to 0.1 milliliters of liver homogenate supernatant. The reaction was initiated with one milliliter of freshly made 30 mM H2O2, and it was stopped as soon as it was finished by adding two milliliters of dichromate-acetic acid reagent (5% K2Cr2O7 in glacial acetic acid). A spectrophotometric shift in absorbance at 620 nm was used to gauge how quickly H2O2 was breaking down. Units/mg protein was used to express catalase activity (25).

Reduced Glutathione (GSH) Assay

The tissue homogenate was collected in 0.1 M phosphate buffer (pH 7.4) in order to determine the GSH content. To precipitate the tissue proteins, equal amounts of homogenate and 20% trichloroacetic acid (including 1 mM EDTA) were combined. After centrifuging the mixture for 10 minutes at 200 rpm, the 200 µl supernatant was moved to a fresh set of test tubes. In parallel, 1.8 ml of Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid) was added to the test tube and produced in 0.3 M phosphate buffer with 1% sodium citrate solution. The test tubes' total volume was then increased to 2 milliliters. Following the completion of the entire reaction, the solutions were measured at 412 nm against a blank. A standard, derived from a standard curve of known GSH, was used to evaluate absorbance values (26).

Results and Discussion

Total phenolic content

Table 1. Total phenolic content of *F. carica*

Sample	TPC (mg/ml)
Hydroalcoholic bark extract of *F. carica*	0.240
Ethyl ether fraction of *F. carica*	0.880

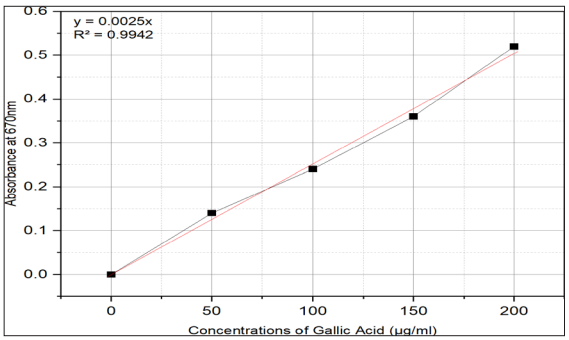

Fig 2. Graphical data of total phenolic content of *F. carica*

Total flavonoid content

Table 2. Total flavonoid content of *F. carica*

Sample	TFC (mg/ml)
Hydroalcoholic bark extract of *F. carica*	0.200
Ethyl ether fraction of *F. carica*	0.100

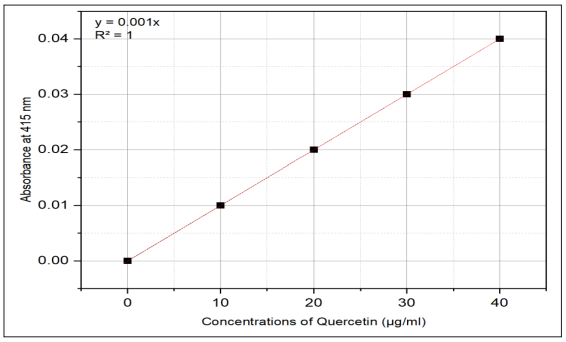

Fig 3. Graphical data of total flavonoid content of *F. carica*

HPTLC finger printing (at 254nm)

Table 3. HPTLC finger printing (at 254nm)

S. N.	F1		F2	
	R_f Value	Area	R_f Value	Area
1	0.11	2593.8	-	-
2	0.16	2015.7	0.29	38823.1
3	0.12	3127.3	0.38	19073.6
4	0.28	1433.4	0.42	16628.1
5	0.37	3527.4	0.47	13732.6
6	0.42	1741.7	0.49	14255.7
7	0.48	2870.2	0.55	24871.2
8	0.51	3481.7	0.71	76234.6
9	0.56	5087.9	0.84	13179.6
10	0.91	299.7	0.93	3970.3

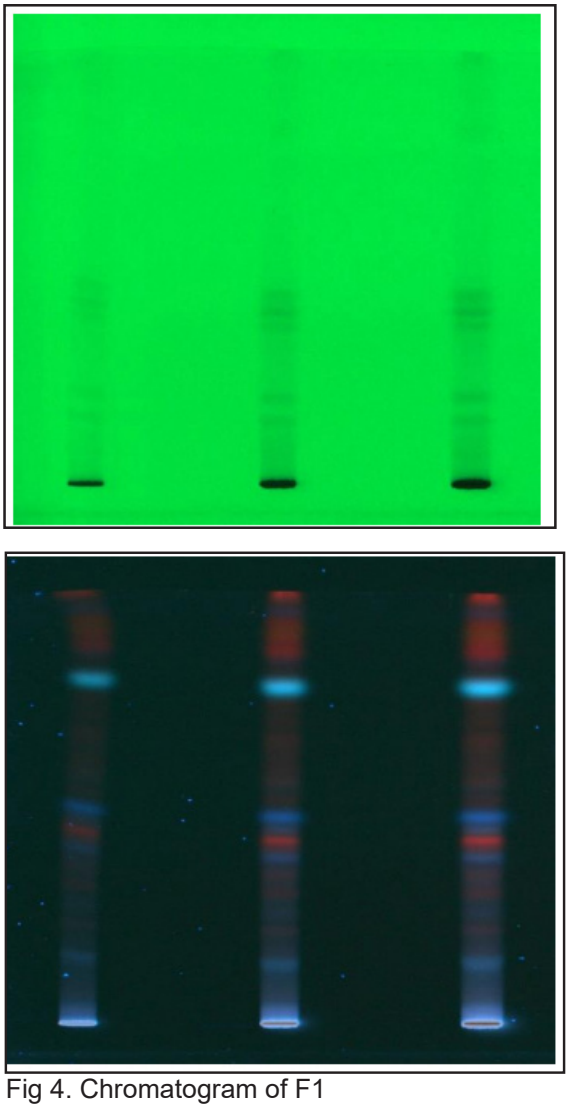

Fig 4. Chromatogram of F1

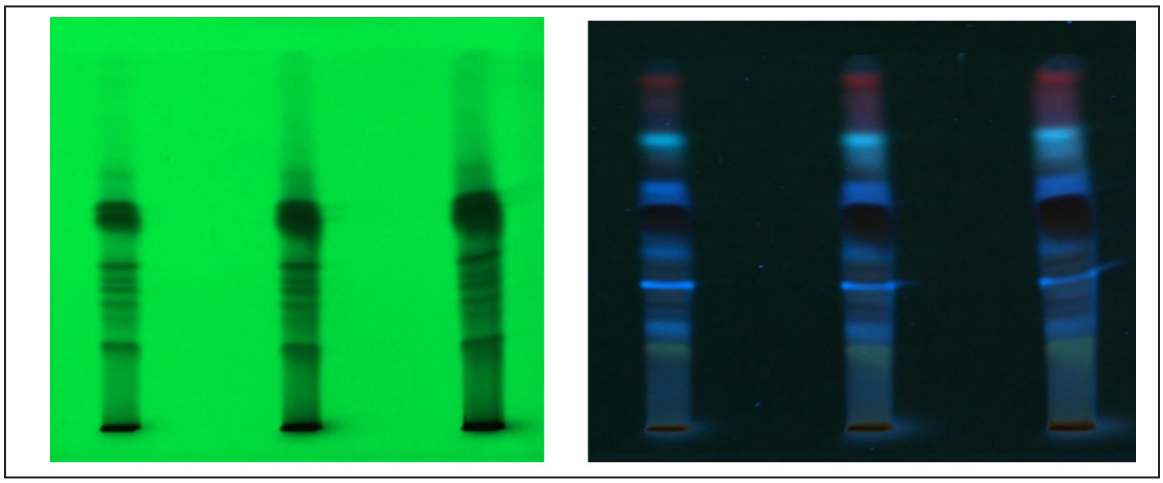

Fig 5. Chromatogram of F2

Table 4. HPTLC finger printing (at 254nm)

S. N.	F3		F4		F5	
	R_f Value	Area	R_f Value	Area	R_f Value	Area
1	0.22	10221.1	0.29	19719.3	0.15	1374.2
2	0.42	3411.8	0.40	1589.0	0.34	266.6
3	0.47	2289.9	0.48	8931.7	0.52	145.5
4	0.59	4211.1	0.53	6546.6	0.69	1124.7
5	0.71	3256.9	0.58	3285.0		
6	0.87	723.0	0.63	9657.1		
7			0.76	8765.1		
8			0.90	4507.3		
9			0.97	190.1		

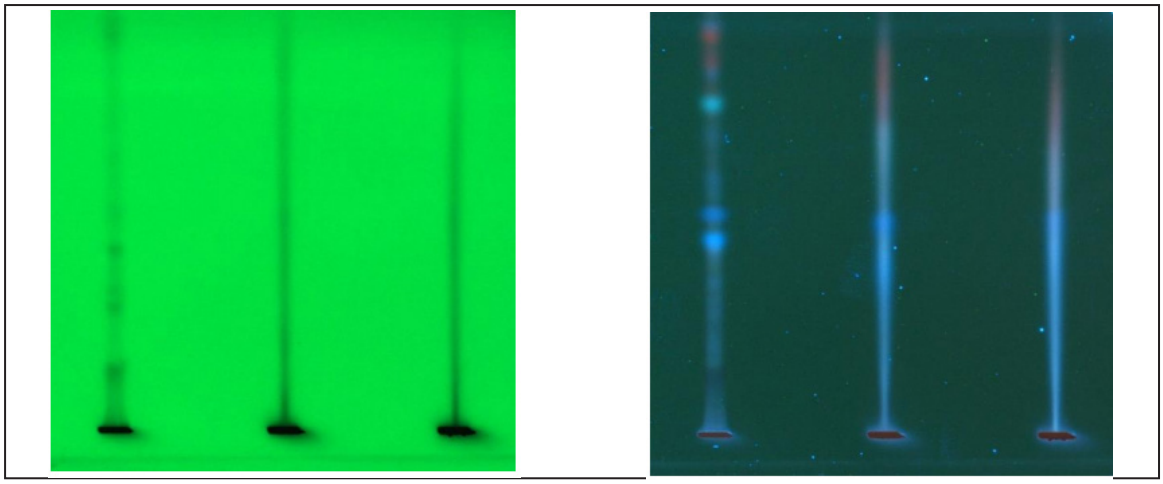

Fig 6. Chromatogram of F3

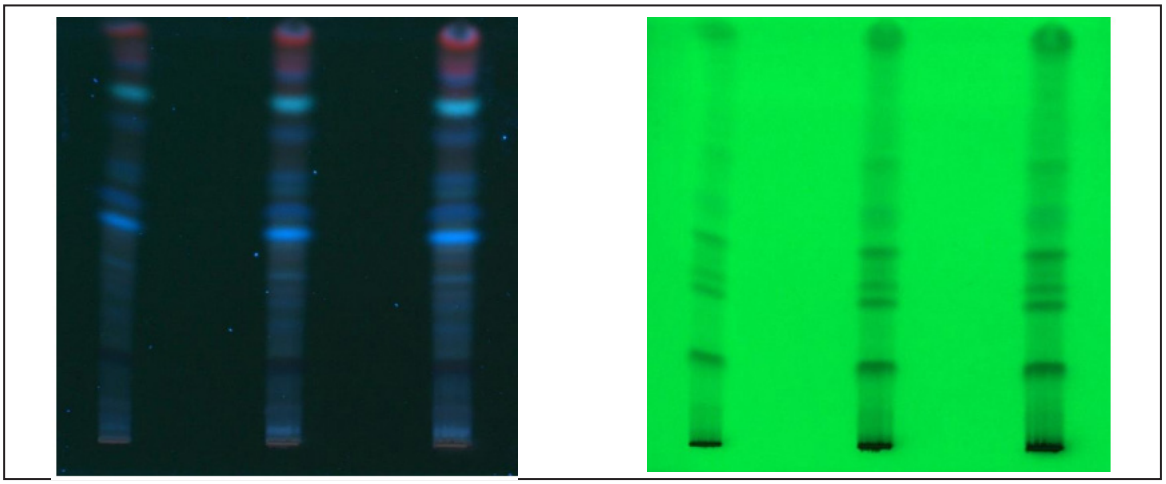

Fig 7. Chromatogram of F4

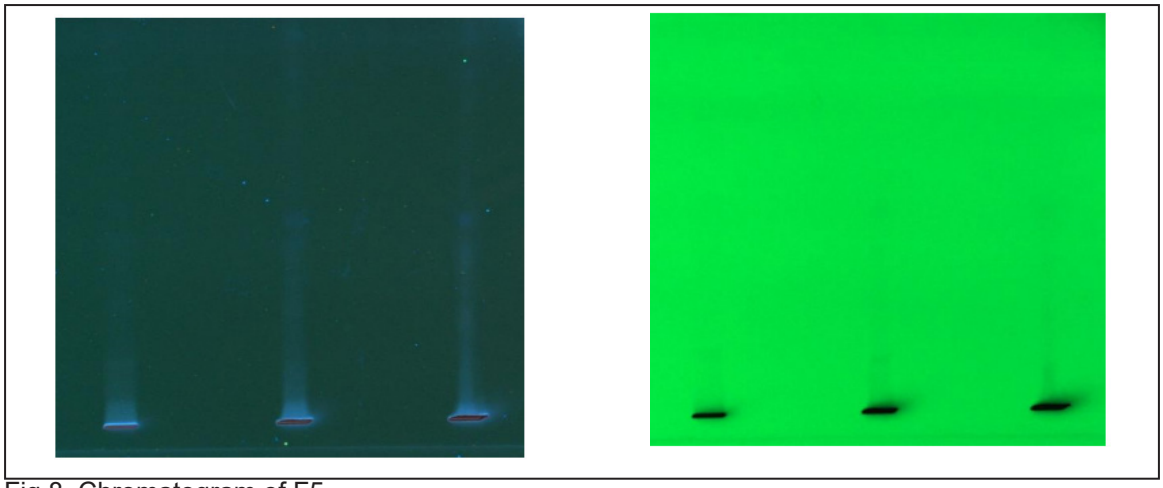

Fig 8. Chromatogram of F5

Pre-formulation studies

Organoleptic properties

Using HPTLC analysis, the formulation compositions i.e., phosphatidylcholine and herbal extracts were determined for organoleptic characteristics. *F. carica* extract was found as brown, solid powder, with characteristic odor. However, Phosphatidylcholine was found as

Table 5. Physical appearance

Sample	Physical state	Color	Odor
F. carica extract	Solid powder	Brown	Characteristic
Phosphatidylcholine	Liquid crystalline	Off-white	Characteristic

Fig 9. Hydroalcoholic bark extract of *F. carica*

Melting point

The capillary tube method was utilized for the determination of the melting point. Phosphatidylcholine and *F. carica* bark extract showed the melting point as 41 ºC and 45ºC, respectively.

Std. calibration curve of F. carica extract

The following table shows the Conc. (µg/ml) of *F. carica* extract and maximum absorption at λ 350 nm.

Table 6. Conc. (µg/ml) of *F. carica* extract and absorption (λ 350 nm)

Conc. (µg/ml)	Absorption (λ 350 nm)
2µg/ml	0.16
4µg/ml	0.30
6µg/ml	0.44
8µg/ml	0.62
10µg/ml	0.84
12µg/ml	0.96

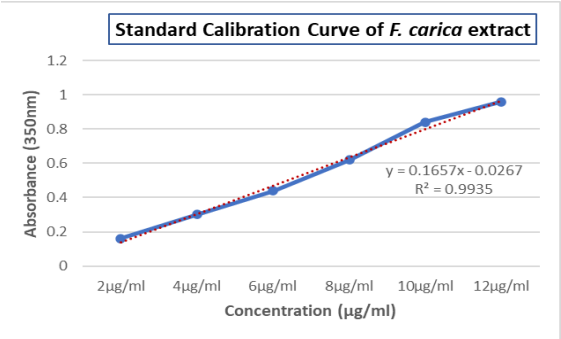

Fig 10. Standard Calibration Curve of *F. carica* extract

Characterization parameters of phytosomes

Organoleptic characteristics

The prepared phytosomes were observed for organoleptic characteristics and found as brown thick viscous material with characteristic odor. Both the formulations showed an identical property.

Table 7. Organoleptic characteristics

Formulation	Physical state	Color	Odor
F1	Thick viscous material	Brown	Characteristic
F2	Thick viscous material	Brown	Characteristic

Particle size, PDI and Zeta potential

The average particle size of F1 and F2 was estimated as 175.7 nm and 352.1 nm, respectively. The PDI was estimated as 0.200 and 0.497 for F1 and F2, respectively. Moreover, Zeta potential was reported to be -16.3 and -31.3. These showed for homogenous particles size in the *F. carica* phytosomes.

Table 8. Particle size, PDI and Zeta potential of phytosomes

Formulation	Particle size	PDI	Zeta potential
F1	175.7	0.200	-16.3
F2	352.1	0.497	-31.3

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Entrapment efficiency

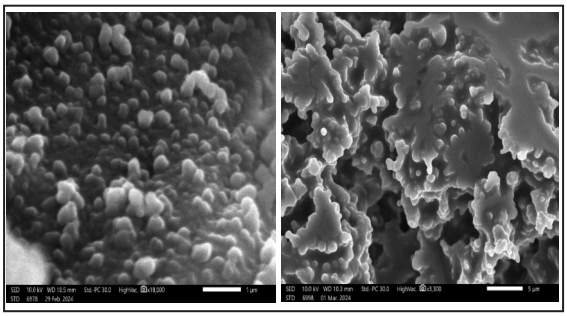
Entrapment efficiency was noted as 86.12±0.29% and 92.43±0.21% in F1 and F2, respectively which demonstrate for the better entrapment efficiency of the *F. carica* phytosomes.

Table 9. Entrapment efficiency

Formulation	Entrapment efficiency (%)
F1	86.12±0.29
F2	92.43±0.21

SEM analysis

The formulated phytosomes were determined for SEM analysis. F1 demonstrated the droplet size of 1µm whereas F2 showed the droplet size of 2µm.



F1 F2

Fig 11. SEM Analysis of *F. carica* phytomes

In vitro drug release

In-vitro drug release was determined at different time intervals i.e., 0, 1, 2, 4, 6, 8, 10, 12 and 24 hours. Both the formulations (F1& F2) demonstrated the optimum and sustained drug release. The highest drug release was estimated at 24 hours, F1 showed the *in vitro* release as 92.3±0.7 and F2 showed the in vitro release as 94.4±0.6.

Table 10. In vitro drug release of phytosomes

Time (hr)	In vitro release± S.D.	
	F1	F2
0	0	0
1	24.1±0.2	26.5±0.1
2	35.7±0.4	33.1±0.2
4	42.4±0.2	45.1±0.3
6	51.4±0.3	53.3±0.6
8	62.5±0.4	65.1±0.2
10	73.4±0.2	74.6±0.1
12	84.2±0.5	86.1±0.3
24	92.3±0.7	94.4±0.6

FTIR Spectroscopy

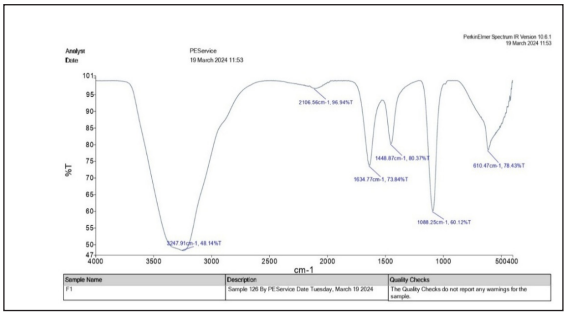


Fig 12. FTIR Spectrum of F1

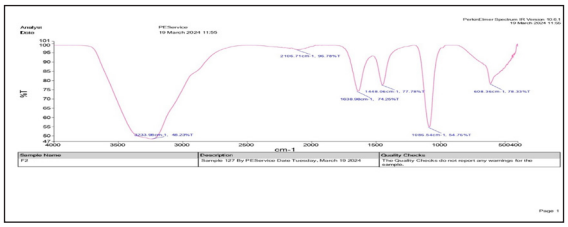


Fig 13. FTIR Spectrum of F2

NMR spectroscopy

The carbons of the phytoconstituents were not apparent in the 13C NMR of the phytoconstituents and the stoichiometric complex with the phosphatidylcholine when it was recorded. The resonance of the fatty acid chains mostly keeps its original sharp line shape, but the signals corresponding to the glycerol and choline sections are widened and some are displaced.

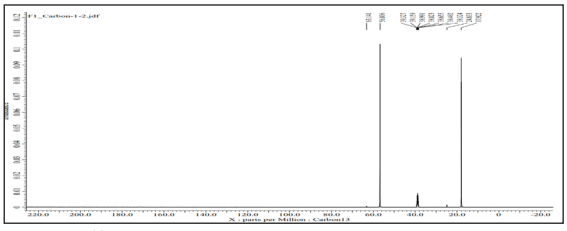


Fig 14. ^{13}C NMR of F1

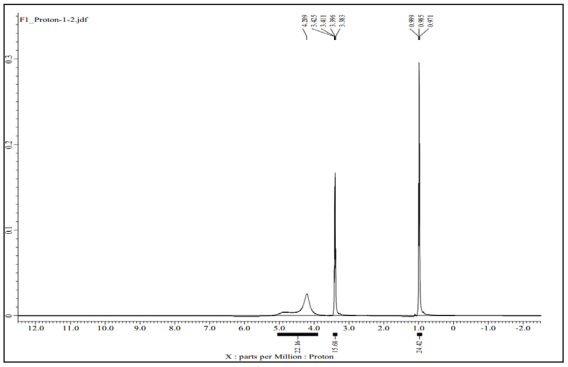


Fig 15. ^{1}H NMR of F1

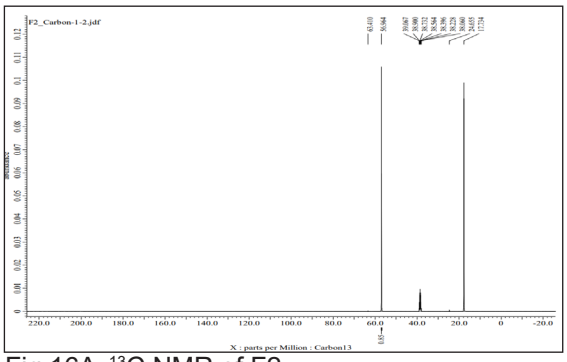


Fig 16A. ^{13}C NMR of F2

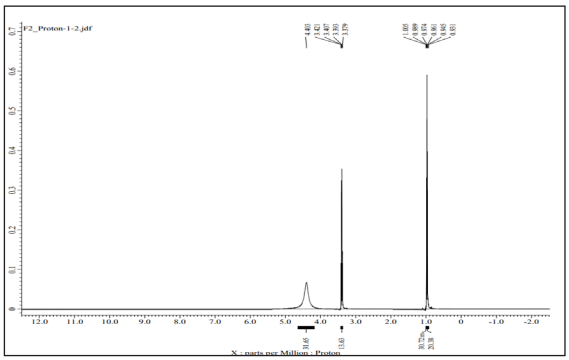


Fig 16B. ^{1}H NMR of F2

Estimation of anti-oxidant enzyme parameters

Superoxide dismutase (SOD) test

The Superoxide Dismutase (SOD) test was performed for both the prepared phytosomes. The SOD was found as 0.894 U and 1.229 U for F1 and F2, respectively.

Table 11. Estimation of Superoxide Dismutase (SOD) level

Tube code	OD at 600nm	% SOD	SOD (U)
F1	0.665	44.722	0.894
F2	0.464	61.430	1.229
Blank	1.203	0	0

Catalase test

In catalase test, F1 and F2 phytosomes of *F. carica* demonstrated the catalase enzyme level as 34.3 U/mg protein and 15.8 U/mg protein, respectively.

Table 12. Estimation of Catalase enzyme level

Formulation	Catalase enzyme level (U/mg protein)
F1	34.3
F2	15.8

Reduced Glutathione (GSH) assay

In Reduced Glutathione (GSH) Assay, the formulated phytosome F1, and F2 demonstrated the GSH enzyme level as 0.463 µmol/ml/min and 2.967 µmol/ml/min, respectively. In different time intervals i.e., 0.5, 1.5, 2.5, 3.5 and 4.5 min, the absorbance was determined.

One study claims that L-Dopa and PBCA nanoparticles coupled to nerve growth factor can penetrate the blood-brain barrier and address the main symptoms of PD (27). Furthermore, SA (Schisantherin A), a medication used to treat Parkinson's disease, was encapsulated in nanoparticles in a different study, which enhanced both its bloodstream circulation and brain absorption. This shown that SA is more effective when delivered via nanoparticles, making it a powerful substance for the treatment of PD (28).

In recent years, an increasing number of studies have focused on naturally occurring phytochemicals that have been demonstrated to be helpful against various age-related neurodegenerative diseases. Due to their toxicity and ability to cause a wide range of

Table 13. Reduced Glutathione (GSH) Assay

Sample	Absorbances					ΔA340 (Sample)-ΔA340 (Blank)	Delta A/min	GSH enzyme level (µmol/ml/min)
	0.5 min	1.5 min	2.5min	3.5 min	4.5 min			
F1	0.242	0.249	0.267	0.2955	0.3135	0.05	0.0111	0.463
F2	0.2825	0.3325	0.3665	0.575	0.6245	0.3205	0.0712	2.967
Blank	0.2135	0.2195	0.2255	0.2315	0.235	0	0	0

serious disorders, synthetic drugs have been shown in clinical studies to be difficult to treat for these illnesses. As a result, natural antioxidants, like polyphenols found in food or nutraceuticals, have emerged as a great way to combat the oxidative damage that free radicals cause to neuronal cells. There is a dearth of study in the area, despite the fact that no significant adverse effects of the currently available phytochemicals have been noted in clinical settings. Furthermore, natural products have been used to treat Parkinson's disease with effectiveness; in particular, the phytochemicals found in foods that occur naturally have the capacity to function as antioxidants and could be a reliable supply of treatment. On the other hand, naturally occurring lipophilic phytochemicals can easily penetrate the blood–brain barrier and enter the brain. These phytochemicals have a higher affinity for the receptors, a quicker metabolism, and increased bioavailability. Furthermore, combining several neurotoxic models of Parkinson's disease shown that herbal medicines might be used to create new treatments for the condition. The effectiveness of plant extracts and their active ingredients in PD models, however, should be studied in real-world settings in the future. Furthermore, there is a continuous need for more thorough descriptions of the components of herbal extracts and the processes by which they work (29). Examined the effects of ethanolic extract of Ficus carica fruit (EEFCF) both acutely and over time in treating PD circumstances brought on by haloperidol. However, the cause of the deterioration remains unclear. Oxidative stress could be a significant factor. The production of toxic free radicals during dopamine metabolism may result in oxidative stress. The substantia nigra pars compacta is more susceptible to oxidative stress and a greater rate of free radical production than the rest of the brain. Oxidative stress alterations in PD patients' brains have been found in a number of investigations (30). The neuroleptic medication haloperidol causes catalepsy by inhibiting post-synaptic striatal dopamine D2 receptors. Numerous investigations have linked reactive oxygen species to the toxicity of haloperidol. Extrapyramidal manifestations of Parkinson's disease may be lessened by medications that decrease motor abnormalities brought on by haloperidol (30).

In rats given haloperidol, ficus carica therapy dramatically reduced catalepsy in a dose-dependent manner. At 100 and 200 mg/kg, the EEFCF showed a protective effect against catalepsy caused by haloperidol, indicating that F. carica may be able to preserve dopaminergic neurotransmission in the striatum. Mitochondrial malfunction, or impairment of mitochondrial complex-1, is the cause of oxidative stress and plays a significant part in the pathophysiology of Parkinson's disease. Malondialdehyde, catalase, and superoxide dismutase levels were measured to assess oxidative stress, while brain tissue's reduced glutathione levels were examined. Catalase, another antioxidant, counteracts hydrogen peroxide's harmful effects. Catalase stops the buildup of precursors to free radical production and transforms H_2O_2 into water and non-reactive oxygen species. Catalase levels

decrease as a result of oxidative stress (31). Superoxide dismutation and the production of hydrogen peroxide and nonreactive oxygen species are catalyzed by another enzyme, SOD. It is an essential antioxidant defense that is found in almost all cells that are exposed to oxygen. It aids in counteracting free radicals' harmful effects. 20 The animals' brains in the control group treated with haloperidol showed a drop in SOD levels, a sign of oxidative stress. GSH, another powerful enzyme, is a key player in the etiology of Parkinson's disease. It is believed that neuronal death is the cause of reduced glutathione depletion in Parkinson's disease, particularly in the substantia nigra. There is proof that glutathione depletion and the degree of neuronal death are related. Reduced glutathione levels may be the cause of impaired neuronal detoxification against hydrogen peroxide, as well as an elevated risk of lipid peroxidation and free radical production. The control animal treated with haloperidol showed a clear decrease in GSH levels (32).

Conclusion

In this investigation, the bark extract of *F. carica* demonstrated the highest levels of antioxidant activity (anti-parkinsonian potential) which may have been caused by the high flavonoid content of this herbal extract. The evaluation of bark extract of *F. carica* showed increasing level of antioxidant enzymes which may function as a natural source of anti-parkinsonian role. A dose-dependent increase was seen in facilitating the release of antioxidant enzymes i.e., SOD, CAT, and Reduced Glutathione by the hydroalcoholic bark extract of *F. carica*.

It suggests to assess the safety and efficacy of *F. carica's* hydroalcoholic bark extract as a potential natural neuromodulator and anti-Parkinson treatment. In order to improve bioavailability and pharmacological response, its physiologically active component would be added in an appropriate dosage form.

Conflict of interest

None.

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