

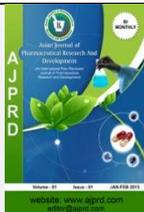
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Research Article

Isolation of Pyran Composition and Anti-Parkinson's Activity of *euphorbia Cyathophora*

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ABSTRACT

Aim of the study: The aim of this research was to investigate the phytochemical profile, isolate the phytoconstituents, Characterised and evaluation of Anti Parkinson activity of ethanolic extract of Indian Medicinal plant of *E.cyathophora*

Materials and methods: Qualitative phytochemical analysis for their phyto constituents. Ethanol was used to extract the crude bio active compound from whole *E. Cyathophora* plant. Phenolic derivative were isolated from the ethanolic extract of *E. cyathophora* by using suitable solvent system and characterised by IR, NMR, MASS spectrophotometric method. The total Neuroprotective activities of *E. Cyathophora* extract were measured by haloperidol induced experimental animal models.

Result: Qualitative phytochemical analysis showed phenolic compounds, Flavonoids, Alkaloids, glycosides, phenolic compounds and Tannins were in high amounts. The isolated compound Phenolic derivative showed various regions from IR, NMR and MASS the functional groups, mass of the molecule and proton were analysed and confirmed. The effects of *E. Cyathophora* (200, 400mg/kg, p.o) were studied using *in vivo* behavioural parameters like catalepsy, muscle rigidity and locomotor activity and its effects on neurochemical parameters in rats. The increased haloperidol-induced cataleptic scores were significantly ($p < 0.01$) found to be reduced, with EEEEC at dose of 200mg/kg and 400mg/kg(ip). EEEEC administration showed significant increase in dopamine level and significant reduced in serotonin and L-glutamate level. Daily administration of EEEEC (400mg/kg) significantly improved motor performance.

Conclusion: Overall results the study proved that *E. Cyathophora* possessed potential components involving in anti - Parkinson treatment significantly attenuated the motor defects and also increased the neuro chemical dopamine level.

Keywords: *E. cyathophora*, Anti-Parkinson's activity, Ethanol extract, L-Dopa, Carbidopa and Haloperidol.

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INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative brain disorder characterized by progressive loss of dopaminergic and other neurons present in the substantia nigra pars compacta resulting in malfunctioning of the cerebral neuronal systems. Clinically the disease may cause slowness of movement, muscle rigidity, and rest tremor¹. Pathologically, depletion of dopamine in brain due to the presence of intracytoplasmic inclusions known as Lewy bodies is caused. These pathological

changes also observed in the locus coeruleus, pedunculopontine nucleus, raphe nucleus and dorsal motor nucleus of the vagal nerve and sympathetic and parasympathetic postganglionic neurons². Drug management of Parkinson's includes drugs like levodopa, carbidopa, orphenadrine, benzotropine, selegiline, pergola which reverses the symptoms of Parkinson's condition but these drugs possess various side effects like respiratory disturbances, hallucinations, discoloration of saliva and urine, dyskinesia convulsions and anxiety, arrhythmia,

mydriasis, dry mouth, sore throat, and transient dizziness on long term use³. *E. cyathophora* is an Euphorbia L-spurge genus belonging to the family Euphorbiaceae. It is the most widely distributed species to Kerala, Andhrapradesh and Tamilnadu. The stem and leaves of *E. cyathophora* are used to Anti-inflammatory, Anti-diabetic, Antimicrobial, Hepatoprotective, Antioxidant, induce lactation. There have been very few scientific studies conducted on *E. cyathophora*, however, a comprehensive and exclusive study of the medicinal properties of *E. cyathophora* is still lacking. Hence, the present study aims to analyse the major phytoconstituent in ethanolic extract of the leaves and stems of *E. cyathophora*.

MATERIALS AND METHODS

Collection of Plant Material:

Fresh leaves and stems of *E. cyathophora* were collected from natural locations in Kollihill Tamilnadu region during July-September. This plant was identified and authenticated by ABS Botanical Conservation Research and Training Centre, Kaaripatty. Voucher number AUT/VMCP/105.

Animals:

Wistar rats (150-200g) of either sex were obtained from Srinivasa Enterprises, Bengaluru, India, housed under standard light/dark cycle and fed with standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee, Chennai, India, approved (IAEC, P.Col/11/2017/IAEC/VMCP) experimental protocols and guidelines were followed in conducting the experiments on animals for the purpose of control and supervision of experiments on animals.

Preparation of Extract:

The leaves were collected, shade dried and coarsely grounded. Coarsely powdered plant material (1000g) was weighed and extracted with 5 lit of solvents like Petroleum ether (60-80°C), Chloroform and ethanol by successive extraction in a Soxhlet apparatus for 72hrs. After each extraction, the solvent was distilled off and concentrated extract was transferred to previously weighed petri dish and evaporated to dryness at room temperature (45-50°C) to obtain dried extracts. The extracts were weighed and the percentage yield of the extracts was calculated as follows:

$$\% \text{ of extractive yield } \left(\frac{w}{w} \right) = \frac{\text{Weight of dried Extract}}{\text{Weight of dried leaves Powder}} \times 100$$

The yield of ethanol extract was 8.95 % (w/w).

Preliminary Phytochemical Studies:

Qualitative chemical tests for establishing the chemical composition profile of given extracts were performed using standard methods/protocols⁴ to detect various phytoconstituents present⁴.

Detection of Alkaloids

Alkaloids were detected by following in which solvent free extract (50mg) was stirred with few ml of dilute hydrochloric acid and filtered. To few ml of filtrate, a drop or two of Mayer's reagent was added by the sides of the test tube. A white creamy precipitate formed indicates the test as positive.

Detection of Carbohydrates:

The extract (100mg) was dissolved in 50ml of distilled water, filtered and subjected to the following test.

Fehling's test:

One ml of filtrate was boiled on water bath with 1ml each of Fehling's solution I and II. A red precipitate indicated the presence of sugar.

Benedict's test:

To 0.5ml of Benedict's reagent was added and heated on a boiling water bath for 2min. a characteristic colored precipitate indicated the presence of sugar.

Detection of glycosides:

50 mg of extract was hydrolyzed with concentrated hydrochloric acid for 2hr on a water bath, filtered and the hydrolysate was subjected to the following test.

Borntrager's test

To 2 ml of filtrate hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. Pink color indicated the presence of glycosides.

Detection of saponins: Foam test

The extract (50mg) was diluted with distilled water and made up to 20ml and the suspension was shaken in a graduated cylinder for 15 min. A 2cm layer of foam indicated the presence of saponins.

Detection of proteins and amino acids:

The extract (100mg) was dissolved in 10ml of distilled water and filtered through Whatman No.1 filter paper and the filtrate was subjected to tests of proteins and amino acids.

Millon's test

To 2ml of filtrate, few drops of millon's reagent were added. A white precipitate indicated the presence of proteins.

Biuret test

An aliquot of 2ml of filtrate was treated with one drop of 2% copper sulphate solution. To this, 1 ml of ethanol (95%) was added, followed by addition of excess of potassium hydroxide pellets. Pink color in the ethanolic layer indicated the presence of proteins.

Detection of Phenolic Compound:

Ferric chloride test

The extract (50mg) was dissolved in 5ml of distilled water. To this few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenolic compounds.

Lead acetate test

The extract (50mg) was dissolved in 5ml of distilled water. To this 3ml of 10% lead acetate were added. A bulky white precipitate indicated the presence of phenolic compounds.

Isolation of compound A

Preparation of Admixture of EEEEC

15g of ethanolic extract of *E. cyathophorawas* admixed with 20 gm silica gel (60/120 meshes) to get uniform mixing.

Column packing for EEEEC

200 gms of silica gel was taken in a suitable column and packed very carefully without air bubbles using benzene as filling solvent. The column was kept aside for 1 hour and allowed for close packing. Admixture was then added at the top of the stationary phase and started separation of compounds by eluting with solvents of different polarity like Hexane, ethyl acetate and methanol, Dichloromethane. All the column fractions were collected separately and concentrated under reduced pressure. Finally the column was washed with ethyl acetate and methanol.

Analysis of the isolated compound from the extract using various analytical techniques

Isolated compounds were characterized and identified by IR, NMR and GC-MS spectrophotometric methods and chemical structure of the compounds were subsequently elucidated.

Spectral analysis of the compounds using FT-IR

IR spectra of the compounds isolated from the extracts were recorded using Shimadzu. The spectral resolution for the Shimadzu was 0.25cm^{-1} , and the spectral data were stored in the database at intervals of 0.5cm^{-1} at $4000\text{-}2000\text{cm}^{-1}$, and of 0.25cm^{-1} at $2000\text{-}400\text{cm}^{-1}$. The samples were measured by using KBr disc method.

Spectral analysis of the compounds using ^1H NMR

^1H NMR spectra of the compound isolated from the extracts were recorded using a Bruker, USA (400 MHz). The measuring conditions for the most of the spectra were as follows: flip angle of $22.5\text{-}30.0$ degrees, pulse repetition time of 30s. The long pulse repetition time and small flip angle is used to ensure precise relative intensities. The ^1H NMR chemical shifts were referred to TMS in organic solvents and TSP in D_2O .

Spectral analysis of the compounds using ^{13}C NMR

^{13}C NMR spectra of the compounds isolated from the extracts were recorded with a Bruker, USA (400 MHz) spectrometer. The measuring conditions for the most of the spectra were as follows: a pulse flip angle of $22.5\text{-}45$ degrees, a pulse repetition time of 4-7 seconds, and a resolution of $0.025\text{-}0.045$ ppm. The spectra whose spectral codes started with "CDS" were reconstructed from peak positions, intensities, and line widths by assuming all resonance peaks were Lorenz lines. The chemical shift was referred to a TMS for all solvents.

Spectral analysis of the compounds using GC-MS

Mass spectra of the compounds isolated from the extracts were recorded with Shimadzu USA by the electron impact method where an electron accelerating voltage 75eV and an ion accelerating voltage of $8\text{-}10\text{nV}$. The reservoir inlet systems were used. The dynamic range for the peak intensities were 3 digits and the accuracy of the mass number was 0.5.

Acute Oral Toxicity of the Extract:

Acute oral toxicity study was performed as per OECD 423 guidelines, albino wistar rats ($n=6$) of either sex selected

by random sampling technique were used for acute toxicity study². The animal were kept fasting for overnight providing only water, after which the extract was administered orally at the starting maximum dose level 2000mg/kg body weight by oral needle and observed for deaths of overt signs of toxicity $\frac{1}{2}$, 1, 2 and 4 h after dosing and subsequently once daily for fourteen days. The signs of changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system, motor activity and behaviour pattern were noted. The toxicity signs of fits, excessive salivation, tremors, diarrhea, lethargy, sleep and coma, as well as the onset of toxicity and signs of toxicity were also noted⁵.

Haloperidol Induced Catalepsy (Model by Elliott and Close in 1990)⁶

Haloperidol causes dysfunctioning of various neurotransmitters such as acetyl choline, serotonin and GABA and induced catalepsy due to increased oxidative stress. Haloperidol, an antipsychotic drug, blocks central dopamine receptor in striatum and also produces a behavioural immobility and postural cataleptic state in animals characterized by muscle rigidity and failure to correct an externally imposed posture, thus haloperidol induced catalepsy model was selected. The method described by Elliott and Close in 1990 was followed for the anti cataleptic activity.

Experimental Design

The animals were divided into five groups ($n=6$). Group I served as vehicle control, group II served as negative control, group III and IV served as test group treated with EEEEC ($200, 400\text{mg/kg}$, i.p.) and group V served as standard L-DOPA+Carbidopa ($100+25\text{mg/kg}$, p.o.) respectively. Haloperidol (1mg/kg , i.p.) induced catalepsy was examined at every 30min interval for 180min using standard bar test. The duration for which the rat retains the forepaws extended and resting on the scale (block-method 0-3.5scale) was considered as cataleptic score.

Behavioural Assessment:

Locomotor Activity:

The effect on locomotor activity was measured for 10 min at every 30 min upto 3 hours using actophotometer. The locomotor activity. It operates on photoelectric cells that are collected in circuit with a counter. can be easily measured by using an actophotometer. When the beam of light falling on the photocell is cutoff by animal, a count is recorded. Actophotometer has either circular or square arena in which animal movements are measured.

Exploratory behaviours

Head dipping

The effect on exploratory behaviour (head dipping) was measured for 10min at every 30min upto 3 hours using hole board. The hole board made of plywood has the size ($60\text{cm} \times 60\text{cm}$, 3mm thick). The mat finished of the upper surface avoids reflections which might alter the behaviour of the animal. The board embodies 9 uniformly distributed holes each of 5 cm in diameter. Each rat was acclimatized for 10 min and number of holes explored through head plunging acts during the total observation time period were noted. Care has to be taken to avoid

multiple events (two or more head plunging in quicker session). A fresh exploration was considered when the animal neatly plunged its head once and did something else in between like grooming, taking a short walk etc., before plunging its head for the next time. One animal at a time was tested for each activity.

Line crossing

The effect on exploratory behaviour (line crossing) was measured for 10 min at every 30 min up to 3 hours using hole board. The hole board made of plywood has the size (60cm X 60cm, 3mm thick). The mat finished of the upper surface avoids reflections which might alter the behaviour of the animal. The board embodies 9 uniformly distributed lines. Each rat was acclimatized for 10 min and the number of line crossing acts, during the total observation period were counted. Care has to take to avoid multiple events.

Dissection and Homogenization:

On the day of experiment rats were sacrificed, whole brain was dissected out and separated the subcortical region (including the striatum). Weighed a specific quantity of tissue and was homogenized in 3ml HCl-butanol in a cool environment. The sample was then centrifuged for 10min at 2000rpm. 0.8ml of supernatant phase was removed and added to an Eppendorf reagent tube containing 2ml of heptane and 0.25ml 0.1M HCl. After 10min, shake the tube and centrifuged under same conditions to separate two phases. Upper organic phase was discarded and the aqueous phase was used for biochemical estimation⁷.

Biochemical Estimation:

Dopamine assay

To 0.02ml of the HCl phase, 0.005ml 0.4ml HCl and 0.01ml EDTA/ Sodium Acetate buffer (pH 6.9) were added, followed by 0.01ml iodine solution for oxidation. The reaction was stopped after 2min by the addition of 0.1ml sodium thiosulphate in 5M Sodium hydroxide. 10 M Acetic acid was added 1.5 min later. The solution was then heated to 100° C for 6 min. Excitation and emission spectra were determined using spectrofluorimeter at 330 - 375nm for the samples at room temperature. Fluorescence (Test - Blank) values, with internal standard (0.005ml distilled water and 0.1ml HCl butanol to 20ng of dopamine standard), were measured.

Serotonine Estimation

Serotonin assay

Tissue extract 0.5ml and 0.625ml of OPT (Orthophthalaldehyde) reagent was heated to 100°C for ten min. After the samples reached equilibrium with the ambient temperature, excitation/emission spectra readings at 360-470 nm were taken.

Estimation of brain glutamate levels by UV assay method

In this study animals were decapitated after the last behavioural session. The brains were immediately excised and cerebellum discarded. The cortex, striatum and the sub-cortical parts were separated and weighed. The sub-cortical region of the brain comprised all the remaining parts of the forebrain after dissection of the cerebral

cortex and striatum, including the hippocampus, thalamus, hypothalamus, amygdale and other sub-thalamic structures. Spectrophotometer-suitable for precise measurement at 430nm.

Deproteinization

Weighed quantity of brain portion was homogenized with 2 parts of perchloric acid by centrifugation for 10 min at 3000 rpm. Pipette out 3.0ml supernatant fluid and make up the pH to 9 with phosphate solution. Allow the solution to stand for 10 min in ice bath and filtered using fluted filter paper. Further, solution allowed warming to room temperature, diluted and taking 1.0 ml for the subsequent assays. Wavelength: 340nm, Light path: 1cm, Final volume: 3.35ml, Room temperature. For blank measurement, water used as blank instead of sample.

Pipette out 2.00ml of Glycine-hydrazine buffer, 1.00ml of Sample, 0.1ml of ADP solution, 0.2 ml of NAD solution. Mix and read the extinction E_1 at 340nm. Then added 0.05 ml of GIDH (Glutamate dehydrogenase) solution. Sample and Blank solutions were allowed to stand for 45 min and measured the extinction E_2 at 340nm. Calculated the difference between E_1 and E_2 for sample and blank (ΔE). $\Delta E_{\text{sample}} - \Delta E_{\text{blank}} = \Delta E_{\text{glutamate}}$ is used for the calculations⁸.

Statistical Analysis

All the values were expressed as mean \pm SEM. The statistical analysis was carried out using analysis of variance ANOVA (between control and drug treatments), followed by Dunnett's 't' test. P values < 0.05 considered as significant.

Histopathological Studies:

Histopathological study on the brains from control and experimental groups were done after fixing with 10% formalin, embedded in paraffin wax and sliced into longitudinal sections of 5 μ m. Further, Histopathology of these sections was observed using stained with hemotoxylin and eosin dye.

RESULTS AND DISCUSSION

Phytochemical Screening:

Table 1: Phytochemical screening of the ethanolic extract of *E.Cyathophora*.

Compound	Result
Alkaloids	+
Glycosides	+
Flavonoids	+
Phenolic compounds and Tannins	+

Acute Toxicity:

The EEEEC dose of 2000mg/kg was found to be the maximal safe dose with no mortality. Therefore, lower dose of 200mg/kg and higher dose of 400mg/kg of EEEEC were fixed as the study dose.

Effect of EEEEC on haloperidol induced catalepsy (0-3.5 scale)

The cataleptic scores are depicted in Table 1. There was a significant difference (P<0.01) between control group (I) and negative control group (II) in catalepsy. The EEEEC treated group's shows significant anticataleptic action. EEEEC at dose level of 400mg/kg particularly, shows

anticataleptic action comparable to standard drug treatment. There was a significant difference ($P < 0.01$) between negative control group (II) and EEEC in catalepsy. EEEC at dose level of 400 mg/kg, showed good anticataleptic action at 30, 150, 180 min after haloperidol challenge.

Effect of EEEC on locomotor activity

The changes in locomotor activity after haloperidol administration are shown in Table 2. There is a significant ($P < 0.01$) decrease in locomotor activity in negative control group (II) when compared with the control group (I). EEEC 400 mg/kg treated animals showed improved locomotor activity when compared with negative control group ($P < 0.01$). EEEC at a dose of 400 mg/kg potentiated the locomotor activity of standard drug. All groups were showing significant difference ($P < 0.01$) when compared with negative control group at all time intervals.

Effect of EEEC on exploratory behaviour

The exploratory behaviour was expressed by head dippings and line crossings. Head dippings are shown in Table 3 and 4. Negative control group (II) indicated decrease in exploratory behaviour i.e. head dippings and line crossings when compared with control group. The results presented by the EEEC treated groups show significant ($P < 0.01$ and $P < 0.05$) increase in head dippings

and line crossings when compared with negative control group at 90, 120, 150, 180 min after haloperidol challenge.

Effect of EEEC dopamine levels

Dopamine levels are significantly ($P < 0.01$) decreased in negative control group (II) when compared with control group. A significant ($P < 0.01$) increase in dopamine value is observed in EEEC treated group animals. The results are showing increased dopamine levels in standard drug treated animals also. The results were shown in Table 5.

Effect of EEEC serotonin levels

The present study shows significant ($P < 0.01$) increase in serotonin levels in negative control group (II) animals. EEEC treated animals at both doses 200mg/kg and 400 mg/kg showed significant ($P < 0.05$ & $P < 0.01$) reduction in serotonin levels when compared with negative control group. The results are shown in Table 5.

Effect of EEEC on glutamate levels

The present study shows significant ($P < 0.01$) increase in glutamate levels in negative control group animals. EEEC treated animals at both doses 200 mg/kg and 400 mg/kg showed significant ($P < 0.05$ & $P < 0.01$) decrease in glutamate level, which is a significant factor in excitotoxicity. The results are shown in Table 5.

Table 2: Effect of EEEC on Catalepsy (0-3.5 scale)

Group	Catalepsy (0-3.5 scale)					
	30 min	60 min	90 min	120 min	150 min	180 min
I	0	0	0	0	0	0
II	2.90±0.13 ^{a**}	3.10±0.18 ^{a**}	3.40±0.15 ^{a**}	3.60±0.17 ^{a**}	3.60±0.15 ^{a**}	3.60±0.14 ^{a**}
III	1.95±0.12 ^{bms}	2.64±0.14 ^{bns}	2.89±0.10 ^{b*}	2.65±0.17 ^{b**}	2.33±0.15 ^{b**}	1.97±0.12 ^{b**}
IV	1.19±0.08 ^{b**}	2.14±0.15 ^{b*}	2.49±0.19 ^{b*}	2.29±0.14 ^{b**}	1.85±0.17 ^{b**}	1.27±0.12 ^{b**}
V	0.80±0.07 ^{b**}	1.05±0.10 ^{b**}	1.24±0.09 ^{b**}	1.05±0.05 ^{b**}	0.65±0.10 ^{b**}	0.42±0.12 ^{b**}

Values are expressed as mean ± SEM, ^{a**} $P < 0.001$, ^{**} $P < 0.01$, ^{*} $P < 0.05$, ^{ms} $P > 0.05$

(One way ANOVA followed by Dunnet's test), n=6

Table 3: Effect of EEECon Locomotor activity

Group	Locomotor					
	30 min	60 min	90 min	120 min	150 min	180 min
I	321.52±7.24	329.22±7.14	325.19±7.62	337.19±5.33	331.28±6.17	334.33±5.29
II	29.19±2.17 ^{**}	32.33±2.41 ^a	30.41±2.64 ^{**}	31.22±1.92 ^{a**}	32.17±2.74 ^{a**}	34.28±2.52 ^{b**}
III	77.29±3.41 ^{b*}	126.67±4.33 ^{b**}	133.27±4.54 ^{b**}	154.22±4.28 ^{b**}	172.87±4.64 ^{b**}	201.52±4.33 ^{b**}
IV	91.24±3.27 ^{b**}	145.21±4.12 ^{b**}	152.17±4.52 ^{b**}	174.29±4.22 ^{b**}	195.67±4.52 ^{b**}	222.17±4.74 ^{b**}
V	128.05±6.27 ^{b**}	228.33±6.47 ^{b*}	244.17±6.54 ^{b*}	267.22±6.14 ^{b*}	272.19±5.21 ^{b*}	312.64±6.39 ^{b*}

Values are expressed as mean ± SEM, ^{**} $P < 0.001$, ^{**} $P < 0.01$, ^{*} $P < 0.05$, ^{ms} $P > 0.05$ (One way ANOVA followed by Dunnet's test),n=6

Table 4: Effect of EEEC on Exploratory Behaviour - Head Dipping

Group	Head Dipping Behaviour					
	30 min	60 min	90 min	120 min	150 min	180 min
I	8.52±0.30	7.84±0.43	8.22±0.54	7.72±0.42	7.52±0.42	7.41±0.21
II	0.49±0.22 ^{a**}	0.45±0.31 ^{a**}	0.21±0.17 ^{a**}	0.52±0.22 ^{a**}	0.37±0.21 ^{a**}	0.59±0.22 ^{a**}
III	2.92±0.52 ^{b*}	4.85±0.33 ^{b**}	5.69±0.14 ^{b**}	6.52±0.37 ^{b**}	6.96±0.28 ^{b**}	7.12±0.33 ^{b*}
IV	4.22±0.32 ^{b**}	5.96±0.12 ^{b**}	7.14±0.41 ^{b**}	7.33±0.24 ^{b**}	7.89±0.28 ^{b**}	8.27±0.24 ^{b**}
V	5.21±0.22 ^{b**}	6.54±0.41 ^{b**}	7.69±0.33 ^{b**}	8.12±0.28 ^{b**}	8.29±0.51 ^{b**}	8.69±0.33 ^{b**}

Values are expressed as mean ± SEM, ^{a**} $P < 0.001$, ^{**} $P < 0.01$, ^{*} $P < 0.05$, ^{ms} $P > 0.05$ (One way ANOVA followed by Dunnet's test),n=6

Table 5: Effect of EEHP on Exploratory Behaviour - Line Crossing

Group	Line Crossing Behaviour					
	30 min	60 min	90 min	120 min	150 min	180 min
I	84.33±3.69	82.67±4.41	85.49±4.57	81.22±3.67	88.42±4.33	89.17±4.17 ^{a**}
II	5.29±0.54 ^{a**}	4.52±0.33 ^{a**}	4.19±0.41 ^{a**}	3.54±0.29 ^{a**}	3.22±0.57 ^{b**}	3.64±0.46 ^{b**}
III	14.17±1.09 ^{bns}	23.54±3.54 ^{b**}	30.29±3.72 ^{b**}	41.19±3.84 ^{b**}	50.22±3.21 ^{b**}	56.33±3.24 ^{b**}
IV	29.17±3.54 ^{b**}	44.19±3.22 ^{b**}	49.67±3.45 ^{b**}	52.13±4.17 ^{b**}	62.57±4.62 ^{b**}	65.39±3.14 ^{b**}
V	39.54±3.13 ^{b**}	59.22±4.80 ^{b**}	68.44±4.46 ^{b**}	74.19±4.75 ^{b**}	77.69±4.49 ^{b**}	79.54±5.28 ^{b**}

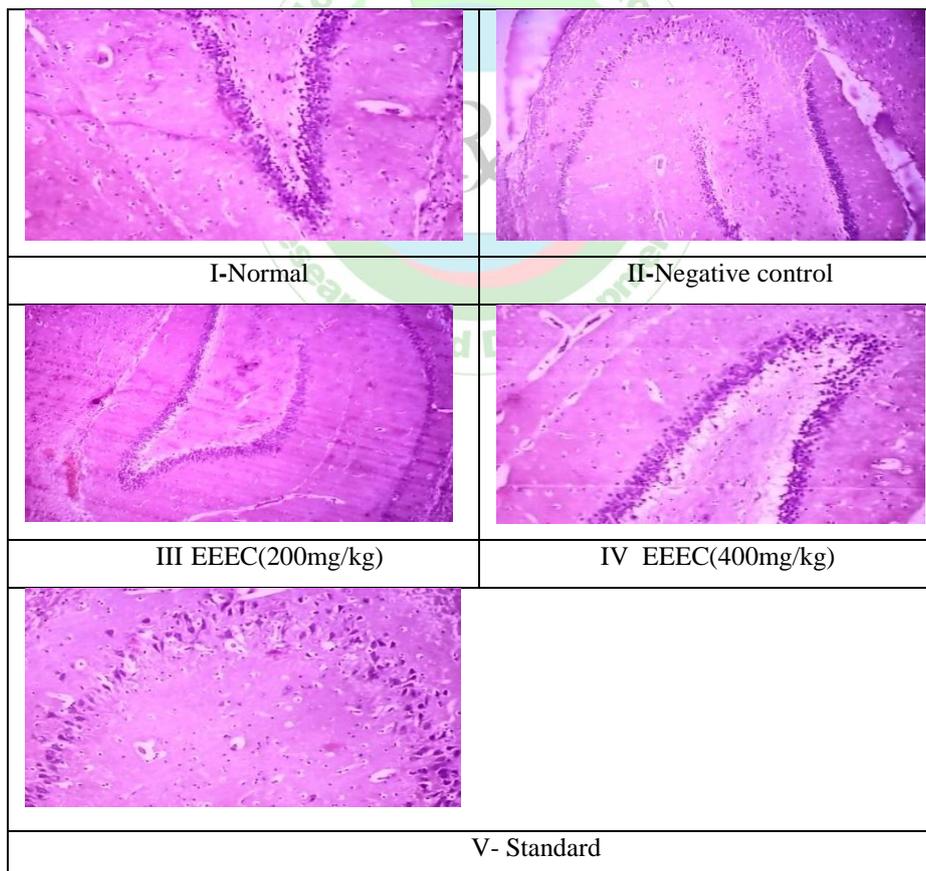
Values are expressed as mean ± SEM, ^{***}P < 0.001, ^{**}P < 0.01, ^{*}P < 0.05, ^{bns}P > 0.05(One way ANOVA followed by Dunnet’s test),n=6

Table 6: Effect of EEEC on brain Neurotransmitter Levels in chronic haloperidol treated rats

Groups	Treatment	Dopamine (pg/mg tissue)	Serotonin (pg/mg tissue)	L-Glutamate (µ mol/gm tissue)
I	Control	754.69±12.47	274.33±5.17	74.19±3.22
II	Negative control (Haloperidol)	267.33±10.14 ^{a**}	412.27±4.67 ^{a**}	52.17±2.33 ^{a**}
III	EEEC (200mg/kg)	514.51±7.49 ^{b**}	372.12±6.47 ^{b*}	55.84±3.69 ^{bns}
IV	EEEC (400mg/kg)	568.64±8.22 ^{b**}	353.22±6.14 ^{b**}	59.44±2.97 ^{b*}
V	L-DOPA (100 mg/kg) + Carbidopa (25 mg/kg) as Standard	682.19±10.22 ^{b**}	307.58±4.69 ^{b**}	69.22±2.54 ^{b**}

Values are expressed as mean ± SEM, ^{***}P < 0.001, ^{**}P < 0.01, ^{*}P < 0.05, ^{bns}P > 0.05(One way ANOVA followed by Dunnet’s test),n=6

Histopathological studies on brain



Characterization of Compound EC I

The isolated compound is light yellowish brown in colour, solid (323 mg), soluble in methanol, ethanol and water. Melting point was found to be 178-183°C. The mass of compound was found as 194[M+1].

S. No	Wave numbers cm^{-1}	Type
1.	3395	OH
2.	3013	C-H
3.	1698	C=O
4.	1412	C-H
5.	1058	C-O-C

IR cm^{-1} : (KBr) Pressed pellet technique (EC I)

^1H NMR (Acetone - d_6 , 400MHz) ppm

δ 3.87 (d, 1H, C2 - H), 3.46 (d, 1H, C6 - H), 3.35 (t, 1H, C3 - H), 3.23 (t, 1H, C4 - H), 3.15 (t, 1H, C5 - H).

^{13}C NMR: (Acetone- d_6 , 400 MHz), ppm

δ 165 C (C - 7), 95 C (C - 2), 75.89 C (C - 4), 75.70 C (C - 3), 70 C (C - 5), 69.55 C (C - 6).

Properties of isolated compound EC I

Name	Compound A
Molecular formula	$\text{C}_6\text{H}_{10}\text{O}_7$
Melting point	185 - 191 $^\circ\text{C}$
Mass (m/z)	194 (M+1)
Colour	Yellowish brown
Nature	Solid
Solubility	Soluble in methanol, ethanol and water
TLC studies	Methanol: DCM (R_f Value 0.21) 80:20 (v/v)

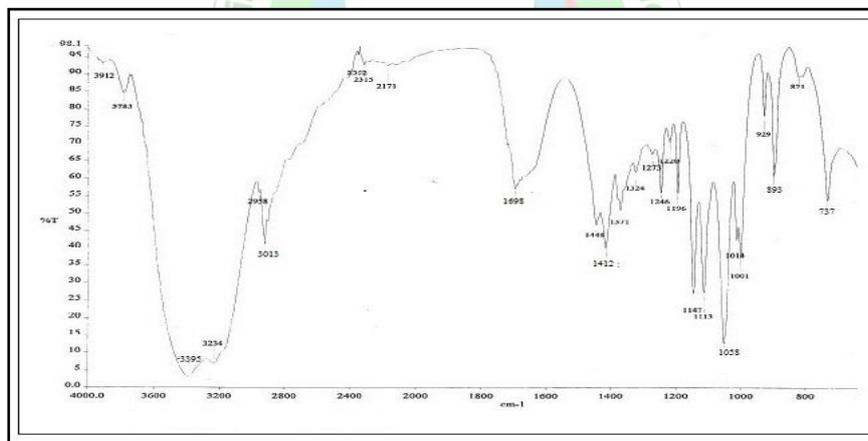


Figure 1: IR Spectrum of EC 1

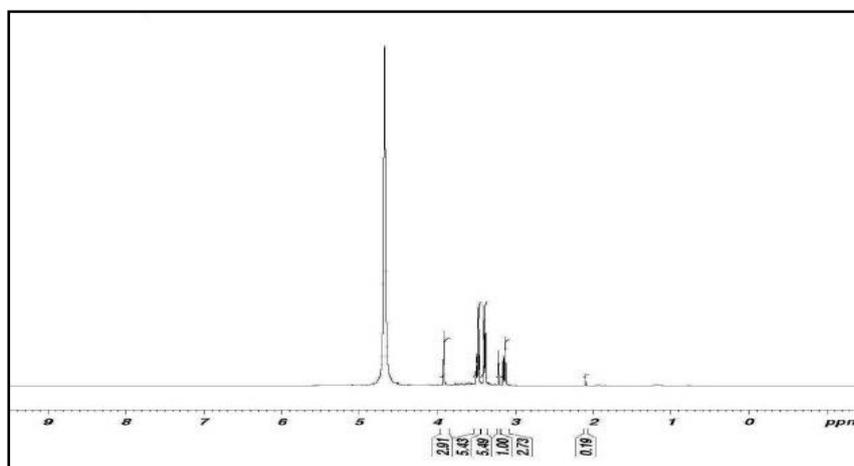


Figure 2: ^1H NMR Spectrum of EC1

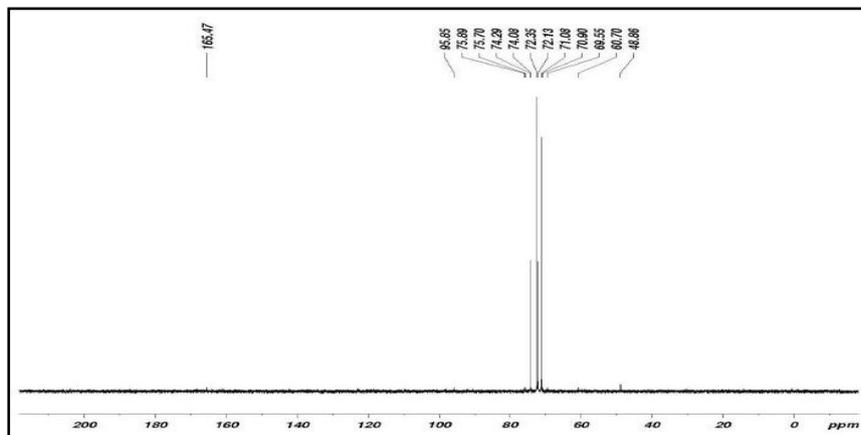


Figure 3: C^{13} NMR of structure EC 1

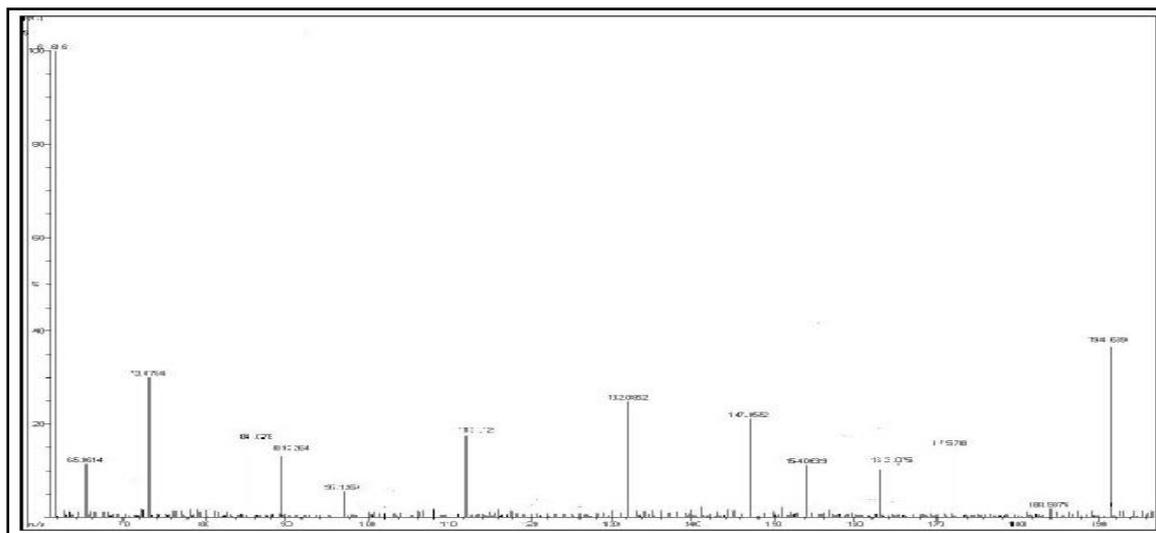


Figure 4: MASS Spectrum of EC1

CONCLUSION

From these results of the present study, it was understood that *E. Cyathophora* has higher anti Parkinson activity it may be understood from the variations in the profiles of the functional groups and phytochemical of the plants. In view of the above facts, we are concluding that ethanolic extract of plant showed to a *Euphorbia cyathophora* promising effect in animals with parkinson's disease. And we appreciate further detailed molecular docking studies with this drug in anti-parkinson's pharmacology and toxicology responsible for Neuroprotective effect.

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