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Antioxidant, Anti-alzheimer and Anti-parkinson activity of *Artemisia nilagirica* Leaves with Flowering Tops

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Abstract

Presently peoples are loaded with stress and leads to various neurodegenerative disorders like anxiety, Alzheimer's disease and Parkinson's disease. The present study was aimed to investigate the antioxidant, anti-alzheimer and anti-parkinson activity of *Artemisia nilagirica* leaves with flowering tops extracts. The ethanol and aqueous extracts of *Artemisia nilagirica* leaves with flowering tops were processed for evaluation of *in vitro* antioxidant activity namely hydrogen-donating activity, superoxide scavenging activity, total polyphenol content, total flavonol content and reducing power assay. The object recognition and Y-Maze test were used to evaluate the anti-alzheimer's activity of extract. The different parameters like catalepsy (bar test), locomotor activity (actophotometer test), and muscle activity (rotarod test) were measured in all animals for anti-parkinson activity. The findings of *in vitro* antioxidant study demonstrated that ethanol extract has maximum antioxidant properties compared to aqueous extract. Hence the ethanol extract of *Artemisia nilagirica* was selected for screening of anti-alzheimer and anti-parkinson activity. The administration of ethanol extract of *Artemisia nilgirica* exhibited significant anti-alzheimer and anti-parkinson activity. The findings of anti-alzheimer and anti-parkinson activity ethanol extract of *Artemisia nilagirica* demonstrated that this plant have neuroprotective properties.

1 Introduction

Neurodegenerative disorders are a diverse group of diseases of the nervous system. Disorders such as Alzheimer's and Parkinson's disease account for a significant and increasing amount of morbidity and mortality in the developed world. Alzheimer's and Parkinson's disease are becoming more common mainly as a result of increased life expectancy and changing population demographics. There is a growing body of evidence that nigral neurons may be damaged by free radicals in this disorder. Free radicals are thought to be produced locally within the basal ganglia and lead to progressive damage and death of substantia nigra neurons in susceptible individuals. Oxidative stress has been implicated in the pathogenesis of Alzheimer's and Parkinson's disease by the finding of several characteristics, such as enhanced lipid peroxidation in specific areas of the brain in postmortem studies[1-3].

Plant extracts and their constituents act as a natural source of antioxidants. The antioxidant activity of several plant extracts is due to several secondary metabolites especially phenolic compounds such as flavonoids, alkaloids and tannins. The potent antioxidant activity of flavonoids may be the most important function of flavonoids responsible for their actions in the body. There are several studies suggesting neuroprotective effect of flavonoids[4,5].

Various synthetic medicines are prescribed for Alzheimer's and Parkinson's disease but they exert side effects. Still there is a challenge to the medical system for Management of Alzheimer's and Parkinson's disease without any side effects. Consequently, the search for natural drugs from medicinal plants is being increased because of its fewer side effects, willingly availability and low cost. Thus the scientific validation of

medicinal plants traditionally used in the treatment and management of Alzheimer's and Parkinson's disease is demanded.

Artemisia nilagirica locally known as Indian wormwood is a wild perennial hardy plant, belongs to family Asteraceae. Traditionally it is used in the treatment of epilepsy, nervous disorders, diuretic, malaria, nerve tonic, inflammation, diabetes, stress, depression, diabetes, anti-inflammatory and various skin diseases[6,7]. The common phytochemicals constituents of *Artemisia nilagirica* are flavonoids, alkaloids, tannins, glycosides, phenol, terpenoids, amino acids, quinines, saponins and polysaccharides[8,9]. *Artemisia nilagirica* contain sesquiterpene lactones, coumarins and acetylenes as the main metabolites. The main constituents of the essential oil of the *Artemisia nilagirica* are camphor, β-eudesmol, limonene, tripinoline and aromadendrene, 1,8-cineole, borneol, artemisia alcohol, camphene, α- gurjunene, p-cymene, terpinen-4-ol and α-pinene[10,11].

The anti-alzheimer and anti-parkinson activity of leaves with flowering tops of *Artemisia nilagirica* has not been experimentally studied. On the basis of literature and documentation of existing uses of *Artemisia nilagirica*, an effort has been made to establish the scientific validity to investigate antioxidant, anti-alzheimer and anti-parkinson activity.

2 Materials and Methods

2.1 Plant material

The leaves with flowering tops of *Artemisia nilagirica* was selected for present work.

2.2 Collection and identification of plant material

The plant was collected from Ooty District, Tamil Nadu, India, and identified by a Tamil Nadu Agriculture University Horticulture Research Station Ooty, Tamil Nadu, India. The plant specimens were deposited in the herbarium of Department of Pharmacognosy, Mahakal Institute of Pharmaceutical Studies, Ujjain, Madhya Pradesh, Voucher no. MIPS/A/36/2011. The plant materials were shade dried, reduced to coarse powder and stored in airtight container till further use.

2.3 Preparation of extracts

The powder of the leaves with flowering tops **of *Artemisia nilagirica*** was packed in the Soxhlet apparatus and successively extracted with petroleum ether, chloroform, ethanol, methanol and distilled water until the completion of the extraction. The extracts were filtered while hot, and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely, and later dried in a desiccator. After that extracts of petroleum ether, ethanol and aqueous were kept in air tight container for further study.

2.4 In vitro antioxidant activity of extract

2.4.1 Hydrogen-donating activity

The methanolic solution of DPPH (100 mM, 2.95 ml), 0.05 ml of each extracts dissolved in methanol was added at different concentrations (50-250 µg/ml). Reaction mixture was shaken and after 30 min at room temperature, the absorbance values were measured at 517 nm and converted into percentage of antioxidant activity (% AA). Ascorbic acid was used as standard. The degree of discoloration indicates the scavenging efficacy of the extract, was calculated by the following equation:

$$\% AA = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100] / Abs_{DPPH}\}$$

2.4.2 Superoxide Scavenging Activity

Superoxide scavenging was carried out by using alkaline Dimethyl sulfoxide (DMSO). Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 h and the solution was filtered immediately before use. Filtrate (200 ml) was added to 2.8 ml of an aqueous solution containing nitroblue tetrazolium (56 mM), EDTA (10 mM) and potassium phophate buffer (10 mM, pH 7.4). Sample extract (1 ml) at various concentrations (50-250 µg/ml) in water was added and the absorbance was recorded at 560 nm against a control in which pure DMSO has been added instead of alkaline DMSO[12-14].

2.4.3 Total polyphenol content

Total polyphenol content was determined using colorimetric method. 1.0 ml of the prepared extract was oxidized using 2.5 ml of Folin- Ciocalteu reagent, and 2.0 ml of sodium carbonate solution (75 g/l) was then added to the reaction mixture. The absorbance readings were taken at 760 nm after incubation at room temperature for 2 h. The amount was calculated using the gallic acid calibration curve. The results were expressed as gallic acid equivalent (GAE) mg per 100 ml of the sample (extract).

Calibration curves of gallic acid

Accurately weighed 100 mg of gallic acid was dissolved in 100 ml of distilled water which gives the concentration of 1000 µg/ml. 10 ml of this solution was taken and made up to 100 ml with gallic acid which contains the concentration of 100 µg/ml. Further 10 ml of this solution was taken and made up to 100 ml with gallic acid which contains the concentration of 10 µg/ml. 1 to 10 ml were taken from this solution and made up to 10 ml to get the concentration ranges of 1 to 10 µg/ml. Calibration curve was plotted by mixing 1 ml aliquots of gallic acid solutions with 2.5 ml of Folin-Ciocalteu reagent and 2.0 ml of sodium carbonate solution (75g/l). The absorbance was measured after incubation at room temperature for 2 h at 760 nm using UV spectrophotometer, against blank solution.

2.4.4 Total flavonol content

Flavones and flavonols contents were analyzed by the colorimetric method. 9.8 ml of the prepared extract was mixed with a 10% solution of aluminum chloride (200 µl). After 30 min, absorption was measured at a 425 nm wavelength. The amount was calculated using quercetin calibration curve. The results were expressed as the quercetin equivalent (QE) mg per 100 ml of the sample.

Calibration curves of quercetin

Accurately weighed 100 mg of quercetin was dissolved in 100 ml of distilled water which gives the concentration of 1000 µg/ml. 10 ml of this solution was taken and made up to 100 ml with quercetin which contains the concentration of 100 µg/ml. Further 10 ml of this solution was taken and made up to 100 ml with quercetin which contains the concentration of 10 µg/ml. 1 to 10 ml were taken from this solution and made up to 10 ml to get the concentration ranges of 1 to 10 µg/ml. Calibration curve was plotted by mixing 9.8 ml aliquots of quercetin solutions with a 10% solution of aluminum chloride (200 µl). The absorbance was measured 30 min at 425 nm using UV spectrophotometer, against blank solution.

2.4.5 Reducing power assay

The relative reducing activity in terms of antioxidant activity of extracts was determined by using individual extracts (5 mg) as well as its combination with equal amount of ascorbic acid The extracts and ascorbic acid were dissolved separately in 1.0 mL of deionized water with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 mL, 10% w/v) were added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared $FeCl_3$ solution (0.5 mL, 0.1%). The absorbance was measured at 700 nm by making 500 µg mL^{-1} extracts aliquot. Increased absorbance of the reaction mixture indicated increased antioxidant activity via reducing power with reference to equal amount of standard ascorbic acid[15].

2.5 Pharmacological activity

2.5.1 Anti-alzheimer's activity

2.5.1.1 Object recognition test

The apparatus consisted of plywood (70 × 60 × 30 cm) with a grid floor that could be easily cleaned with hydrogen peroxide after each trial. The apparatus was illuminated by a 40 W lamp suspended 50 cm above the box. The objects to be discriminated were also made of plywood in two different shapes of 8 cm height colored black.

The day before test, mice were allowed to explore the box (without any object) for two min. On the day of the test in the first trial (T1) two identical objects were placed in two opposite corners of the box and the amount of time taken by each mouse to complete 20 sec of object exploration was recorded. Exploration was considered directing the nose at a distance <2 cm to the object and/or touching it with the nose. During the second trial (T2, 90 min after T1) one of the objects presented in trial T1 was replaced by new object and the mice were left in the box for 5 min. The time spent in exploration of familiar (F) and the new object (N) were recorded separately and discrimination index (D) was calculated (N-F/N+F). Care was taken to avoid place preferences and olfactory stimuli by randomly changing the role (familiar or new object) and the position of the two objects during T2 and cleaning the apparatus with hydrogen peroxide.

Swiss albino mice of either sex were selected and divided into four groups of six animals each and treated as follows:

- Group I: Administered propylene glycol (5 ml/kg body weight), served as vehicle group
- Group II: Administered extract at the doses of 100 mg/kg body weight intraperitonially
- Group III: Administered extract at the doses of 200 mg/kg body weight intraperitonially
- Group IV: Received Piracetam (100 mg/kg body weight)

The mice were treated with vehicle, extract (100 and 200 mg/kg, i.p.) and Piracetam (100 mg/kg, i.p.) 30 minutes before the first trial. The second trial was performed 90 min after the first trial. Each group consisted of 6 animals.

2.5.1.2 Y-Maze test

Swiss albino mice of either sex were selected and divided into four groups of six animals each and treated as follows:

- Group I: Administered propylene glycol (5 ml/kg body weight), served as vehicle group
- Group II: Administered extract at the doses of 100 mg/kg body weight intraperitonially
- Group III: Administered extract at the doses of 200 mg/kg body weight intraperitonially
- Group IV: Received diazepam (10 mg/kg body weight) as standard drug

The test was performed in albino mice at 30, 60, 90 and 120 min after treatment. The mice were placed individually symmetrical Y-shaped runway (33 x 38 13cm) for 3 min and the number of times, a mice entered in the arm of the maze with all 4 ft (an 'entry') were counted[16,17].

2.5.2 Anti-parkinson activity

Swiss albino mice of either sex were divided into four groups of six animals each and treated as follows:

- Group I: Administered propylene glycol (5 ml/kg body weight), served as vehicle group
- Group II: Administered chlorpromazine (3 mg/kg body weight) intraperitonially for a period of 21 days
- Group III: Administered Chlorpromazine (3 mg/kg body weight) and extract at the doses of 100 mg/kg body weight intraperitonially for a period of 21 days
- Group IV: Administered (3 mg/kg body weight) and extract at the doses of 200 mg/kg body weight intraperitonially for a period of 21 days
- Group V: Received Chlorpromazine (3 mg/kg body weight) and combination of carbidopa + levodopa (1 : 10 ratio) (10 mg/kg body weight) intraperitonially served as standard drug for a period of 21 days

Chlorpromazine was given 30 minutes prior to standard and test drug. Body weight changes and behavioral assessments were carried out before the start of the treatment. Various parameters like catalepsy (bar test), locomotor activity (actophotometer test), and muscle activity (rotarod test) were measured in all animals[18,19]. After the 21 days, animals were sacrificed and their brains were removed and weighed. A 10% tissue homogenate was prepared in 0.1 M phosphate buffer (pH 8) for TBARS, GSH, nitrites, and total protein.

2.5.2.1 Biochemical estimation

2.5.2.1.1 Lipid Peroxidation Assay (TBARS)

Thiobarbituric acid reactive substances (TBARS) measurement is an index of lipid peroxidation in brain. For the estimation of TBARS, ten percent (w/v) tissue homogenate was mixed with sodium dodecyl sulfate, acetate buffer (pH 3.5), and aqueous solution of thiobarbituric acid. After heating at 95°C for 60 min, the red pigment produced was extracted with n-butanol-pyridine mixture and estimated by the absorbance at 532 nm. As an external standard, tetramethoxypropane was used, and lipid peroxide level was expressed in terms of nmol malondialdehyde[20].

2.5.2.1.2 Estimation of Reduced Glutathione (GSH)

For the estimation of reduced glutathione the 1 ml of tissue homogenate was precipitated with 1 ml of 10% trichloroacetic acid (TCA). To an aliquot of the supernatant 4 ml of phosphate solution and 0.5 ml of 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB) reagent were added and absorbance was taken at 412 nm. A standard curve of reduced glutathione was prepared and the concentration of GSH in the supernatant was determined from the standard curve[21].

2.5.2.1.3 Estimation of Nitrite

The production of nitric oxide (NO) in the brain may occur due to oxidative stress and it can be determined by estimation of nitrite level. The nitrite level was determined spectrophotometrically with Griess reagent (0.1% N-1-naphthyl ethylene amine dihydrochloride, 1% sulphanilamide, and 2.5% phosphoric acid). Brain homogenate and Griess reagent were mixed equally and this mixture was incubated for 10 min and the absorbance was measured at 546 nm. The standard curve of sodium nitrite was prepared and the concentration of nitrite in the supernatant was determined from standard curve[22].

2.5.2.1.4 Estimation of Protein

For the estimation of protein content of brain, Lowry method was used. Standard curve was determined using bovine serum albumin[23].

5.2.3 Statistical analysis

Results were analyzed using one way analysis of variance (ANOVA) followed by the tukey's test by using statistical software package, Graph Pad Prism; version 5.03. Values were expressed as mean ± SEM and the $p < 0.05$ were considered as statistically significant.

3 Results and Discussions

The current available drug treatments for Alzheimer's disease and Parkinson possess various side effects. Therefore, herbal therapies should be considered as alternative/complementary medicines for therapeutic approach. In the present study, *Artemisia nilagirica* was selected for the screening of antioxidant, anti-alzheimer and anti-parkinson activity.

3.1 Antioxidant activity

3.1.1 Hydrogen-donating activity

The ethanol and aqueous extracts of *Artemisia nilagirica* strongly scavenged DPPH radical with the IC_{50} being 116.96 and 145.41 µg/ml, respectively (Table 1; Fig 1 and 2). The scavenging was found to dose dependent. The standard drug ascorbic acid scavenged DPPH radical was found to be 90.72.

3.1.2 Superoxide scavenging assay

Superoxide free radical scavenging activity was performed with the ethanol and aqueous extracts of *Artemisia nilagirica* and was expressed as IC_{50} value. The IC_{50} value for ethanol and aqueous extracts of *Artemisia nilagirica* were 84.18 and 138.81, respectively (Table 2; Fig. 3 and Fig. 4). The superoxide radical scavenging activity was found to dose dependent. The standard drug ascorbic acid scavenged superoxide radical with the IC_{50} being 93.14.

From results, it was found that the extracts showed moderately to strongly free radical scavenging activity. The extracts donated their electrons to the superoxide and scavenge them to prevent their further interaction with NBT followed by inhibition of formation of blue colored formazan product[12,13]. The outcomes of results revealed that the ethanol extracts displayed

high content of flavonoids, which was significantly correlated with the superoxide radical scavenging activity.

Table 1: Free radical scavenging capacity of *Artemisia nilagirica* extract

Conc. (µg/ml)	DPPH Scavenging %		
	Ethanol Extract	Aqueous Extract	Ascorbic Acid
50	26.12±0.52	16.24±0.86	90.72±0.14
100	41.05±0.43	32.13±0.41	-
150	63.72±0.82	53.57±0.76	-
200	80.53±0.91	69.72±0.58	-
250	101.31±0.38	86.49±0.23	-
IC_{50}	116.96	145.41	-

Values are mean ± SEM of six determinations

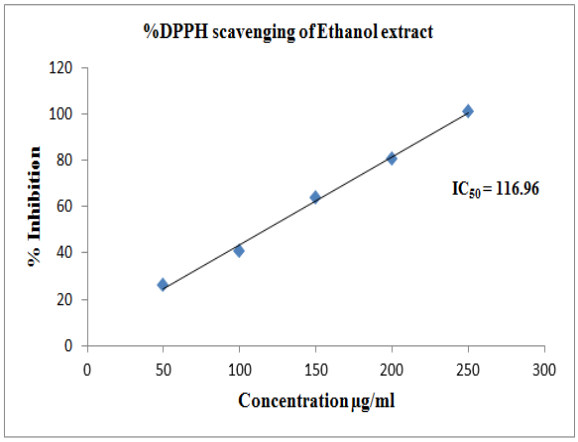

Fig. 1: IC_{50} values of ethanol extracts of *Artemisia nilagirica*

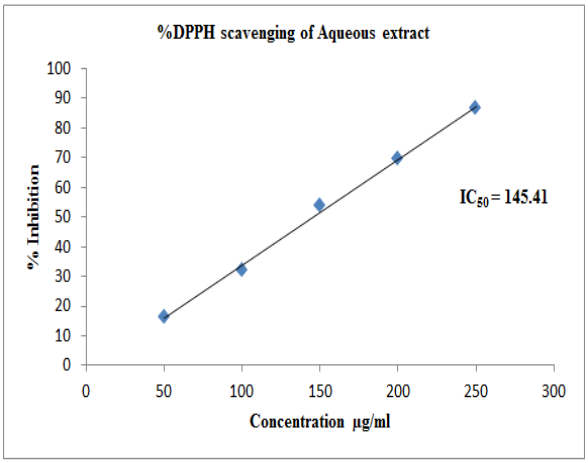

Fig. 2: IC_{50} values of aqueous extracts of *Artemisia nilagirica*

From the result of DPPH and superoxide radical scavenging activity it was observed that the ethanol extracts of *Artemisia nilagirica* showed highest DPPH and superoxide radical scavenging activity. It indicates the presence of antioxidant components in crude extracts of *Artemisia nilagirica*. From these results it can be concluded that antioxidant activity of extracts depends on the presence of quality of active constituents, because each *in vitro* antioxidant model has different mechanism to reduce free radicals.

Table 2: Superoxide scavenging capacity of *Artemisia nilagirica* extract

Conc. (µg/ml)	Superoxide Scavenging %		
	Ethanol Extract	Aqueous Extract	Ascorbic Acid
50	35.16±0.47	22.54±0.63	93.14±0.73
100	58.21±0.32	36.14±0.72	-
150	77.43±0.41	51.86±0.49	-
200	91.38±0.95	69.73±0.68	-
250	115.76±0.18	88.17±0.34	-
IC_{50}	84.18	138.81	-

Values are mean ± SEM of six determinations

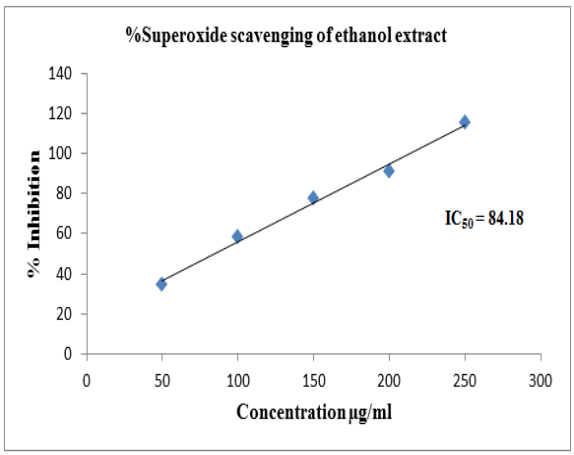

Fig. 3: IC_{50} values of ethanol extract of *Artemisia nilagirica*

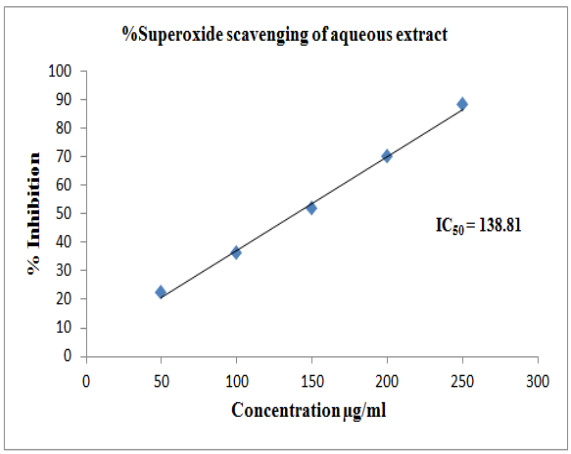

Fig. 4: IC_{50} values of aqueous extract of *Artemisia nilagirica*

Earlier many researchers have reported that the antioxidant activity of extracts is directly proportional to the phenolic and flavonol contents. Following it as a guidelines total flavonol and polyphenol content was determined in ethanol and aqueous extracts.

3.1.3 Total phenolic content

The ethanol and aqueous extract of *Artemisia nilagirica* was evaluated for investigation of the total phenolic content concentrations in extracts. Standard curve of gallic acid was calculated and plotted in distilled water for determining absorption data. From this Beer's law range and regression coefficient was determined. The linear equation of gallic acid was found to be y = 0.0383 x +0.0021 (Fig 5). The total phenolic content of ethanol and aqueous extract of *Artemisia nilagirica* were 79.32 and 56.81 GAE mg/gm, respectively (Table 3). The ethanol extracts exhibited highest amount of total polyphenol content compared to aqueous extracts.

Table 3: Determination of total polyphenol content of *Artemisia nilagirica*

Extract	Total polyphenol content (GAE mg/gm)
Ethanol	79.32±0.59
Aqueous	56.81±0.47

Data expressed as gallic acid equivalent (GAE) mg per gm of the extract, Values are mean ± SEM of triplicate determinations

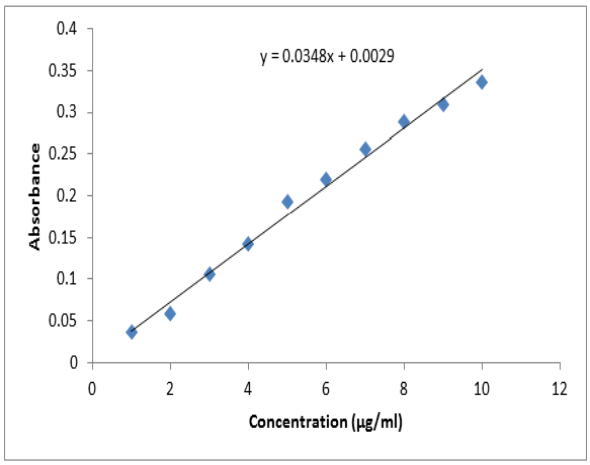

Fig 5: Calibration curve of gallic acid in distilled water

3.1.4 Total flavonol content

The content of flavonoids was expressed in terms of quercetin equivalents. Standard curve of quercetin was calculated and plotted in distilled water for determining absorption data. From this Beer's law range and regression coefficient is determined. The linear equation of quercetin was found to be y = 0.0382 x +0.0097 (Fig 6). The content of flavonoids identified in the tested extracts is shown in table 4. The concentrations of flavonoids in ethanol and aqueous extract of *Artemisia nilagirica* were 56.73 and 41.53 QE mg/gm, respectively. The ethanol extracts exhibited highest amount of flavonoids content compared to aqueous extracts.

Table 4: Determination of total flavonol content of *Artemisia nilagirica*

Extract	Total flavonol content (QE mg/gm)
Ethanol	56.73±0.43
Aqueous	41.53±0.81

Data expressed as quercetin equivalent (QE) mg per gm of the extract, Values are mean ± SEM of triplicate determinations

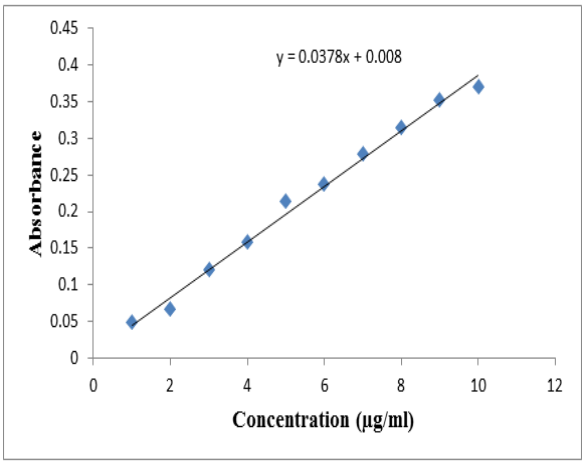

Fig 6: Calibration curve of quercetin in distilled water

It is well documented that plant flavonoids and phenols in general, are greatly effective free radical scavenging and antioxidants. Polyphenol and flavonoids are used for the prevention and cure of various diseases, which are mainly associated with free radicals. The phenolic compounds have been recognized as antioxidant and have been known to show medicinal activity as well as for exhibiting physiological functions. It has been reported that compounds such as the flavonoids, which contain hydroxyl, are responsible for the radical scavenging effects of most plants. The mechanism of action of the flavonoids is through scavenging or chelating processes. It is well known that plant phenolics, in general are highly effective in free radicals scavenging, and they are antioxidants[24].

The findings of total polyphenol and flavonol content of ethanol and aqueous extract of *Artemisia nilagirica* supports the study of DPPH and superoxide scavenging capacity of extracts.

3.1.5 Reducing power assay of

The absorbance value of ascorbic acid was considered to be 100% antioxidant activity. The higher the absorbance of the reaction mixture, the higher would be the reducing power. Table 5 revealed that the antioxidant activity of ethanol and aqueous extract of *Artemisia nilagirica*. The reducing power of the ethanol and aqueous extract of *Artemisia nilagirica* were found to be 55.40% and 37.50%, respectively.

Table 5: Antioxidant activity determination of *Artemisia nilagirica*

Particulars	Absorbance at 700 nm	Antioxidant activity (%)
Ascorbic acid	0.832±0.15	100.00
Ethanol extract	0.461±0.53	55.40
Aqueous extract	0.312±0.21	37.50

Values are mean ± SEM of triplicate determinations

The reducing power of ascorbic acid was found to be higher than ethanol and aqueous extract of *Artemisia nilagirica*. It has been reported that the reducing power of substances is probably because of their hydrogen donating abilit. The ethanol extract of *Artemisia nilagirica* might, therefore, contain high amount of reductions than aqueous extract. The result indicates that extracts act as electron donors and could react with free radicals to convert them into more stable products and then terminate the free radical chain reactions. During study it was found that antioxidant activity was produced due to the presence of phenolic compounds.

The reducing power assay is generally used to estimate the ability of an antioxidant to donate an electron which is an important mechanism of phenolic antioxidant action. It has been documented that the polyphenol and flavonol component present in extract imparts antioxidant activity. The extent of antioxidant activity of extracts is directly proportional to the phenolic and flavonol contents of plant extracts[25]. Hence reducing power assay justify that ethanol extract of *Artemisia nilagirica* contain the maximum amount of the total polyphenol and flavonol. The researchers used the plant extracts containing antioxidant as free radical scavengers to prevent neurodegenerative disorders.

The findings of antioxidants demonstrated that ethanol extract of *Artemisia nilagirica* produces higher antioxidant activity compared to aqueous extract. It can treat the number of oxidative stress induced neurodegenerative disorders with higher efficiency compare to aqueous extract. The *in vitro* antioxidant activity was performed for accurate selection of extracts from *Artemisia nilagirica* for the treatment of Alzheimer's and Parkinson's diseases. Hence the ethanol extract of *Artemisia nilagirica* was selected for screening of *in vivo* Anti-Alzheimer's and Anti-Parkinson's activity.

3.2 Pharmacological activity

The *in vitro* studies of the ethanol and aqueous extract of *Artemisia nilagirica* indicates that ethanol extract incorporating higher quantity of flavonoids and polyphenol. Hence the *in vivo* activities were performed with ethanol extract of *Artemisia nilagirica***.**

3.2.1 Anti-alzheimer's activity

3.2.1.1 Object recognition test

In the object recognition test, the mice spent more time to explore the objects in the first trial (T1 session). In the second trial (T2 session), when a new object replaced a familiar object, ethanol extract of *Artemisia nilgirica* and piracetam significantly reduced the time to explore the familiar object as compared with the time to explore the new object (Table 6). Moreover, ethanol extract of *Artemisia nilgirica* also showed significant increase in discrimination index (Table 7).

Table 6: Effect of *Artemisia nilagirica* on exploration time in Object recognition test in mice

Group	Exploration time (sec)	
	Familiar object	New object
Normal propylene glycol (5 ml/kg)	78.14±2.51	89.23±1.05
Ethanol extract (100 mg/kg)	49.34±1.73*	79.38±2.47
Ethanol extract (200 mg/kg)	35.48±2.18*	71.64±2.31
Piracetam (100 mg/kg)	28.61±1.24*	70.47±1.83

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with normal control group

Table 7: Effect of *Artemisia nilagirica* on discrimination index in Object recognition test in mice

Group	Discrimination index
Normal propylene glycol (5 ml/kg)	0.21±0.02
Ethanol extract (100 mg/kg)	0.52±0.46*
Ethanol extract (200 mg/kg)	0.69±0.72*
Piracetam (100 mg/kg)	0.73±0.39*

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with normal control group

3.2.1.2 Y-Maze test

In the Y-maze test the animals treated with the extract in tested doses have shown a marked decrease in exploratory behaviour compared with controls (Table 8). Thus, ethanol extract of *Artemisia nilgirica* showed significant decrease in exploratory behaviour indicating facilitator action on learning and memory.

The results of object recognition test and Y-maze test confirmed the Anti-alzheimer's activity of ethanol extract of *Artemisia nilgirica*.

The high metabolic activity of nervous tissues attached with lipid present, and leads to susceptible to oxidative damage. Additionally, catecholamines present in brain showed more sensitive for production of free radical. The catecholamines such as adrenaline, noradrenaline and dopamine can spontaneously break down (auto-oxidize) to free radicals, or can be metabolized to radicals by the endogenous enzymes such as monoamine oxidase. The antioxidants studies revealed that flavonoids containing substance protect nervous tissue from damage by oxidative stress. Consequently, clinical studies exhibit that Alzheimer's are accomplished of exciting the generation of free radicals and depletion of antioxidant levels[22-4].

The reactive oxygen species imparts chief role in the pathogenesis of Alzheimer's diseases. Various researches reported that antioxidant containing plants are neuroprotective and hence may have a role in improving memory in aging and neurodegenerative diseases. The Y-maze task and object recognition test is a specific and sensitive test of spatial recognition memory in experimental animals. The animals treated with ethanol extract of *Artemisia nilgirica* showed significant cognitive improvement as shown by the decrease in transfer latency in Y-maze test and increase in discrimination index in object recognition test[25,26]. Thus, ethanol extract of *Artemisia nilgirica* has a neuroprotective effect and hence may have a role in improving cognition. It suggests the Anti-alzheimer's activity of *Artemisia nilgirica* is due to presence of flavonoid and polyphenol component.

3.2.2 Anti-parkinson activity

3.2.2.1 Catalepsy study

All the animals were evaluated for catalepsy using bar test for 2–4 sec during weekly observation and at last on the 21st day of treatment. The cataleptic behavior (inability to correct abnormal posture) of Chlorpromazine treated animals was found to increase significantly every weak compared to vehicle treated normal control group anials. The animals pretreated with the ethanol extract of *Artemisia nilgirica* showed a significant decrease in catalepsy score compared to Chlorpromazine treated animals (Table 9). The animals treated with levodopa and carbidopa group significantly prevented the increase in catalepsy compared to Chlorpromazine treated animals on the 21st day.

Table 8: Effect of *Artemisia nilagirica* on Y-Maze test in mice

Group	Time			
	30 min	60 min	90 min	120 min
Normal propylene glycol (5 ml/kg)	9.23±0.32	9.43±0.23	9.45±0.43	9.12±0.23
Ethanol extract (100 mg/kg)	6.56±0.12	6.45±0.02	6.23±0.23	6.98±0.43
Ethanol extract (200 mg/kg)	5.76±0.08*	5.43±0.12*	5.34±0.23*	5.65±0.07*
Diazepam (10 mg/kg)	3.56±0.08*	3.45±0.02*	3.78±0.03*	3.26±0.06*

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with normal control group

Table 9: Effect of *Artemisia nilagirica* on chlorpromazine induced catalepsy in mice

Group	Time (sec)			
	0 Day	7th Day	14th Day	21st Day
Normal propylene glycol (5 ml/kg)	1.9±0.14	2.2±0.35	2.1±0.19	2.3±0.47
Chlorpromazine (3 mg/kg)	3.4±0.43	9.8±0.18*	15.7±0.91*	19.2±0.57*
Ethanol extract (100 mg/kg)	2.8±0.81	5.3±0.25[a]	8.6±0.47[a]	9.5±0.61[a]
Ethanol extract (200 mg/kg)	2.5±0.52	4.9±0.46[a]	7.2±0.64[a]	7.9±0.49[a]
Carbidopa + levodopa (10 mg/kg)	2.2±0.36	4.1±0.68[a]	6.1±0.24[a]	6.5±0.73[a]

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with normal control group; significantly different at [a]P<0.05 when compared with Chlorpromazine treated group

3.2.2.2 Locomotor activity (Actophotometer test)

Locomotor activity of animals was evaluated using actophotometer. Locomotor activity of vehicle treated normal control group was found to be 72–76 counts/5 min for all the four weeks of treatment. The administration of Chlorpromazine to animals demonstrated significant decrease in the locomotor

activity compared to vehicle treated normal control group animals. The animals pretreated with the ethanol extract of *Artemisia nilgirica* showed significant changes in locomotor activity compared to Chlorpromazine treated animals (Table 10) after first week. The animals treated with levodopa and carbidopa group significantly increases the locomotor activity compared to Chlorpromazine treated animals on the 21st day.

3.2.2.3 Muscle activity (Rotarod test)

Muscle rigidity of animals was evaluated by using the rotarod apparatus. The mean fall-off time of vehicle treated normal control group animals from the rotarod was found to be 119–121 seconds during weekly observation of the treatment. The administration of Chlorpromazine to animals demonstrated significant decrease in the rotarod readings (muscle rigidity) compared to vehicle treated normal control group animals. The animals pretreated with the ethanol extract of *Artemisia nilgirica* showed significant increase in rotarod readings compared to Chlorpromazine treated animals (Table 11) after first week. The animals treated with levodopa and carbidopa group significantly increases the decreases in rotarod readings compared to Chlorpromazine treated animals on the 21st day.

Table 10: Effect of *Artemisia nilagirica* on chlorpromazine induced hypolocomotion in mice

Group	Locomotor activity (counts/min)			
	0 Day	7th Day	14th Day	21st Day
Normal propylene glycol (5 ml/kg)	73.1±1.02	75.6±0.84	72.3±0.1.47	76.5±1.53
Chlorpromazine (3 mg/kg)	70.6±1.35	51.2±1.09*	39.1±0.81*	28.4±1.36*
Ethanol extract (100 mg/kg)	74.8±0.73	65.1±0.64	55.8±0.43[a]	51.7±1.28[a]
Ethanol extract (200 mg/kg)	71.5±1.42	68.2±1.38[a]	61.2±0.69[a]	59.4±1.61[a]
Carbidopa + levodopa (10 mg/kg)	72.9±0.59	70.4±1.21[a]	68.7±1.39[a]	65.2±1.74[a]

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with normal control group; significantly different at [a]P<0.05 when compared with Chlorpromazine treated group

Table 11: Effect of *Artemisia nilagirica* on chlorpromazine induced muscle rigidity in mice

Group	Mean fall-off time (sec)			
	0 Day	7th Day	14th Day	21st Day
Normal propylene glycol (5 ml/kg)	121.4±1.63	119.7±1.08	121.2±1.86	120.7±1.54
Chlorpromazine (3 mg/kg)	114.3±1.28	88.5±1.86*	71.1±1.42*	48.4±1.53*
Ethanol extract (100 mg/kg)	116.7±1.43	97.9±1.38[a]	91.2±1.63[a]	82.7±1.25[a]
Ethanol extract (200 mg/kg)	119.4±1.62	101.2±1.39[a]	95.8±1.79[a]	89.4±1.08[a]
Carbidopa + levodopa (10 mg/kg)	118.3±0.85	108.7±1.49[a]	97.1±1.31[a]	92.5±1.57[a]

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with normal control group; significantly different at [a]P<0.05 when compared with Chlorpromazine treated group

3.2.2.4 Biochemical estimation

Lipid Peroxidation Assay (TBARS)

The animals treated with Chlorpromazine showed significant increases in TBARS level in brain compared to normal control group animals. The TBARS level in animals treated with ethanol extract of *Artemisia nilgirica* and standard drug was significantly decreased compared to Chlorpromazine treated animals (Table 12).

Estimation of Reduced Glutathione (GSH)

The animals treated with Chlorpromazine showed a significant decrease in GSH levels in brain compared to normal control group animals. The GSH level in animals treated with ethanol extract of *Artemisia nilgirica* and standard drug was significantly increased compared to Chlorpromazine treated animals (Table 12).

Estimation of Nitrite

The animals treated with Chlorpromazine showed significant increases in nitrite level in brain compared to normal control group animals. The nitrite level in animals treated with ethanol extract of *Artemisia nilgirica* and standard drug was significantly decreased compared to Chlorpromazine treated animals (Table 12).

Estimation of Protein

The animals treated with Chlorpromazine showed a significant decrease in protein levels in brain compared to normal control group animals. The protein level in animals treated with ethanol extract of *Artemisia nilgirica* and standard drug was significantly increased compared to Chlorpromazine treated animals (Table 12).

Table 12: Effect of *Artemisia nilagirica* on the different parameters in mice brain

Group	GSH level (nM/mg protein)	Nitrite level (nM/mg of protein)	Total protein level (mg/mL)	TBARS level (nM/mg protein)
Normal propylene glycol (5 ml/kg)	6.28±0.63	2.05±0.18	14.29±0.51	1.24±0.19
Chlorpromazine (3 mg/kg)	1.15±0.42*	9.47±0.61*	4.58±0.41*	8.63±0.38*
Ethanol extract (100 mg/kg)	5.02±0.31[a]	3.21±0.17[a]	11.18±0.38[a]	2.38±0.12[a]
Ethanol extract (200 mg/kg)	5.68±0.49[a]	2.96±0.57[a]	12.42±0.65[a]	2.01±0.96[a]
Carbidopa + levodopa (10 mg/kg)	6.72±0.68[a]	2.83±0.43[a]	13.63±0.83[a]	1.92±0.11[a]

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with normal control group; significantly different at [a]P<0.05 when compared with Chlorpromazine treated group

Parkinson's disease is a chronic neurodegenerative disorder characterized by loss of dopamine neurons of the SNpc. The pathogenesis of Parkinson's disease includes oxidative stress, protein accumulation like a-synuclein, mitochondrial dysfunction, apoptosis, and neuronal excitotoxicity. Among all, oxidative stress is a crucial pathological mechanism for Parkinson's disease. SNpc is more vulnerable to reactive oxygen species as it contains more amount of dopamine.

Chlorpromazine is one of the antipsychotic drugs listed as essential drugs by WHO in 2003 to treat both acute psychosis and chronic psychosis. It has been associated with various side effects. Chronic treatments with Chlorpromazine increase the dopamine receptor binding site in neostriatum and in mesolimbic region, which could account for dopamine hypersensitivity that induced tardive dyskinesia. Chlorpromazine induced Parkinsonism by interfering with the storage of catecholamines in intracellular granules which may cause monoamine depletion in nerve terminals and in the induction of hypolocomotion and muscular rigidity[26-29]; thus, Chlorpromazine was produced by the Parkinson disease-like symptoms followed by chronic treatments of rats for 21 days. It results in an increase level of oxidative stress that may cause the reduction in antioxidant enzymes which were also seen with the atypical agent's ziprasidone, risperidone, and olanzapine. Except olanzapine both typical and atypical agents increase lipid peroxidation after chronic dosing. There was a significant increase in catalepsy, a decrease in movements, and a decrease in body weight following chlorpromazine administration to rats.

The present data suggested that chlorpromazine developed Parkinson's disease-like behavioral symptoms in rats. The oxidative stress was measured through determination of levels of TBARS, reduced glutathione, and nitrite level in brain tissue. Lipid peroxidation is a sensitive marker of oxidative stress. Lipid peroxidation occurs due to attack by radicals on double bond of unsaturated fatty acid and arachidonic acid which generate lipid peroxyl radicals. These radicals have further attacks on other unsaturated fatty acids. Increased levels of the lipid peroxidation product have been found in the substantia nigra of Parkinson's disease patients. In the present study the same result was observed in the brain homogenates of chlorpromazine treated control animals. Brain protects against oxidative stress by SOD, catalase, and glutathione peroxidase and thus these antioxidant enzymes protect brain from neurodegeneration. Glutathione peroxidase protects brain from neurodegeneration by scavenging H_2O_2 generated by cellular metabolism and balance formation and decomposition of H_2O_2 in normal condition[30-32]. It is obvious that reduced glutathione is the limiting factor in the removal of H_2O_2.

Neuronal cell loss may cause the depletion of reduced glutathione in the substantia nigra in Parkinson disease. Nitric oxide production can be determined by nitrite determinations in biological material. Nitric oxide has been involved in the cytotoxicities by activation of macrophages or excess stimulation of neurons by glutamate. In further study on glutamate stimulation causes neurotoxicity in primary cultures of rat fetal cortical, striatal, and hippocampal neurons[33-35]. Chlorpromazine group showed a significant increase in the level of TBARS and gradual decrease in GSH levels in brain as compared control group. All observations showed that chlorpromazine increases the oxidative stress in the brain of animals. The administration of ethanol extract of *Artemisia nilgirica* were used in the chlorpromazine model in rats; both doses were found to be significant in reducing the catalepsy, increasing the locomotor activity (actophotometer), and increasing the muscle activity (rotarod test) in a chlorpromazine model of Parkinson in mice which indicates ethanol extract of

Artemisia nilgirica has potential effects against Parkinson's disease-like symptoms produced in various experimental models.

The antioxidative properties of ethanol extract of *Artemisia nilgirica* reduced the duration of the catalepsy that decreased the elevated levels of lipid peroxidation in the chlorpromazine treated animals.

4 Conclusion

The leaves with flowering tops of *Artemisia nilagirica* extract were evaluated for anti-alzheimer and anti-parkinson activity. The ethanol extract of *Artemisia nilagirica* demonstrated significant anti-alzheimer and anti-parkinson activity. The findings of *in vitro* antioxidant confirmed the neuroprotective effect of ethanol extract of *Artemisia nilgirica* is due to presence of high phenolic and flavonoid content.

5 Conflict of interest

The authors declared that there are no conflicts of interest.

6 Author's contributions

PP and AKG performed the experimental work and draft the manuscript.

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