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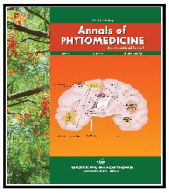

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Antioxidant, anti-inflammatory and anti-neurodegenerative activities of *Jatropha integerrima* Jacq. floral methanolic extract

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Abstract

The medium sized drought-tolerant perennial flowering shrub, *Jatropha integerrima* Jacq. which belongs to the family Euphorbiaceae, have traditionally been used to treat various conditions like herpes, pruritis, toothaches, rheumatism, scabies, tumors and eczema. The study presented here was to find out the antioxidant, anti-inflammatory, and anti-neurodegenerative potentials of the methanolic extract of the flowers of the plant. The phytocompounds present in the methanolic extract of *J. integerrima* flowers were investigated using standard biochemical procedures. The antioxidant capability was examined using DPPH scavenging assay, FRAP and H_2O_2 scavenging assay and the anti-inflammatory potential was found out by albumin denaturation and heat-induced hemolysis methods. To find out the anti-neurodegenerative activity, the flower extract was subjected to the acetylcholine esterase inhibition and tyrosinase assays. *J. integerrima* flower methanolic extract displayed the presence of saponins, flavonoids, phenols, quinone and proteins. The extract also showed promising concentration-dependent antioxidant activities and potentials against albumin denaturation and heat induced hemolysis as evidence of its anti-inflammatory properties. The flower extract was also able to effectively inhibit the acetylcholine esterase and tyrosinase in the *in vitro* assays showing its anti-neurodegenerative capabilities. So, the authors recommend for further investigations to make use of the promising antioxidant, anti-inflammatory and anti-neurodegenerative potentials of the *J. integerrima* flowers to develop it for medicinal applications.

1. Introduction

Mankind has reaped enormous benefits by utilizing medicinal plants in the treatment of disease as they are generally safer, more accessible and occasionally have improved treatment effectiveness than synthetic pharmaceuticals. Several conventional natural products are now garnering more attention and so the therapeutics derived from plants is still a valuable resource, particularly in developing nations, for treating serious illnesses. Herbal remedies are still used to treat common ailments by between 62 and 80% of the world's population (Zhang, 2004).

The Euphorbiaceae family, one of the largest families in the Angiosperms, contains over 7,800 species that are dispersed throughout about 300 genera and five subfamilies in different parts of the world. These species favour tropical and subtropical regions as their natural habitats (Webster, 1994; Alves 1998). *Jatropha* is a genus belonging to the family Euphorbiaceae comprising varieties of succulent plants, trees, and shrubs. The genus *Jatropha* additionally serves as a significant source of phytochemicals that can be used in the food, medicinal, and agricultural sectors. Extracts from several parts of the *Jatropha* plant, including the roots, stem, leaves and bark have traditionally been utilized in folk medicines (Sharma and Singh, 2012).

Jatropha species have been used in folkloric traditional medicine to treat a variety of maladies in Latin America, Asia and Africa as energy crops and decorative plants. Its use as a traditional healthcure is most prevalent among four fifth of the global population in Latin America, Asia and Africa, and it has also been shown to have fewer adverse effects. The seed oil from *Jatropha* species is renowned for its purgative properties and so it has been designed to treat disturbances in the digestive system including dysentery, diarrhoea, stomach-ache, and vomiting (Sabandar *et al.,* 2013). Moreover, the *Jatropha* plant has anti-inflammatory compounds that can be used to treat urine discharge, reduce skin inflammation, and treat venereal illnesses. Several compounds from this genus have been isolated, including coumarins, cyclic peptides, alkaloids, flavonoids, lignans, and terpenes (Mohammed *et al.,* 2021).

Jatropha integerrima Jacq. is a medium sized drought-tolerant perennial belonging to the family Euphorbiaceae and it flowers through the entire year making it a significant decorative species planted in tropical and subtropical regions for its magnificent crimson blooms. The flowers of *J. integerrima* seemed to have a significant antioxidant property among the wild and edible flowers in China (Xu *et al.,* 2015) and the traditional uses of different parts of *J. integerrima* include purgative, styptic, and emetic properties for

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the treatment of tumors, herpes, warts, pruritis, toothaches, rheumatism, scabies, ringworm and eczema. The latex of *J. integerrima* has exhibited anticancer action, and its leaves and branches have been proven to possess cholinesterase activity (Kolawole *et al.,* 2017).

Therefore, considering all those benefits of *J. integerrima*, the present investigation was conducted a study to find out the antioxidant, anti-inflammatory, and anti-neurodegenerative potentials of the methanolic extract of *J. integerrima* flowers.

2. Materials and Methods

2.1 Sample collection

Jatropha integerrima Jacq. flowers were obtained from a local nursery. The collected flowers were washed with distilled water and then dried at room temperature in the shade. It was then powdered with a mechanical blender and stored till the extraction in an air-proof container.

2.2 Extract preparation

The ground *J. integerrima* flowers (20 g) was placed in "thimble" which was placed in the Soxhlet thimble chamber apparatus. 200 ml of the solvent methanol was added to the extractor and at a temperature of 60°C, the system was left for a period of 6 h. After the solvent evaporation, the extract was collected in sterile containers.

2.3 Phytochemical screening

To detect the presence of phytochemicals (like phenols, tannins, catechins, saponins, alkaloids, flavonoids, triterpenes, flavanols, reducing sugars, steroids, xanthones and flavanones), a preliminary screening was performed as per the standard protocols (Jyothiprabha and Venkatachalam, 2016; Boggula and Peddapalli, 2017).

2.4 Antioxidant activity

2.4.1 DPPH radical scavenging assay

The DPPH radical (1,1-Diphenyl-2-Picrylhydrazyl) scavenging potential of the methanolic extract of *J. integerrima* flowers was measured as per the protocol of Formagio *et al.* (2014). The extract in various concentrations (25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml, 250 µg/ml, 500 µg/ml, 750 µg/ml and 1000 µg/ml) was mixed with 0.1 mM methanolic DPPH solution (3 ml). The test tubes containing the reaction mixtures prepared were incubated for a period of 30 min at room temperature and the absorbance (OD values) was read against the blank using a UV Vis spectrophotometer at a wavelength of 517 nm.

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{(\text{Absorbance of Sample})} \times 100$$

2.4.2 Ferric reducing antioxidant power (FRAP) assay

Ferric reducing antioxidant power assay (FRAP) was done to estimate the reducing potentials of *J. integerrima* flowers. 3 ml of the FRAP reagent solution which consisted of 10/mM 2,4,6-Tri-(2-pyridyl)-5-triazine) (TPTZ), 300/mM sodium acetate buffer and 20/ mM $FeCl_3.6H_2O$ in a 1:10:1 ratio was added to the varying aliquots of methanolic extract of *J. integerrima* flowers. The reaction mixture prepared was incubated at 37°C for a 30 min period and the absorbance was measured at the wavelength of 610 nm spectrophotometrically (Benzie and Strain, 1996).

2.4.3 Hydrogen peroxide scavenging assay

The capability of the methanolic extract of *J. integerrima* flowers to scavenge hydrogen peroxide (H_2O_2) was fanout as per the standard protocol (Ruch *et al.,* 1989). Different concentrations of methanolic extract of *J. integerrima* flowers were taken in each test tube and mixed with H_2O_2 solution (40 mM), which was prepared using phosphate buffer (0.1 M, pH 7.4). The optical density was determined against the blank at 230 nm.

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{(\text{Absorbance of Sample})} \times 100$$

2.5 Anti-inflammatory potentials

2.5.1 Albumin denaturation assay

The albumin denaturation assay was performed as per the protocols presented by Bougandoura *et al.* (2016) with a slight modification. Various aliquots of methanolic extract of *J. integerrima* flowers were mixed with 3 ml of 5% aqueous bovine albumin. The reaction mixture was incubated for a period of 20 min at a temperature of 37°C and then heated for 20 min at 50°C. The test tubes were allowed to reach to the room temperature and then the OD values at 660 nm were noted.

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{(\text{Absorbance of Sample})} \times 100$$

2.6 Heat-induced hemolysis assay

RBC suspension at 10% v/v was made with normal saline using previously described procedures (Sakat *et al.,* 2010).

The heat-induced hemolysis assay was performed to find out the anti-inflammatory activities of the methanolic extract of *J. integerrima* flowers, as per the protocols described by Obluchinskaya *et al.* (2022) with minor modifications. 3 ml of RBC solution was mixed with various concentrations of *J. integerrima* flowers methanolic extract, followed by incubation for 30 min at 55^0C. The prepared reaction mixture was then centrifuged for 10 min and then the supernatant was collected. The OD values of the collected supernatant was determined at λ=560 nm.

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{(\text{Absorbance of Sample})} \times 100$$

2.7 Anti-neurodegenerative activity

2.7.1 Acetylcholinesterase (AChE) inhibition assay

The reaction mixture comprising 20 µl of DTNB (5,5'-dithio-bis (2-nitrobenzoic acid) solution, 140 µl of 0.1 mol^{-1} sodium phosphate buffer (pH 7) and 20 µl of 5 Uml^{-1} acetylcholinesterase solution in 20 mmol l^{-1} Tris-HCl buffer (pH 7.5) was prepared. Different aliquots of methanolic extract of *J. integerrima* flowers (25, 50, 75,100, 250, 500, 750, and 1000 µg/ml) were added to the each of the test tube containing the prepared reaction mixture and the optical

densities were measured at the wavelength of 412 nm (Ellman *et al.,* 1961).

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{(\text{Absorbance of Sample})} \times 100$$

2.7.2 Tyrosinase inhibition assay

The tyrosinase inhibitory activity of the methanolic extract of *J. integerrima* flowers was examined using slightly modified method explained by Masuda *et al.* (2005). Different concentrations of test extract were mixed with 80 µl of 0.1 mol l^{-1} sodium phosphate buffer (pH 7), and 40 µl of 46 Ul^{-1} tyrosinase solution. L-DOPA buffer solution (40 µl) was added to each test tube respectively and then incubated at a temperature of 25^0C for a period of 30 min. Then, the absorbance values were measured at λ = 475 nm.

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{(\text{Absorbance of Sample})} \times 100$$

3. Results

3.1 Phytochemical analysis

The preliminary analysis for the phytochemical of the methanolic extract of *Jatropha integerrima* Jacq. flowers showed the existence of phytochemicals such as flavonoids, saponins, phenols, quinone, and protein, whereas the steroids, glycosides and alkaloids were absent. The results are given in Figure 1 and Table 1.

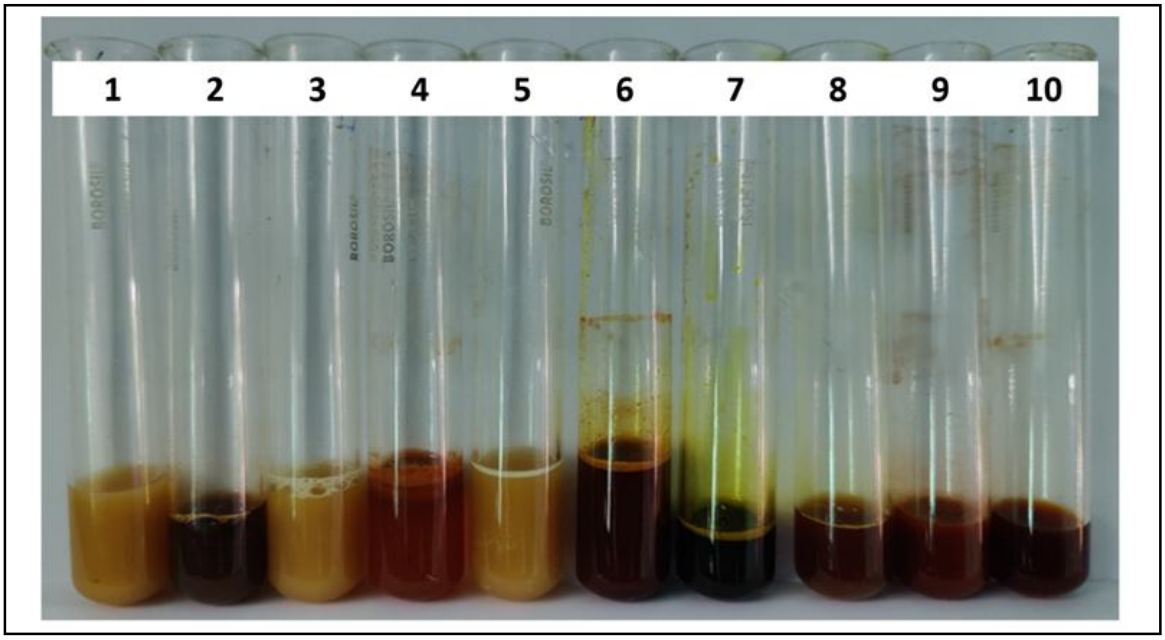

Figure 1: Phytochemical screening of the methanolic extract of *Jatropha integerrima* Jacq. flowers.

Table 1: Phytochemical screening of the methanolic extract of *J. integerrima* flowers

Phytochemicals	Methanolic extract of *J. integerrima* flowers
Tannins	+
Flavonoids	+
Saponins	+
Steroids	-
Terpenoids	+
Glycosides	-
Phenols	+
Alkaloids	-
Quinones	+
Proteins	+

3.2 Antioxidant activity

3.2.1 DPPH radical scavenging assay

The methanolic extract of *J. integerrima* flowers exhibited concentration-dependent DPPH radical scavenging activity. The maximum inhibition percentage obtained was 89.28% at the extract concentration of 1000 µg/ml, whereas the minimum inhibition percentage was found to be 16.89% (Figure 2 and Table 2). The IC_{50} value of *J. integerrima* flowers methanolic extract was 4.67 µg/ml.

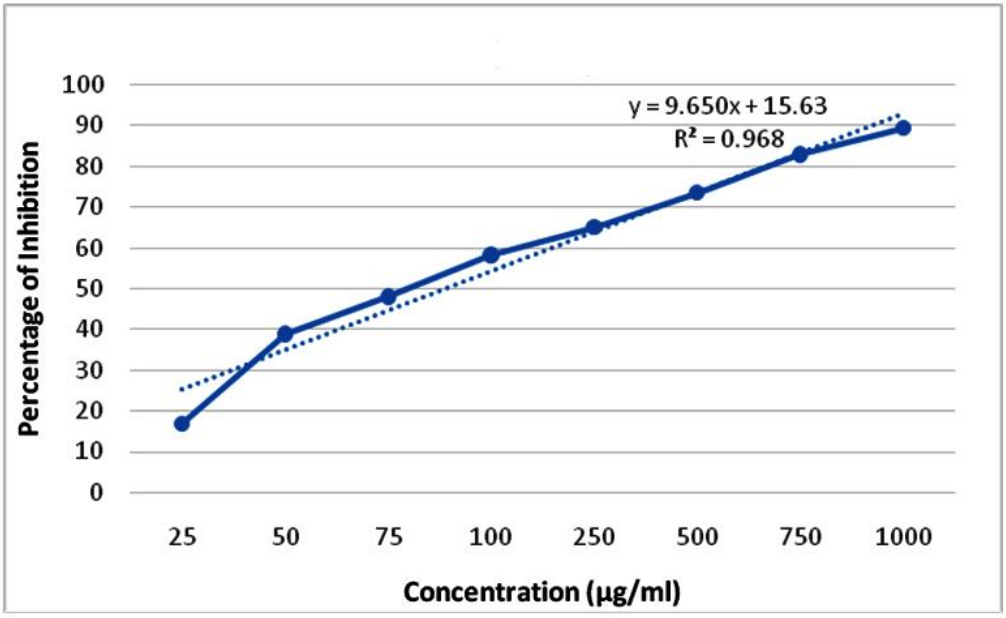

Figure 2: DPPH scavenging assay.

3.2.2 Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power of eight different concentrations of *J. integerrima* flower extract was depicted in the Figure 3 and Table 2. The highest FRAP value recorded was 49.78% (IC_{50} = 4717.6 µg/ml).

Table 2: Antioxidant activity of methanolic extract of *J. integerrima* flowers

Concentration (µg/ml)	Percentage of inhibition		
	DPPH	FRAP	H_2O_2
25	16.89	7.18	11.19
50	38.79	11.81	19.17
75	47.96	17.71	26.19
100	58.12	20.90	39.22
250	65.08	24.91	46.99
500	73.47	27.79	52.71
750	82.87	41.6	67.00
1000	89.28	49.78	76.89
IC_{50} (µg/ml)	4.67	4717.6	2799.9

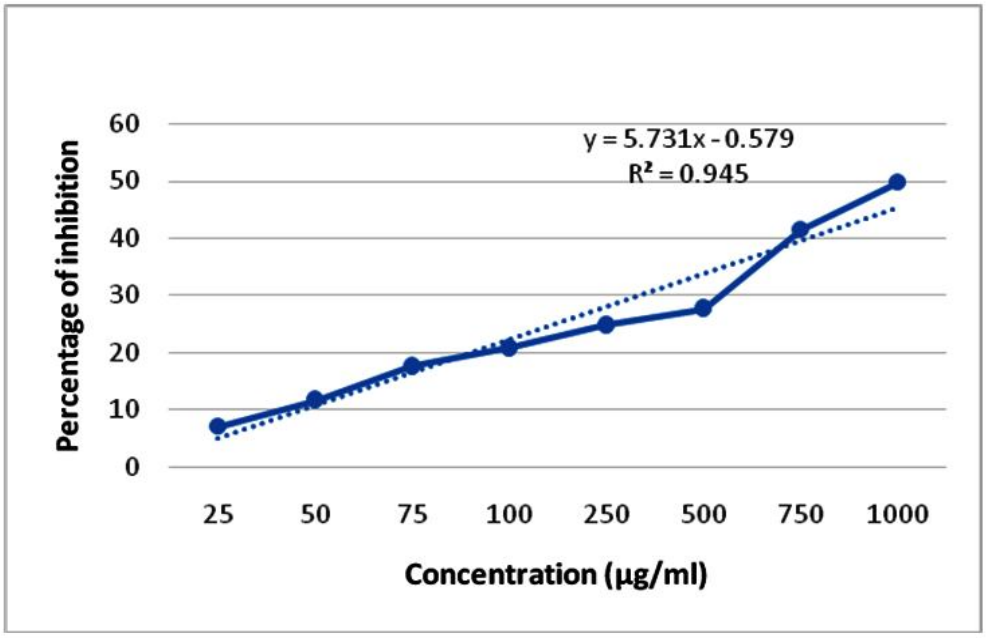

Figure 3: FRAP assay.

3.2.3 Hydrogen peroxide scavenging assay

The data showed that the methanolic extract of *J. integerrima* flowers displayed a concentration related to hydrogen peroxide radical scavenging activity, as shown in Figure 4. The highest percentage of inhibition was found to be 67% at 1000 µg/ml of *J. integerrima* flower extract, and the lowest inhibition percentage was 11.19% at the concentration of 25 µg/ml.

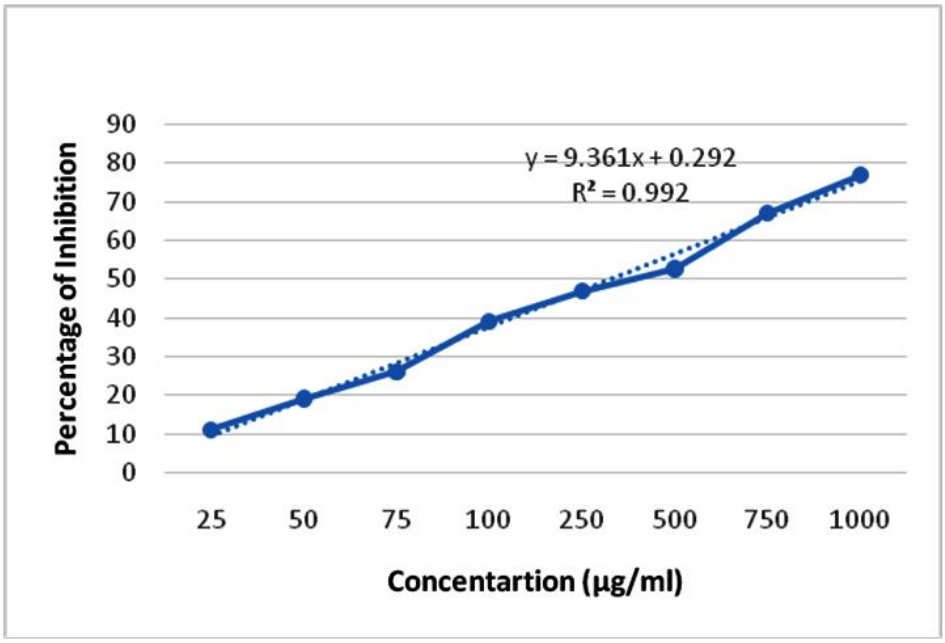

Figure 4: Hydrogen peroxide scavenging activity.

3.3 Anti-inflammatory activity

3.3.1 Albumin denaturation assay

The protein albumin was denatured by the methanolic extract of *J. integerrima* flowers in a concentration-dependent manner. The maximum inhibitory percentage obtained was 51.03% for the 1000 µg/ml concentration of *J. integerrima* extracts. The IC_{50} value was determined to be 104.42 µg/ml.

3.4 Heat-induced hemolysis assay

The anti-inflammatory capability of *J. integerrima* flowers methanolic extract was found out by heat-induced hemolysis has been given in Figure 5 and Table 3. All the tested concentrations have inhibited the heat-induced hemolysis and the percentage of inhibition was found to be enhancing upon an increase in concentration. The highest inhibitory percentage was obtained to be 41.37%.

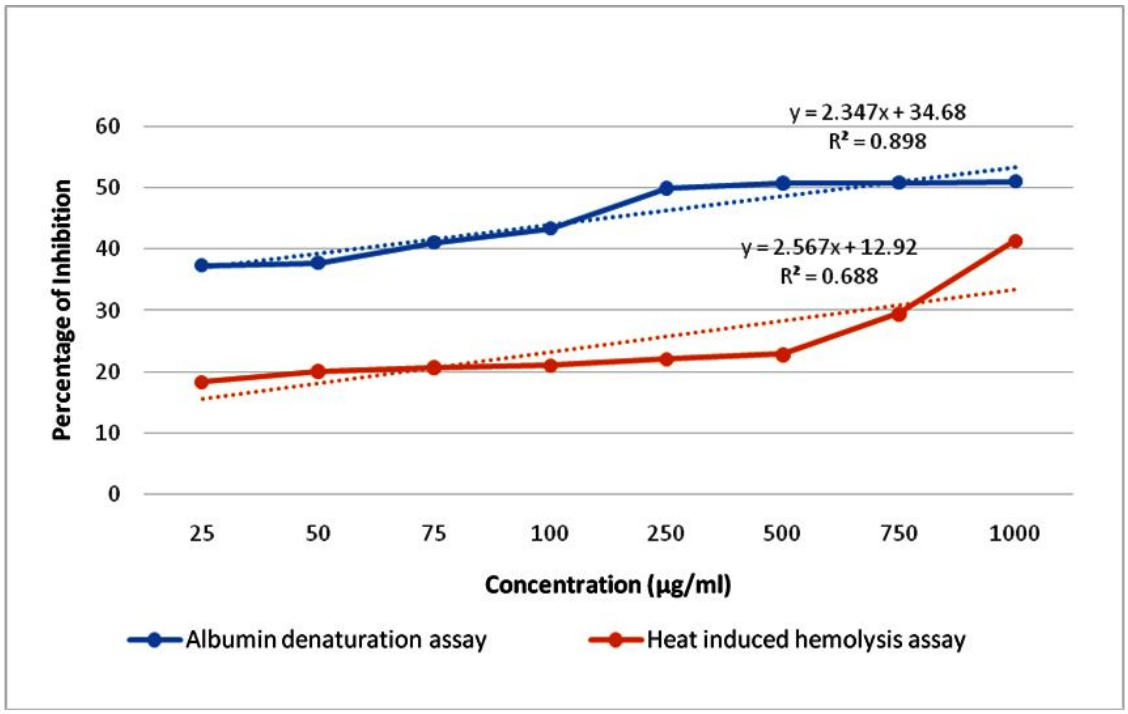

Figure 5: Anti-inflammatory activity of *Jatropha integerrima* Jacq. flowers.

Table 3: Anti-inflammatory activity of *J. integerrima* flowers

Concentration (µg/ml)	Percentage of inhibition	
	Albumin denaturation assay	Heat-induced hemolysis assay
25	37.36	18.35
50	37.70	20.04
75	41.06	20.69
100	43.32	21.07
250	49.93	22.02
500	50.75	22.82
750	50.86	29.47
1000	51.03	41.37
IC_{50} (µg/ml)	104.42	1851.24

3.5 Anti-neurodegenerative activity

3.5.1 Acetylcholinesterase (AChE) inhibition assay

The methanolic extract of *J. integerrima* flowers showed moderate inhibition against acetylcholinesterase (IC_{50} value = 442.82 µg/ml). The most potent inhibitory percentage was obtained at the concentration of 1000 µg/ml (53.23%), and the least inhibitory percentage was determined to be 0.21% at the concentration of 25 µg/ml. the result has been shown in Figure 6, Table 4.

3.5.2 Tyrosinase inhibition assay

All the tested concentrations of methanolic extract of *J. integerrima* flowers effectively inhibited the enzyme Tyrosinase with the IC_{50} value of 86.20 µg/ml (Figure 6, Table 4). The maximum tyrosinase inhibitory value recorded was 61.67%.

Table 4: Anti-neurodegenerative activity of *J. integerrima* flowers

Concentration (µg/ml)	Percentage of inhibition	
	Acetylcholinesterase (AChE) inhibition assay	Tyrosinase inhibition assay
25	0.21	3.28
50	0.86	11.84
75	1.93	22.36
100	5.34	28.38
250	17.98	47.63
500	36.38	48.20
750	48.26	57.32
1000	53.23	61.67
IC_{50} (µg/ml)	442.82	86.20

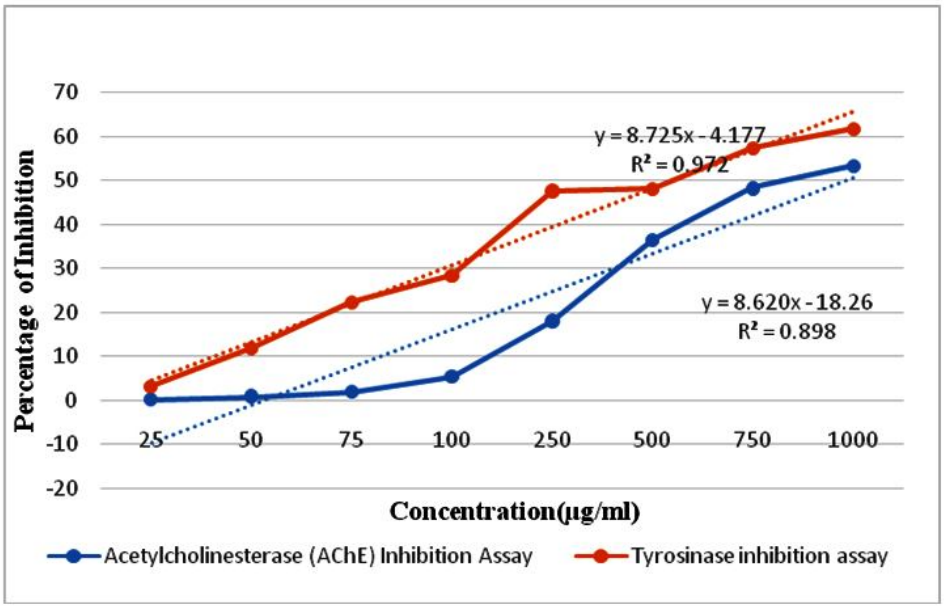

Figure 6: Antineurodegenerative activity of *Jatropha integerrima* Jacq. flowers.

4. Discussion

Native inhabitants in tropical and subtropical countries have employed the *Jatropha* species as medicinal plants. *Jatropha* genus pharmacological research has shown relationships between secondary metabolites and a variety of bioactivities, including antimalarial, antibacterial, antiparasitic, antispasmodic, anticarcinogenic, antioxidant, analgesic, gastroprotective and cytotoxic properties (Cavalcante *et al.,* 2020).

The present study could reveal the presence of phytochemicals such as saponins, phenols, flavonoids, quinone and protein in the methanolic floral extract of *J. integerrima*. The analysis for potential phytochemicals in the aqueous extract of *J. integerrima* flowers showed the existence of phenol, protein, saponin, anthocyanin, tannin, carbohydrate, coumarin, and glycoside (Suriyakala *et al.,* 2022), whereas the alkaloids were absent, as reported in the current study. The work done by Kuspradini *et al.* (2016), quantified the phenol, flavonoid, and anthocyanin content of the *J. integerrima* flowers extracted using ethanol and 1% HCl - ethanol mixture. However, alkaloids and steroids were reported in the methanol and ethyl acetate extract of *J. integerrima* leaves (Rampadarath *et al.,* 2014).

The concentration-dependent manner antioxidant activity was observed for the methanolic extract of *J. integerrima* flowers in DPPH, FRAP, and H_2O_2 assay. Likewise, the IC_{50} value obtained for the ethanol and 1% HCl-ethanol extract of *J. integerrima* flowers was found to be 11.67 and 119.65 ppm, respectively (Kuspradini *et al.,* 2016). The FRAP value of the water-soluble as well as fat-soluble fractions of *J. integerrima* flowers were reported to be 148.52 ± 6.11 and 71.42 ± 3.97 $\mu lmol^{-1}$ Fe (II)/g, respectively (Li *et al.,* 2014). Interestingly, the methanol and aqueous extract of *J. multifida* flowers effectively scavenged the DPPH radicals (Narayanaswamy and Balakrishnan, 2011). The *J. integerrima* flower extracts displayed the highest activity of 79 ± 0.15% for DPPH radical scavenging capacity (Vankar and Srivastava, 2010). The antioxidant potentials of the ethanolic extract of leaves of *J. curcas* and *J. multifida* was examined as DPPH radical scavenging and reducing power capability (MK and Louis, 2017) and found to be effective. The study of Oloyede *et al.,* (2012), proved that the aqueous of the *J. curcus* leaves inhibited the free radicals such as DPPH (IC_{50} value = 21.24 mg/ml), and hydrogen peroxide (15.67 mg/ml).

The current study showed the inhibitory capability of the methanolic extract of the *J. integerrima* flowers against albumin denaturation and heat-induced hemolysis, which thereby proved its anti-inflammatory property. Likewise, the oral administration and topical application of an ethanolic extract of the *J. integerrima* leaves showed significant anti-inflammatory activity. The presence of 65 components in the metabolomics profile of *J. integerrima* extract, including a high concentration of terpenoids, flavonoids, and oxygenated fatty acids, can be associated with anti-inflammatory action (Mahrous *et al.,* 2022). The findings of Khalifa *et al.* (2021), showed the anti-inflammatory potential of different varieties of *Jatropha* plants cultivated in Egypt such as *J. integerrima* Rosea, *J. integerrima* Jacq, *J. multifida* Linn, *J. curcas* Linn, *J. pandurifolia* Andrews and *J. gossypifolia* Linn using HRBC hemolysis and membrane stabilization assay. The *J. gossypifolia* extracts derived using the methanol and petroleum ether solvents exhibited potent anti-inflammatory activity (Panda *et al.,* 2009).

The methanolic extract of the *J. integerrmia* flowers effectively inhibited the acetylcholine esterase and tyrosinase, which plays a role in neurodegenerative diseases. Similarly, the existence of bioactive metabolites in the extracts of *J. gossypyfolia*, along with substantial inhibitory potential against the acetylcholinesterase (AChE) and butyrylcholinesterase, validating the long-standing use of this plant in the treatment of Alzheimer's disease (Saleem *et al.,* 2016). Natural AChE (acetylcholinesterase) inhibitors of plant origin, particularly compounds of polyphenolic like flavonoids with inhibitory potential like that of currently recommended AChE inhibitor drugs, have recently been the focus of research for the prevention and the treatment of Alzheimer's disease. That substance has the benefit of

being more readily accepted and less expensive due to its frequent occurrence in foods and its antioxidant properties (Santos *et al.,* 2018). Interestingly, the seed oil of *J. curcas* Linn entrapped in niosomes disclosed the potent tyrosinase inhibitory activity with the IC_{50} value of 40.43 ± 2.97 $mgml^{-1}$ (Manosroi *et al.,* 2011). Tyrosinase involves the conversion of tyrosine to melanin, which is crucial in the development of dopamine toxicity in neurodegenerative disorders like Parkinson's disease (Orhan *et al.,* 2017). It might be essential to investigate medicinal plants for their ability to inhibit tyrosinase with the intent to find a beneficial new source of medications for neurodegenerative diseases (Li *et al.,* 2013).

5. Conclusion

The study presented here could find that the methanolic extract of *J. integerrima* flowers contains several useful phytochemical constituents like flavonoids, saponins, phenols, quinone, and protein. The potent antioxidant activity, anti-inflammatory, and anti-neurodegenerative activity were exhibited by the methanolic extract of *J. integerrima* flowers. Additionally, the *J. integerrima* flowers will be a potent source of bioactive compounds to develop innovative, risk-free plant-based therapies or pharmacologically applicable natural products for the treatment of inflammatory or neurodegenerative conditions.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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