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NEUROPROTECTIVE EVALUATION OF FLAVONOID FRICTION OF CYPERUS ROTUNDUS K

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ABSTRACT

INTRODUCTION: *Cyperus rotundus, or Purple Nutsedge, is a perennial, glossy-green, grass-like Eurasian sedge or weed with an erect triangular stem branching into three stems of purple, antenna-like seedpods*

A medicinal herb traditionally used to treat various clinical conditions at home such as diarrhea, diabetes, malaria, disorders.

Flavonoids, a group of natural substances with variable phenolic structures, are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine.

These natural products are well known for their beneficial effects on health and efforts are being made to isolate the ingredients so called flavonoids.

Alzheimer's disease is an irreversible progressive neurode- generative disease.aggregation and deposition of amyloid beta protein (Aβ) in the brain may be closely correlated with the development of Alzheimer's disease Symptoms of the disease include decreases in verbal ability and motor function

MATERIALS METHODS:

- *Plant abstract*
- *Xanthine oxidase*
- *Beta amyloid protein*

RESULTS:

Cyperus rotundus shows neuroprotective evaluation against neuroprotective diseases

CONCLUSION:

Cyperus rotundus's potential as a natural treatment for neurodegenerative diseases has been demonstrated by the neuroprotective evaluation of the plant, which has produced encouraging results.

Keyword: Neuroprotective evaluation , Amyloid protein , Oxidative stress , Inflammation, Tau protein

INTRODUCTION

Neurodegenerative diseases are a class of debilitating disorders that are characterized by the progressive degeneration of neurons in specific areas of the central nervous system (CNS). Due to their prevalence, complexity, and lack of effective treatments, these disorders, which include Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and Amyotrophic Lateral Sclerosis (ALS), pose significant challenges to the world's healthcare systems. It is critical to discover efficient neuroprotective therapies that can halt the progression of the disease or lessen symptoms because neurodegenerative diseases are becoming more prevalent as the world's population ages.[1]

During the pathogenesis of neurodegenerative diseases, genetic, environmental, and lifestyle factors interact in a multifactorial manner, leading to the accumulation of misfolded proteins, oxidative stress, neuroinflammation, and mitochondrial dysfunction. In the end, these processes lead to the death of neurons and the loss of synaptic connectivity and neuronal function. Finding neuroprotective treatments has thus become a top priority in the search for disease-modifying drugs.[2]

For a variety of neurodegenerative diseases, many studies have provided insightful information on potential neuroprotective methods. One such strategy involves targeting amyloid-beta (Aβ) and tau proteins in AD. Monoclonal antibodies against A have been tested in clinical trials, such as the one carried out by [3] in an effort to lessen its aggregation and deposition in the brain. Even though some of these trials have shown encouraging results in slowing cognitive decline, the complexity of AD pathology necessitates a multifaceted approach to achieve significant therapeutic efficacy.[3,4]

Enhancing dopaminergic function and lowering oxidative stress and inflammation have been the main goals of neuroprotective treatments for Parkinson's disease. Neurotrophic factors, such as glial cell line-derived neurotrophic factor (GDNF), have been shown to support and promote the survival of dopaminergic neurons, according to [5]. Initial clinical trials produced encouraging preclinical results, but the ability to successfully deliver drugs to the brain has made it difficult to translate these results into effective clinical treatments.

Cyperus rotundus is a herbaceous perennial plant that has been used for centuries in traditional medical systems all over the world. It is also known as "Nutgrass" or "Purple Nutsedge." This plant species, which is a member of the Cyperaceae family, is well known for having a wide range of therapeutic benefits. This is proven by the fact that it is widely used in numerous traditional medical systems, including Ayurveda, Traditional Chinese Medicine (TCM), and traditional African medicine. The complex phytochemical makeup of Cyperus rotundus, which has recently drawn more attention from researchers, is what gives it its therapeutic potential. [6]

Several ancient texts and historical records mention the use of Cyperus rotundus in traditional medicine. For instance, Cyperus rotundus is referred to as a potent "Medhya Rasayana" in Ayurveda and is noted for its advantages on memory improvement and cognitive function. Similar to Western medicine, Traditional Chinese Medicine uses this herb in herbal preparations to treat a variety of health issues, such as inflammation, dysmenorrhea, and gastrointestinal disorders.[7]

Studies on phytochemistry have shown that Cyperus rotundus contains a variety of bioactive substances, each of which contributes to the plant's medicinal properties. In a study by(Ahmed et al. (2016)), the phytochemical profile of the plant was thoroughly analysed, revealing significant components like flavonoids, alkaloids, saponins, tannins, and phenolic compounds. These substances have shown a range of pharmacological effects, such as antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and neuroprotective effects. The aim of the study is to study the neuroprotective evaluation of Cyperus rotundus. The objective of the study is to understand and evaluate the neuroprotective evaluation of cyperus rotundus

MATERIALS & METHODS

Plant abstract preparation

The rhizomes of Cyperus rotundus were purchased from M/s. SKM Siddha and Ayurvedic medicines India private limited, Tamil Nadu. The rhizomes were cleaned under running tap water and then shade dried at ambient temperature. Thereafter the dried rhizomes were pulverized into a coarse powder and ready for extraction.

Preparation of Total Oligomeric Flavonoids (TOFs)

The Total Oligomeric Flavonoids (TOFs) was prepared from the rhizomes of Cyperus rotundas as per the method of (Kilani et al. 2008). The rhizome of Cyperus rotundus was shade dried and made into a coarse powder. It was then macerated in 1:2 (v/v) ratio of water/acetone and incubated in dark for 6 h with intermittent stirring. Acetone was evaporated under low pressure and the extract was filtered. Filtrate was precipitated with excess NaCI for 24h at 50°C to separate tannins. The supernatant was collected and extracted with ethyl acetate, concentrated and precipitated with excess chloroform. Precipitation was separated and the TOFs fraction was dissolved in water for further investigation.

Chemicals and Reagents

Xanthine, acetylthiocholine iodide, acetylcholine enzyme (0.3U/ml) were procured from Sigma-Aldrich, USA. Quercetin was purchased from TCI Chemicals, India. Donepezil hydrochloride was purchased as a tablet from a

local pharmacy. All other chemicals, reagents and solvents used were of analytical grade and purchased from SRL chemicals, India.

Xanthine Oxidase Inhibitory Activity

The XO inhibitory activity was assayed spectrophotometrically under aerobic conditions, based on the procedure reported by (Bustanji et al. 2011). The substrate and the enzyme solutions were freshly prepared. The assay mixture, consisting of 50uL of different concentrations C.rotundus (10-320 ug/ml) flavonoid fraction, different concentrations of Quercetin (10-320 M), 35 L of 0.1mM phosphate buffer (pH=7.5) and 30uL of enzyme solution (0.01units/ml of XO in 0.1mM phosphate buffer, pH=7.5), was prepared immediately before use. After 30 mins of incubation at 25°C, the reaction was initiated by the addition of 60mL of substrate solution (150mM of Xanthine in 0. 1mM Phosphate buffer). The absorption at 295 nm, indicating the formation of uric acid at 25°C, was monitored and the initial rate was calculated. A blank was prepared in the same manner. One unit of XO was defined as the amount of enzyme required to produce 1 mmol of uric acid/minute at 25 °C.

XO inhibitory activity is expressed as the percentage inhibition of XO in the above system, calculated as (1-B/A) × 100, where A and B are the activities of the enzyme without and with different concentrations of C.rotundus and Quercetin. ICso values were calculated from the mean values of data from three determinations. Quercetin was used as a reference standard.

In vitro acetylcholinesterase (AChE) inhibition assay (Ellman et al., 1961)

The flavonoid fraction of C.rotundus and standard Donepezil hydrochloride was examined for its AChE inhibitory activities at different concentrations of 10-320uM and 10-320 ug/ml respectively. 200ul of the different concentrations C Rotundus (10-320 ug/ml) flavonoid fraction and standard Donepezil hydrochloride (10-320 ug/ml) were prepared using 0.05M tris base. Briefly, in this method, 200ul of acetylthiocholine iodide (15mM), 1000ul of DTNB (3mM), and 200ul of C.rotundus flavonoid fraction and Donepezil at different concentrations were mixed and incubated for 15 min at 30°C. Then, the mixture was monitored spectrophotometrically at 412m 10 times, each 13 s. After that, 200ul of AChE (0.3U/ml) solution was added to the initial mixture, to start the reaction and then the absorbance was determined.

Control contained all components except the tested extract. The percentage of AChE inhibitory activity (% IA) was calculated by using the following equation:

IA (%) = (Activity of Control - Activity of Test)/ Activity of Control x 100

Assessment of Aß (1-42) Concentration

Preparation of AB solution

The Aß solution was prepared according to the method of Miyazaki et al., 2019.

Briefly, synthetic B-Amyloid Peptide 1-42 (AßI-42) (PP69, Sigma Merck, USA) was dissolved in 0.1% ammonia solution at a final concentration of 250 uM and sonicated in ice-cold water for a total of 5 min (1 min × 5 times) to avoid pre-aggregation. For preparation of the Aß solution, aliquots of Aß were diluted to 25M in 50mM phosphate buffer (pH 7.5) and 100mM NaCI.

Thioflavin T fluorescence assay

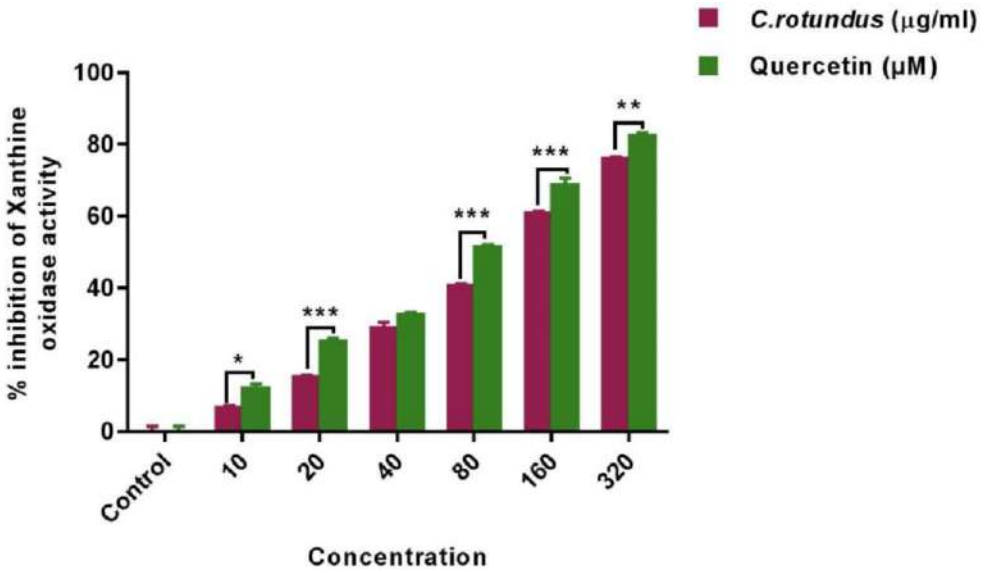
The thioflavin T (ThT) fluorescence assay was performed as Miyazaki et al., 2019.

AB solution (8uL) was mixed with the different concentrations of C.rotundus (10-320 ug/ml) flavonoid fraction and Donepezil (10-320 ug/ml) and the mixture was then added to 1.6mL of ThT solution containing 5pM ThT and 50mM NaOH-glycine-buffer (pH 8.5). The samples were incubated at 37°C and the fibrillogenesis rate was monitored by using ThT fluorescence assays. The samples ThT fluorescence levels were evaluated by using Biotek Synergy H4 hybrid multimode reader (USA). The respective excitation and emission wavelengths were 446 nm and 490 nm.

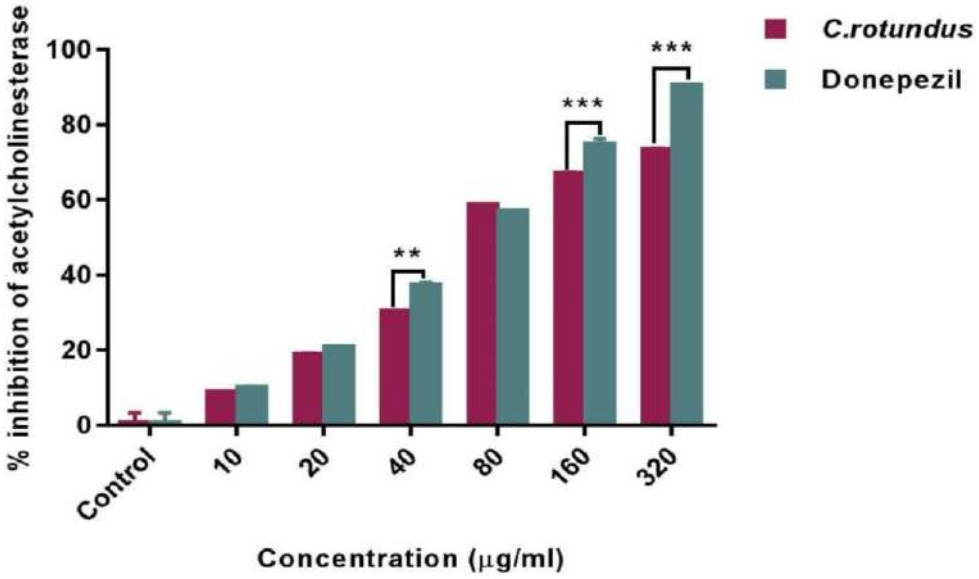
STATISTICAL ANALYSIS

Data were analyzed using Graphpad prism (version 7.0). The results were expressed as Mean SEM and the ICs values were obtained from the linear regression plots. Two-way ANOVA was used to assess differences between means at $p<0.001$ level of significance. The means were compared with standard groups using the Holm-Sidak Test.

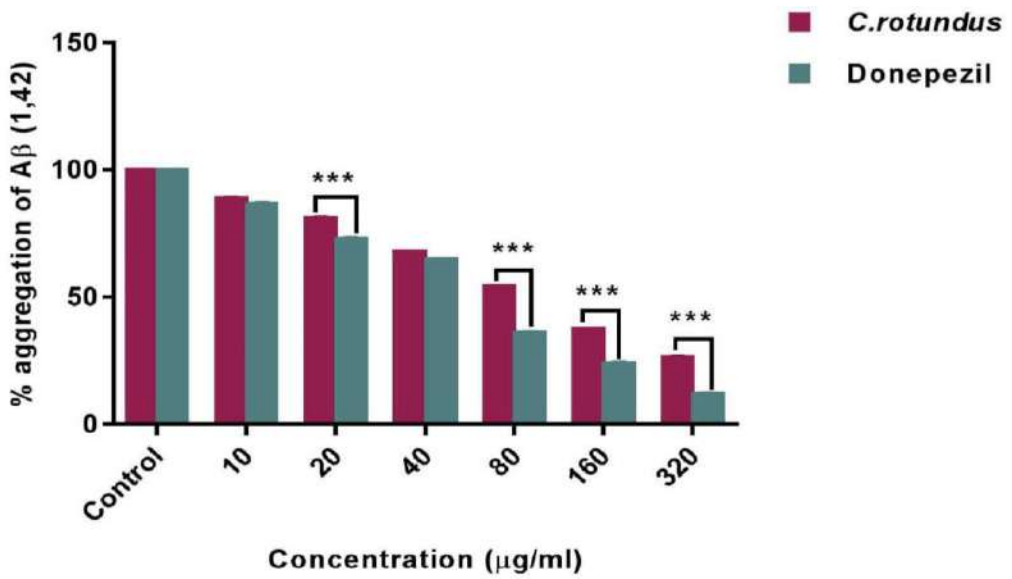
RESULTS



Picture 1: It indicates percentage inhibition of xanthine oxidase activity



Picture 2: It indicates percentage inhibition of acetylcholinesterase activity



Picture 3: It indicates the percentage aggregation of A protein

DISCUSSION

Researchers have become increasingly interested in the neuroprotective properties of Cyperus rotundus as evidence of its therapeutic efficacy comes from the plant's widespread use in traditional medical practises. This analysis summarises the results of numerous investigations into the neuroprotective abilities of Cyperus rotundus and discusses its potential use as a complementary or alternative therapy for neurodegenerative diseases.

Oxidative stress, which causes the production of free radicals and ensuing damage to neurons, is one of the main mechanisms underlying neurodegeneration. The antioxidant capacity of Cyperus rotundus has been the subject of numerous studies. (Ahmed et al. (2016)) found that the plant contained a variety of bioactive substances, including flavonoids and phenolic compounds with potent antioxidant properties. These antioxidants can scavenge free radicals, minimising the neuronal damage brought on by oxidative stress. Cyperus rotundus may therefore provide neuroprotection by reducing oxidative brain damage, making it a potential candidate for slowing the progression of neurodegenerative diseases.[8]

Another important element in neurodegenerative processes that contributes to the aggravation of neuronal damage is inflammation. (Asad et al. (2017)) investigated the anti-inflammatory properties of Cyperus rotundus and showed that it could suppress inflammatory cytokines and markers in animal models of inflammation. Neuroinflammation is a common component of disorders like Alzheimer's and Parkinson's, and Cyperus rotundus may have neuroprotective effects in these diseases by modulating inflammatory responses. [9]

In Alzheimer's disease, the accumulation of amyloid-beta (Aβ) plaques and tau protein tangles plays a central role in neuronal degeneration. In a study by (Singh et al. (2015)), the effects of Cyperus rotundus were examined in a mouse model of Alzheimer's disease, and it was discovered that there were notable improvements in cognitive function, along with decreased A deposition and tau phosphorylation. These results imply that Cyperus rotundus might exert neuroprotective effects by concentrating on the pathogenic mechanisms underlying Alzheimer's disease.[10]

The degeneration of dopaminergic neurons in the substantia nigra, on the other hand, is a hallmark of Parkinson's disease. In a rat model of Parkinson's,h Li et al. (2018)) assessed the neuroprotective potential of Cyperus rotundus and found that treatment with an extract of the plant significantly slowed the degeneration of dopaminergic neurons. The protective effects of the extract on dopaminergic neurons were responsible for its anti-

inflammatory and antioxidant properties. These findings suggest that Cyperus rotundus may be able to prevent Parkinson's disease-related neuronal loss.[4]

In addition to having neuroprotective effects on Alzheimer's and Parkinson's diseases, Cyperus rotundus has shown promising results in other neurodegenerative diseases. In a transgenic mouse model of Huntington's disease, treatment with Cyperus rotundus extract improved motor coordination and decreased neuroinflammatory markers, according to a study by (Das et al. (2021)). (Rahman et al.'s (2019)) cytoprotective effects against oxidative stress-induced neurotoxicity in an in vitro model were also demonstrated.[11]

Numerous neurodegenerative diseases have been studied extensively with regard to the use of antioxidants as a neuroprotective strategy. The polyphenol curcumin, which is present in turmeric, has been shown in a study by (Schubert et al. (2018)) to protect against A-induced toxicity in AD models. Curcumin's antioxidative qualities reduced oxidative stress and neuronal damage, demonstrating the substance's potential as a natural neuroprotective agent.[12]

Many neurodegenerative diseases share the common feature of mitochondrial dysfunction, which causes energy deficits and increased reactive oxygen species production. As a result, methods for improving mitochondrial function have become more popular. PGC-1, a crucial regulator of mitochondrial biogenesis, was the subject of a study by that examined its neuroprotective effects in ALS model organisms. PGC-1 overexpression improved mitochondrial function and prolonged motor neuron survival, offering a potential ALS treatment option. [13]

The antioxidant activity of Cyperus rotundus is one of its most researched properties. used various in vitro models to conduct research on the plant's antioxidant potential. The findings emphasized its capacity to neutralize free radicals and shield cells from oxidative damage, which is essential in preventing a number of degenerative diseases linked to oxidative stress.[14]

Furthermore, extensive research has been done on Cyperus rotundus's anti-inflammatory properties. In a study conducted on animal models of inflammation, (Asad et al. (2017)) examined the anti-inflammatory effects of a Cyperus rotundus extract. Its potential therapeutic use in inflammatory conditions is suggested by the findings, which showed significant drops in inflammatory markers and cytokine levels.[15]

Researchers have paid a lot of attention to Cyperus rotundus' potential for neuroprotection. In a rat model of cerebral ischemia, examined the neuroprotective properties of a standardized Cyperus rotundus extract.(19) The extract's effectiveness as a neuroprotective agent was demonstrated by the researchers' observations of a decrease in infarct size and improvements in neurological deficits.[16] Additionally, research has been done to determine whether Cyperus rotundus has any antimicrobial properties that could be used to fight different pathogens. A study by showed that Cyperus rotundus has potent antimicrobial properties against a range of bacterial and fungal strains. Its potential as an additional therapeutic agent for infectious diseases was suggested by the researchers.[16, 17]

The anti-diabetic effects of Cyperus rotundus have also been investigated, in addition to its antimicrobial and neuroprotective effects. In a study conducted on diabetic rats, looked into the hypoglycemic potential of a Cyperus rotundus extract and discovered significant drops in blood glucose levels.(20) Despite the abundance of encouraging research on the therapeutic benefits of Cyperus rotundus, it is crucial to use caution when using it, taking into account any possible negative side effects and drug interactions. To ensure consistency and safety in its therapeutic applications, standard extraction techniques and quality control measures should also be established. [18]

CONCLUSION

Cyperus rotundus's potential as a natural treatment for neurodegenerative diseases has been demonstrated by the neuroprotective evaluation of the plant, which has produced encouraging results. Preclinical data support the various mechanisms of action, such as antioxidant, anti-inflammatory, and anti-amyloid properties. To determine the safety, effectiveness, and therapeutic potential of Cyperus rotundus in human subjects, rigorous clinical trials

are necessary. If these studies are successful, Cyperus rotundus may provide an important neuroprotective treatment option to lessen the burden of neurodegenerative diseases and improve the quality of life for those who are afflicted.

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STATEMENT OF CONFLICT OF INTEREST

The author declares that there is no conflict of interest in the present study.

REFERENCES

1. Shakerin Z, Esfandiari E, Ghanadian M, Razavi S, Alaei H, Dashti G. Therapeutic effects of Cyperus rotundus rhizome extract on memory impairment, neurogenesis and mitochondria in beta-amyloid rat model of Alzheimer's disease. Metab Brain Dis. 2020 Mar;35(3):451–61.
2. Screening of natural compounds from Cyperus rotundus Linn against SARS-CoV-2 main protease (Mpro): An integrated computational approach. Comput Biol Med. 2021 Jul 1;134:104524.
3. Khazdair MR, Kianmehr M, Anaeigoudari A. Effects of Medicinal Plants and Flavonoids on Parkinson's Disease: A Review on Basic and Clinical Evidences. Advanced Pharmaceutical Bulletin. 2020;11(2):224–32.
4. Neuroprotective effects of Cyperus rotundus on SIN-1 induced nitric oxide generation and protein nitration: Ameliorative effect against apoptosis mediated neuronal cell damage. Neurotoxicology. 2013 Jan 1;34:150–9.
5. Shakerin Z, Esfandiari E, Razavi S, Alaei H, Ghanadian M, Dashti G. Effects of *Cyperus rotundus* extract on spatial memory impairment and neuronal differentiation in rat model of Alzheimer's disease. Adv Biomed Res. 2020 Jan 1;9(1):17.
6. In vitro evaluation of antibacterial, antioxidant, cytotoxic and apoptotic activities of the tubers infusion and extracts of Cyperus rotundus. Bioresour Technol. 2008 Dec 1;99(18):9004–8.
7. Hudaib MM, Tawaha KA, Mohammad MK, Assaf AM, Issa AY, Alali FQ, et al. Xanthine oxidase inhibitory activity of the methanolic extracts of selected Jordanian medicinal plants. Pharmacogn Mag. 2011 Oct;7(28):320–4.
8. Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961 Jul;7:88–95.
9. Miyazaki H, Okamoto Y, Motoi A, Watanabe T, Katayama S, Kawahara SI, et al. Adzuki bean (Vigna angularis) extract reduces amyloid-β aggregation and delays cognitive impairment in Drosophila models of Alzheimer's disease. Nutr Res Pract. 2019 Feb 1;13(1):64–9.
10. Effect of Cyperus rotundus tubers ethanolic extract on learning and memory in animal model of Alzheimer. Biomedicine & Aging Pathology. 2013 Oct 1;3(4):185–91.

11. Al-Snafi AE. Medicinal plants possessed anti-Parkinsonian effects with emphasis on their mechanisms of action. GSC Biological and Pharmaceutical Sciences. 2021;17(1):232–7.

12. Total oligomeric flavonoids of Cyperus rotundus ameliorates neurological deficits, excitotoxicity and behavioral alterations induced by cerebral ischemic–reperfusion injury in rats. Brain Res Bull. 2011 Apr 5;84(6):394–405.

13. Oxidative stress and mitochondrial dysfunction in neurodegenerative diseases. Neuroscience. 2007 Apr 14;145(4):1233–48.

14. Nagulendran KR, Velavan S, Mahesh R, Begum VH. *in vitro* Antioxidant Activity and Total Polyphenolic Content of *Cyperus rotundus* Rhizomes. J Chem Chem Eng. 4:440–9.

15. Preclinical study of the topical anti-inflammatory activity of Cyperus rotundus L. extract (Cyperaceae) in models of skin inflammation. J Ethnopharmacol. 2020 May 23;254:112709.

16. Sutalangka C, Wattanathorn J. Neuroprotective and cognitive-enhancing effects of the combined extract of Cyperus rotundus and Zingiber officinale. BMC Complement Altern Med. 2017 Mar 3;17(1):135.

17. Brahmachari G. Neuroprotective Natural Products: Clinical Aspects and Mode of Action. John Wiley & Sons; 2017. 372 p.

18. Krisanapun C, Wongkrajang Y, Temsiririrkkul R, Kongsaktrakoon B, Peungvicha P. Anti-diabetic effect and acute toxicity of the water extract of Cyperus rotundus L. in rats. The FASEB Journal. 2012 Apr 1;26:686.8–686.8.

19. Sneka S, Preetha Santhakumar. Antibacterial Activity of Selenium Nanoparticles extracted from Capparis decidua against Escherichia coli and Lactobacillus Species. Research Journal of Pharmacy and Technology. 2021; 14(8):4452-4. doi: 10.52711/0974-360X.2021.00773

20. Vishaka S, Sridevi G, Selvaraj J. An in vitro analysis on the antioxidant and anti-diabetic properties of Kaempferia galanga rhizome using different solvent systems. J Adv Pharm Technol Res. 2022 Dec;13(Suppl 2):S505-S509. doi: 10.4103/japtr.japtr_189_22.