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SCREENING OF *HABENARIA PLANTAGINEA* LINDL. FOR ANTI-OXIDANT AND *INVIVO* ANTI-ALZHEIMER POTENTIAL

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Abstract

The present work reported the anti-oxidant potential of various extracts of the plant Habenaria plantaginea (Hp) of the Orchidaceae family. To ascertain the anti-oxidant potential of the Hp extracts various pharmacological assays were conducted. The antioxidant potential of different fractions of Hp plant was evaluated through DPPH, ABTS and H_2O_2 anti-oxidant assays. In DPPH assay Hp.Chf fraction exhibited highest antioxidant activity with IC_{50} value of 2.9 µg/ml followed by Hp.EtAc with IC_{50} 4.0 µg/ml. In H_2O_2 inhibitory assay Hp.Chf exhibited highest activity (IC_{50} :14 µg/ml), subsequently followed by Hp.EtAc (IC_{50} : 17 µg/ml) , Crude (IC_{50} :18 µg/ml using the standard Ascorbic acid (IC_{50} :10 µg/ml).In behavioral assessment test, Open field arena test, Highest exploratory, grooming and rearing behavior was seen for Hp.Chf fraction followed by Hp.EtAc and Hp.Crude respectively when compared to the standard diazepam. Similarly, in shallow water paddling test, lowest latency time was exhibited by Hp.Chf fraction followed by Hp.EtAc and Hp.Crude fractions respectively against the standard drug galatamine, which indicated enhanced memory in the animals treated with the above mentioned fractions, in the dose dependent manner. From the results of the study it can be concluded that the aforementioned plant has great potential to treat Alzheimer's disease through multiple mechanisms. Hence, further studies are recommended on this potentially valuable plant to develop safe and effective therapeutic treatment for AD.

INTRODUCTION

Oxidative stress is a key pathological factor involved in the onset and progression of numerous chronic disorders, including cardiovascular diseases, diabetes, neurodegenerative conditions, inflammation, and cancer [1-2]. It arises from an imbalance between the generation of reactive oxygen species (ROS) and the ability of endogenous antioxidant defense systems to neutralize them. Excessive ROS can damage cellular macromolecules such as lipids, proteins, and nucleic acids, ultimately leading to cellular dysfunction and disease development. Consequently, the search for effective antioxidants capable of scavenging free radicals has become an important focus in pharmaceutical and biomedical research [3-4].

Natural products, particularly medicinal plants, have long been recognized as valuable sources of bioactive compounds with antioxidant potential. Plant-derived secondary metabolites such as

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flavonoids, phenolic acids, alkaloids, tannins, and terpenoids exhibit strong free radical scavenging properties and offer advantages over synthetic antioxidants due to their lower toxicity and better biocompatibility. This has stimulated growing interest in exploring underutilized and traditionally important plant species for their antioxidant activity and therapeutic relevance [5-6].

The family Orchidaceae is one of the largest and most diverse plant families, known not only for its ornamental value but also for its medicinal importance in traditional systems of medicine. Several orchid species have been reported to possess pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and antidiabetic effects, which are often attributed to their rich phytochemical composition. However, despite their potential, many orchid species remain scientifically unexplored [7].

Habenaria plantaginea is an orchid species traditionally used in folk medicine, yet systematic scientific data regarding its antioxidant properties are limited. Preliminary evidence suggests that the plant may contain a wide range of bioactive phytochemicals that could contribute to its therapeutic effects [8-9]. In this context, the present study was designed to investigate the phytochemical profile and evaluate the in vitro antioxidant potential of different solvent fractions of *Habenaria plantaginea* using established free radical scavenging assays, including DPPH, ABTS, and hydrogen peroxide (H_2O_2) inhibition models. This work aims to provide a scientific basis for the antioxidant efficacy of *Habenaria plantaginea* and support its potential use in the development of natural antioxidant agents.

1.0 MATERIALS AND METHODS

2.1 Chemicals and reagents

Potassium hydroxide, ethyl acetate, AchE, BchE, agarose, EDTA, Acetylthiocholine iodide, Butyrylthiocholine iodide, galantamine and boric acid.

2.2 Collection and Extraction of Plants

Midway through April, *H. plantaginea* (Hp) was acquired from the Dir (L) KPK, Pakistan and identified by Prof. Muhammads Ilyas, Department of Botany, University of Swabi, Swabi KPK, Pakistan. The plant specimen was preserved and documented in the herbarium under voucher number H.UOS.20-2.The aerial part of the plant (15Kg) was rinsed and shade dried for 21 days. The dried plant was then ground to coarse powder and subsequently macerated in 26 L of methanol (80 %) for 21 days. After maceration marc was removed by filtration through muslin cloth with subsequent filtration with Whatman filter paper. The resultant filtrate was dried at 40°C using rotavap. The methanolic extract thus obtained weighed 650 g with somber green hue [1].

2.3 Fractionation

The crude methanolic extract of H.P was reconstituted with distilled water and extracted with organic solvents viz *n*-hexane, ethyl acetate, chloroform, and butanol. First *n*-hexane fraction was formed by taking 200 mL of *n*-hexane in a separatory funnel (500 mL). Both *n*-hexane and aqueous layers of ethanolic extracts were shaken and swirled and left overnight. The upper layer of *n*-hexane was removed from the top of separatory funnel in a beaker. This process was repeated thrice each time pouring fresh 200ml of *n*-hexane in aqueous solution. At the end all fractions were mixed and dried by rotavap. The process was repeated for other solvents to obtain their respective fractions. The fractions thus obtained were stored at 4 °C for further investigation [10].

2.4 Antioxidant studies

2.4.1 DPPH radical scavenging assay

In DPPH radical scavenging assay, a stable purple DPPH free radical (insoluble in water) is used to determine the anti-oxidant potential of Hp extracts according to previously reported method. Concisely, DPPH solution (0.1 mM) was prepared by taking crystalline DPPH (0.004 g) in analytical grade methanol (100 mL) placed at 4 °C.Stock solutions of various fractions of the plant were prepared and subsequent required dilutions (1000,500, 250, 125, 62.5) via serial dilution method were prepared using methanol as solvent. 2 mL of each plant extract dilution was poured

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into a test tube and DPPH solution (3ml) was subsequently added into it.The mixtures of DPPH with each sample concentration were incubated for 30 min in dark because DPPH is unstable in light. After incubation absorbance is recorded using a UV–Vis Spectrophotometer at 517 nm [11]. Ascorbic acid was used as reference standard in this assay. 3 mL of DPPH (0.1 mM in 100ml methanol) was used as control. all processes were performed in triplicates. The percent of inhibition were plotted against concentration from which IC_{50} values were calculated.

DPPH % inhibition = $A_{control} - A_{sample} / A_{control}$

$A_{control}$ = Absorbance of the DPPH solution in methanol.

A_{sample}= the mixture of sample extract and DPPH solution

2.4.2 ABTS radical scavenging decolorizing assay

In ABTS inhibitory assay a blue green radical cation is neutralized into yellow solution based on the antioxidant capacity of the sample. The color of the solution fades as the sample scavenges the ABTS radical. ABTS assay was carried out in three steps. In the first step ABTS radical cation was generated by mixing 10ml of 7mM of aqueous ABTS with 10ml of 2.45mM of potassium persulfate and allowing in dark for 16 hours until a blue-green color appeared. The absorbance of the resulting solution was adjusted to 0.7 at 734nm using ethanol. In step 2 stock solutions of various extracts were prepared and subsequently the required concentration dilutions were prepared using the serial dilution method and added to 96-well microtiter plate (10 microlitre of each sample concentration and 195 µg/ml of ABTS solution. In the blank well 195 µg/ml mixed with 10 µg/ml of the solvent. The reaction mixture was incubated for 30 minutes and absorbance was measured at 734nm [12]. Trolox, a water soluble Vitamin E analogue, was used as astandard in this assay. The studies were repeated three times.

2.4.3 Hydrogen peroxide scavenging assay

200 ml of phosphate buffer (pH 7.4) was prepared by taking 50 ml KH_2PO_4 and 39.1 ml NaOH (0.2 M) and making up the final volume (200 ml) with distilled water. 50 ml of phosphate buffer was mixed with 50 ml of hydrogen peroxide and set aside at ambient temperature for 5 minutes. 1ml of each concentration of the extract made in distilled water was mixed with H_2O_2 (0.6 ml). Afterwards, the absorbance was determined in spectrophotometer using a wavelength of 230 nm against buffer solution (blank) [13-14]. % scavenging of H_2O_2 by extracts was determined as follows:

% scavenging potential (H_2O_2) = AC—ASS/ AC × 100

AC = absorbance of the control

ASS = absorbance of the sample and standard.

2.5 Acute toxicity studies

This assay confirms the safety profile of the plant sample used. Acute toxicity studies were carried out to check any dose-dependent adverse effects and mortality rate in albino mice of the plant extracts [15].

2.6 In-vivo study

Neurodegenerative symptoms of Alzheimer's disease were induced in animal models via administering $AlCl_3$ (100 mg/kg body weight) Intraperitonially for 60 days.

2.7 Behavioral assessments

Various behavioral assessment tests were carried out to explore the anti-Alzheimer potential of HP plant.

2.7.1 Open Field Test (OFT)

The open field test is a behavioral assay that was used to assess the animal's exploratory behavior and anxiety-like responses in a new environment. In this assay the animals were Placed in the centre of brightly lit open arena and different parameters such as Peripheral locomotoractivity (associated with increased anxiety or fear), centre locomotor activity (associated with reduced anxiety-like behavior), rearings, and grooming Time were observed [16].

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2.7.2 Shallow water (paddling) variants of water maze tests

In this behavioral assay a variant of the Water Maze test, termed Shallow Water Paddling test, was used, in which the water level is low enough (2cm) that the test animal paddle rather than swim to escape, as it had been recorded by the researchers that some strains of mice would float or dive when tested in the Morris water maze. This was not surprising when considering the natural habitat of rats that swim naturally, unlike the mice which had evolved on dry land.In this assay an octagonal pool with a single true exit and seven false exit, was used. The pool was made with transparent plastic to make it aversive for the animals an filled with water upto 2cm deep. The mouse was placed in the centre of the pool and was given 60 sec to find the true exit. The true exit was provided with black color pipe that lead to dry place where as the false exits were blocked with black plugs. The time took by each mouse to find the true exit was recorded [17].

2.7.3 Statistical analysis

The data were analysed by ANOVA following multiple comparison Post-Hoc tests using GraphPad Prism-8. Results are presented in mean ± standard deviation (S.D.) with statistical significance $p < 0.05$.

2.0 RESULTS

3.1 Antioxidant studies

The antioxidant potential of the crude and subsequent fractions were evaluated using ABTS, DPPH, and H_2O_2 free radicals scavenging assays.In DPPH assay Hp.Chf fraction displayed greatest antioxidant potential with IC_{50} value of 2.9 followed by Hp.EtAc with IC_{50} 4.0 , Crude having IC_{50} of 8. Hp.Hex, Hp.Bt, Hp.Aq with IC_{50} values of 250, 410 and 520 against the standard Ascorbic acid (IC_{50} value of 02).In ABTS inhibitory assay Hp.Chf displayed highest activity (IC_{50} value of 5.9), followed by Hp.EtAc (IC_{50} value of 7), Crude (IC_{50} value of 10), Hp.Hex (IC_{50} value of 280), Hp.Bt (IC_{50} value of 470) and Hp.Aq (IC_{50} value of 650) respectively against the standard Ascorbic acid 04. In H_2O_2 inhibitory assay Hp.Chf exhibited highest activity (IC_{50} value of 14) subsequently followed by Hp.EtAc (IC_{50} value of 17) , Crude (IC_{50} value of 18), Hp.Hex (IC_{50} value of 780), Hp.Bt (IC_{50} value of 1281) and Hp.Aq (IC_{50} value of 1450) against the standard Ascorbic acid (IC_{50} value of 10). As depicted in Table 1.

Table 1:The antioxidant activity of fractionsof H. plantaginea lindl.

S.No.	Conc (µg/ml)	ABTS		DPPH		H_2O_2	
		% inhibition	IC_{50} (µg/ml)	% inhibition	IC_{50} (µg/ml)	% inhibition	IC_{50} (µg/ml)
Crude	1000	82.52±0.73***	10	85.37±0.08***	08	77.82±0.86***	18
	500	78.42±0.43***		81.65±0.44***		72.67±0.67***	
	250	73.77±1.52***		76.35±1.42***		67.46±1.67***	
	125	69.68±0.12***		72.21±0.39***		63.58±0.92***	
	62.5	65.48±0.11***		67.66±0.78***		61.36±1.15*	
Hp.Chf	1000	89.30±1.20***	5.9	92.30±0.70***	2.9	88.00±1.150	14
	500	83.72±1.01**		87.70±0.80**		84.67±0.670	
	250	77.56±1.06***		82.91±0.88**		76.85±1.850	
	125	73.91±0.88*		78.80±0.90**		71.33±1.330	
	62.5	68.90±1.00*		73.00±0.60**		66.67±1.670	
Hp.EtAc	1000	87.62±0.58***	07	89.24±0.79***	04	81.45±1.32**	17
	500	82.35±0.23***		85.43±1.39***		75.75±0.86***	
	250	78.36±0.84***		80.48±0.25***		71.92±0.67*	
	125	73.62±0.25**		76.47±0.04***		67.15±1.67ns	
	62.5	69.16±0.16ns		71.47±0.44***		62.42±1.46ns	

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Hp.Hex	1000	65.30±1.42***	280	63.52±0.66***	250	55.45±1.15***	780
	500	61.78±0.45***		59.48±0.60***		51.42±0.67***	
	250	55.44±0.86***		52.54±0.46***		47.48±1.67***	
	125	49.72±0.89***		50.34±0.63***		41.82±1.08***	
	62.5	42.29±0.64***		46.30±0.64***		37.29±0.92***	
Hp.Bt	1000	63.30±1.42***	470	65.52±0.66***	410	51.45±1.67***	1281
	500	57.78±0.45***		59.48±0.60***		45.86±0.68***	
	250	52.44±0.86***		54.54±0.46***		41.67±0.88***	
	125	42.72±0.89***		44.34±0.63***		37.82±1.15***	
	62.5	34.29±0.64***		35.30±0.64***		22.38±2.08***	
Hp.Aq	1000	66.79±0.63***	650	67.73±0.03***	520	53.62±1.15***	1450
	500	59.67±0.61***		57.42±0.12***		47.47±1.67***	
	250	41.69±0.77***		47.39±0.35***		43.56±0.68***	
	125	35.54±0.50***		41.36±0.71***		37.72±1.11***	
	62.5	29.00±0.30***		29.15±0.22***		33.89±0.92***	

***:p <0.001, **:p <0.01, and *:p <0.05; ns: not significantly different from the positive control.

3.2 Acute toxicity

Acute toxicity testing of unadulterated methanolic extract revealed that during the initial two hours of observation, no significant behavioral alterations were observed. Furthermore. Based on the mortality percentage data, it was determined that 1200 mg/kg was the 50% lethal dose at which mortality transpired in half of the animals in the cohort (Table 2).The *95% confidence interval is enclosed in parentheses. No change in behavior was observed during initial two hours of the acute toxicity test upto 200mg/kg of the bw. No mortality was reported in animals receiving doses ranging from 50 to 200 mg/kg. A mortality rate of 16.6% was observed when 400 mg/kg of the test substance was administered. 50 percent death rate was observed at doses more than 1600 mg/kg. 100 percent mortality rate was observed at 3000 mg/kg.

Table 2: Test for acute toxicity

Dose (mg/kg)	Number of animals= 6mice/group		% Mortality (%)	LD_{50} (mg/kg)
	No. of mice dead	No. survived animals		
50	0	6	00.00	1213.89 (922.421-1534.33)
100	0	6	00.00	
200	0	6	00.00	
400	1	5	16.66	
800	1	5	16.66	
1600	2	4	33.33	
2000	3	3	50.00 LD	

*: p < 0.05, **: p < 0.01, ***: p < 0.001, ns: values that do not differ significantly from the +ve control.

3.3 Behavioral assessment tests

Chloroform, crude extracts and ethyl acetate were further evaluated for anti-Alzheimer potential using behavioral tests including the Open Field Arena test andShallow Water test.

3.3.1 Open Field Arena test

Mice treated withHp.Chf fraction at a dose of 8 mg/kg, did not exhibit a statistically significant increase in the time spent in the centre of the arena. However, at doses of 16 and 32 mg/kg the time spent in the centre of the arena was

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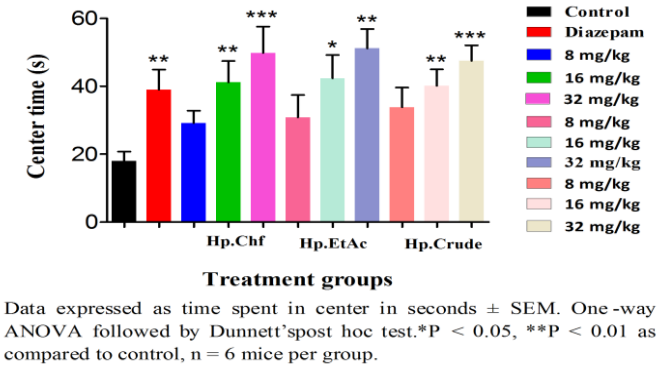
significantly higher as depicted in Figure 1. the exploratory, grooming and rearing behaviors were also enhanced in animals administered with Hp.EtAc and Hp.crude extracts at similar doses. Diazepam was used as standard in this behavioral assay.

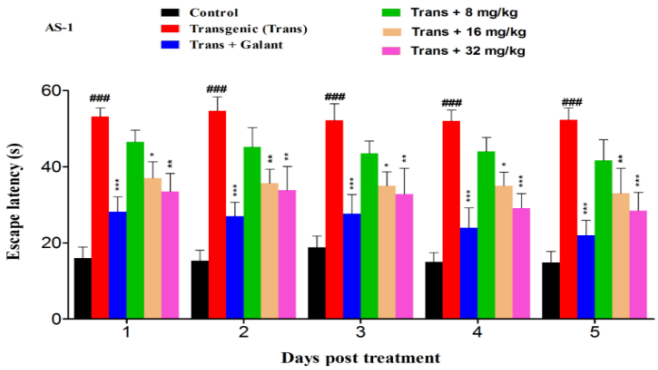


Data expressed as time spent in center in seconds ± SEM. One-way ANOVA followed by Dunnett'spost hoc test.*P < 0.05, **P < 0.01 as compared to control, n = 6 mice per group.

Figure 1:illustrates the impact of different concentrations of tested potent fractions on the duration of time spent in the apparatus's center during the open field test in Aluminum chloride induced Neurogeration animals.

3.3.2 Shallow Water Paddling Variant of Water Maze Tests

A dose-dependent decrease in the escape latency was observed in AD induced animal group when administered with Hp-Chf fractions (8,16,32 mg/kg) over 5 days of treatment compared to the standard drug Galantamine (Figure 2).



Data expressed as mean escape latency in second's ± SEM. Two-way ANOVA followed by Bonferroni's*post hoc*test. ###$P < 0.001$as compared to non-transgenic control mice group,*$P < 0.05$, **$P < 0.01$,***$P < 0.001$as compared to transgenic untreated group, $n = 6$ mice per group.

Figure 2: The influence of HP.Chf on escape time as measured by the SWM paradigm

4. DISCUSSION

The hallmarks of the AD, namely, Aβ peptide and τ proteins, are mainly initiated and enhanced by oxidative stress [18]. Therefore, it is suggested that slowing down the progression of oxidative stress may check the formation of the aforementioned moieties in the brain. The antioxidant potential of different fractions of Hp plant was evaluated through DPPH, ABTS and H_2O_2 anti-oxidant assays [19]. In DPPH assay Hp.Chf fraction exhibited highest antioxidant activity with IC_{50} value of 2.9 µg/ml followed by Hp.EtAc with IC_{50} 4.0 µg/ml, Crude having IC_{50} of 8 µg/ml, against the standard Ascorbic acid (IC_{50} value of 02 µg/ml). In ABTS inhibitory assay Hp.Chf displayed highest activity (IC_{50} :5.9 µg/ml),

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followed by Hp.EtAc (IC_{50} :7 µg/ml), Crude and (IC_{50} : 10 µg/ml respectively against the standard Ascorbic acid 04 µg/ml. In H_2O_2 inhibitory assay Hp.Chf exhibited highest activity (IC_{50}:14 µg/ml) subsequently followed by Hp.EtAc (IC_{50} :17 µg/ml) , Crude (IC_{50} : 18 µg/ml using the standard Ascorbic acid (IC_{50} : 10 µg/ml).Persistent decline in the cognitive capabilities is the characteristic feature of AD, owing to the widespread death of neuronal cells. Translational therapeutic interventions can be developed by investigating the effect of agents on the behavioral phenotypes. Extensive research has shown mice an excellent resource for the conduction of behavioral assays for the evaluation of new therapeutic compounds [20]. Using mouse model molecular targets can be easily identified and screening compounds with potential therapeutic effects utilizing mice paves the way for translational medicine.In the present study two behavioral assessment test were conducted on mouse model, namely, Open Field Arena test and shallow water paddling variant of water maze test, to investigate the anti-AD potential of the most potent fractions, selected after in vitro studies.

5.0 CONCLUSION

The results of both the *in vitro* investigations conducted on various fractions of the plant *Habenaria plantaginea* strongly suggest that the aforementioned plant has excellent potential of treating Alzheimer's disease through multiple mechanisms. Hence, further *invivo* studies are recommended on this potentially valuable plant to develop safe and effective treatment for AD.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

6.0 REFERENCES

1. Khan, A., et al., *Phytochemical profiling, anti-inflammatory, anti-oxidant and in-silico approach of cornus macrophylla bioss (Bark).* Molecules, 2022. **27**(13): p. 4081.
2. Ejaz, I., et al., *Rational design, synthesis, antiproliferative activity against MCF-7, MDA-MB-231 cells, estrogen receptors binding affinity, and computational study of indenopyrimidine-2, 5-dione analogs for the treatment of breast cancer.* Bioorganic & Medicinal Chemistry Letters, 2022. **64**: p. 128668.
3. Javed, M.A., et al., *Evaluation of pyrimidine/pyrrolidine-sertraline based hybrids as multitarget anti-Alzheimer agents: In-vitro, in-vivo, and computational studies.* Biomedicine & Pharmacotherapy, 2023. **159**: p. 114239.
4. Jan, M.S., et al., *Synthesis of pyrrolidine-2, 5-dione based anti-inflammatory drug: in vitro COX-2, 5-LOX inhibition and in vivo anti-inflammatory studies.* Latin Am J Pharm, 2019. **38**(11): p. 2287-2294.
5. Khan, J., et al., *Mechanistic evaluation of a novel cyclohexenone derivative's functionality against nociception and inflammation: an in-vitro, in-vivo and in-silico approach.* European Journal of Pharmacology, 2021. **902**: p. 174091.
6. Zaman, M., et al., *Anxiolytic and Antidepressant Like Effects of a Novel Synthetic Compound in Animal Models.* Phytopharmacology Research Journal, 2025. **4**(1): p. 77-82.
7. Hussain, F., et al., *Screening of Habenaria plantaginea Lindl for anti-Alzheimer potential.* Phytopharmacology Research Journal, 2025. **4**(3): p. 101-107.
8. Almasoudi, H.H., et al., *Phenolic phytochemistry, in vitro, in silico, in vivo, and mechanistic anti-inflammatory and antioxidant evaluations of Habenaria digitata.* Frontiers in pharmacology, 2024. **15**: p. 1346526.
9. Zaman, M., et al., *Exploring the In vivo Neuropsychopharmacological Potential of Polyamine Scaffolds.* Phytopharmacology Research Journal, 2024. **3**(3): p. 68-72.
10. Mahnashi, M.H., et al., *GC-MS Analysis and Various In Vitro and In Vivo Pharmacological Potential of Habenaria plantaginea Lindl.* Evidence-Based Complementary and Alternative Medicine, 2022. **2022**(1): p. 7921408.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

11. Alshehri, O.M., et al., *Succinimide Derivatives as Antioxidant Anticholinesterases, Anti-α-Amylase, and Anti-α-Glucosidase: In Vitro and In Silico Approaches.* Evidence-Based Complementary and Alternative Medicine, 2022. **2022**(1): p. 6726438.
12. Waheed, B., et al., *Synthesis, antioxidant, and antidiabetic activities of ketone derivatives of succinimide.* Evidence-Based Complementary and Alternative Medicine, 2022. **2022**(1): p. 1445604.
13. Hussain, F., et al., *Exploitation of the multitarget role of new ferulic and gallic acid derivatives in oxidative stress-related Alzheimer's disease therapies: design, synthesis and bioevaluation.* RSC advances, 2024. **14**(15): p. 10304-10321.
14. Masood, N., et al., *Antioxidant, carbonic anhydrase inhibition and diuretic activity of Leptadenia pyrotechnica Forssk. Decne.* Heliyon, 2023. **9**(12).
15. Zafar, R., et al., *Prospective application of two new pyridine-based Zinc (II) amide carboxylate in management of alzheimer's disease: Synthesis, characterization, computational and in vitro approaches.* Drug Design, Development and Therapy, 2021: p. 2679-2694.
16. Zaman, M., et al., *Preclinical Evaluation of a Novel Tris Amine Derivative in Animal Models of Anxiety and Depression.* Phytopharmacology Research Journal, 2025. **4**(2): p. 142-146.
17. Zaman, M., et al., *Anxiolytic and Antidepressant Effects of a Tris (2-aminoethyl) amine Derivative: A Preclinical Study.* Phytopharmacology Research Journal, 2025. **4**(1): p. 72-76.
18. Rauf, A., et al., *Hypoglycemic, anti-inflammatory, and neuroprotective potentials of crude methanolic extract from Acacia nilotica L.–results of an in vitro study.* Food Science & Nutrition, 2024. **12**(5): p. 3483-3491.
19. Alam, W., et al., *In vitro 5-LOX inhibitory and antioxidant potential of isoxazole derivatives.* Plos one, 2024. **19**(10): p. e0297398.
20. Mahnashi, M.H., et al., *Exploration of succinimide derivative as a multi-target, anti-diabetic agent: In vitro and in vivo approaches.* Molecules, 2023. **28**(4): p. 1589.