Acta Scientific Neurology (ASNE)

Volume 9 Issue 3 March 2026

Research Article

Anti-Alzheimer Activity of *Fragaria vesca* L. Leaf extract in Scopolamine Induced Amnesia in Mus Musculus

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Received: January 23, 2026

Published: February 28, 2026

DOI: 10.31080/ASNE.2026.09.0899

Abstract

Background: Alzheimer's disease is a growing global health challenge, particularly in developing countries such as Pakistan. Older adults are the most affected, and effective curative treatments remain unavailable, highlighting the need to explore natural therapeutic options.

Methods: This study evaluated the antioxidant and phytochemical properties of wild strawberry leaf extracts and their potential protective effects against scopolamine-induced memory impairment in mice. Behavioural assessments and biochemical analyses were conducted to support the findings.

Results: Wild strawberry leaf extracts significantly improved memory performance and antioxidant status compared to the standard drug piracetam. GC–MS analysis identified bioactive compounds that may contribute to the observed neuroprotective effects.

Conclusion: These findings indicate that wild strawberry leaves may serve as a promising, natural, and cost-effective source for the prevention and management of Alzheimer's disease.

Keywords: Malondialdehyde; Reduced Glutathione; Acetylcholinesterase; Scopolamine Hydrobromide; Alzheimer Disease

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that accounts for approximately 70% of all dementia cases worldwide [36]. The global burden of AD is increasing rapidly, with projections indicating that more than 115 million individuals will be affected by the year 2050 [34]. In Pakistan alone, nearly two million people are currently living with this disorder. Elderly individuals, particularly those aged 65 years and above, represent the most vulnerable population [34,35].

Despite extensive research, limited effective treatment options are available for Alzheimer's disease, prompting the search for novel therapeutic strategies. Cholinergic dysfunction has been identified as a major pathological factor in the progression of AD, primarily due to deficits in acetylcholine levels within the brain [23,26].

Acetylcholine plays a critical role in learning, memory, and cognitive function; however, increased acetylcholinesterase (AChE) activity in AD patients leads to excessive breakdown

of this neurotransmitter. Consequently, the inhibition of AChE is considered a promising approach for the management of Alzheimer's disease [3,6,10,12].

Although several synthetic AChE inhibitors, such as piracetam, donepezil, and tacrine, are currently used in clinical practice, their therapeutic application is limited due to high cost and the risk of severe side effects, which may be life-threatening if misused [2,13]. Therefore, there is pressing need to identify safer, affordable, and naturally derived alternatives for the prevention and treatment of neurological disorders such as AD.

Scopolamine, a well-known anticholinergic agent, is commonly used to induce cognitive impairment in experimental animal models. It disrupts normal learning and memory processes by blocking muscarinic acetylcholine receptors, making it a reliable tool for evaluating the anti Alzheimer and nootropic potential of therapeutic agents in mice [25,30].

Fragaria vesca L., commonly known as wild strawberry, belongs to the family Rosaceae is enrich source of phytochemicals and secondary metabolites. The plant has been traditionally used for various medicinal purposes, including as a stimulant, diuretic, detoxifying agent, and in the treatment of diarrhoea [1,4,22,31]. However, its potential neuroprotective and cognitive-enhancing properties have not been extensively explored.

Therefore, the present study was designed to investigate the nootropic and anti-Alzheimer effects of *Fragaria vesca* L. leaf extracts against scopolamine-induced memory impairment in mice using behavioural paradigms, particularly the passive avoidance test. Biochemical parameters, including total protein content, malondialdehyde (MDA) levels, reduced glutathione (GSH) levels, and acetylcholinesterase activity, were also assessed. Additionally, GC–MS analysis was performed to identify bioactive compounds that may contribute to the therapeutic potential of *Fragaria vesca* in the management of Alzheimer's disease.

Materials and Methods

Experimental animals

Ninety-six (96) male albino mice (3 months old; body weight 20–30 g) were procured from the Institute of Veterinary Research (IVR), Lahore. The animals were acclimatized under standard laboratory conditions at Lahore College for Women University, Lahore, with a controlled temperature of 23 ± 2 °C, relative humidity of 50 ± 5%, and a 12 h light/dark cycle. All experimental procedures were conducted in accordance with the ethical guidelines of the EEC Directive (1986).

Preparation of *Fragaria vesca* L. Leaf Extract

Fresh leaves of *Fragaria vesca* L. were collected from Jinnah Garden, Lahore. The plant material was authenticated and a voucher specimen was deposited at the Prem Madan Herbarium, Lahore College for Women University (Voucher No. LCWU-15-127). Methanolic and ethanolic leaf extracts were prepared using the double maceration technique with 70% methanol and 70% ethanol as solvents. The extraction process was repeated two to three times to ensure complete extraction. The solvents were evaporated under reduced pressure using a rotary evaporator, and the dried extracts were stored at 4 °C until further use.

Phytochemical screening

Qualitative phytochemical screening of the extracts was performed to detect the presence of alkaloids, phenolic acids, flavonoids, terpenoids, and tannins using standard protocols [11,14,24]. The development of characteristic colour changes or precipitate formation was considered indicative of positive results.

Determination of total phenolic and flavonoid content

Total phenolic content was determined using the Folin–Ciocalteu reagent according to the method described by [7]. Total flavonoid content was estimated following the modified method of [9].

Antioxidant activity

The antioxidant potential of methanolic and ethanolic leaf extracts was evaluated using the DPPH radical scavenging assay. Concentration-dependent activity was assessed at four different concentrations (0.125, 0.25, 0.5, and 1 mg/mL). Ascorbic acid, vitamin E, and butylated hydroxytoluene (BHT) were used as positive controls. IC_{50} values were calculated for each extract [20].

Experimental design

Prior to behavioural studies, acute toxicity of methanolic and ethanolic leaf extracts was assessed according to OECD guideline 423. Three doses (75, 150, and 300 mg/kg, p.o.) were administered to mice, and behavioural changes, skin and fur condition, and mortality were monitored for 14 days.

The nootropic potential of *Fragaria vesca* L. leaf extracts was evaluated using a scopolamine-induced memory impairment model in mice through the passive avoidance test. Animals were divided into seven groups (n = 6 per group):

- Group I: Distilled water (1 mL/100 g, p.o.)
- Group II: Normal saline (i.p.)
- Group III: Scopolamine hydrobromide (0.4 mg/kg, i.p.)
- Group IV: Methanolic or ethanolic leaf extracts alone (75, 150, and 300 mg/kg, p.o.)
- Group V: Methanolic or ethanolic leaf extracts plus scopolamine
- Group VI: Piracetam alone (200 mg/kg, p.o.)
- Group VII: Piracetam (200 mg/kg) plus scopolamine (0.4 mg/kg)

All treatments were administered for 21 days. Step-down latency (SDL) was recorded 90 minutes after administration on day 21, and retention was assessed after 24 hours.

Biochemical estimation

Following completion of behavioural studies on day 21, animals were sacrificed by cervical dislocation. Brains were immediately removed, placed on an ice-cold surface, washed, and homogenized in ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenates were used for the estimation of malondialdehyde (MDA), reduced glutathione (GSH), and acetylcholinesterase (AChE) activity using standard protocols [15,17,27].

GC–MS analysis

Based on the observed nootropic activity, the ethanolic leaf extract of *Fragaria vesca* L. was subjected to GC–MS analysis to identify bioactive compounds. Analysis was performed using an Agilent DB-5 column (5% diphenyl dimethyl polysiloxane; 30 m × 0.25 mm × 0.25 µm). Helium was used as the carrier gas at a flow rate of 1 mL/min. The oven temperature was initially set at 110 °C for 2 minutes and then increased to 280 °C over 9 minutes. A 2 µL aliquot of the ethanolic extract was injected, with the injector temperature maintained at 250 °C. Mass spectra were recorded over a 36-minute run and compounds were identified using the NIST mass spectral library (Version 2.0, 2005) [33].

Statistical analysis

All data were statistically analysed and expressed as mean ± standard deviation (SD). Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA), followed by post hoc Tukey's and Bonferroni tests where appropriate. Graphical presentation and statistical analyses were carried out using IBM SPSS Statistics and Microsoft Excel 2010. Differences were considered statistically significant at $P < 0.05$ when compared with the control groups [21,28].

Results

Phytochemical analysis

Qualitative phytochemical screening confirmed the presence of major secondary metabolites in both methanolic and ethanolic leaf extracts of *Fragaria vesca* L. The extracts were rich in terpenoids, phenolics, flavonoids, alkaloids, and tannins. The abundance of these bioactive compounds suggests a strong antioxidant and therapeutic potential of the plant extracts.

Total phenolic and total flavonoid content

Both methanolic and ethanolic leaf extracts of *F. vesca* L. exhibited high total phenolic and flavonoid contents. However, the ethanolic extract showed significantly higher levels compared to the methanolic extract, indicating ethanol as a more efficient solvent for the extraction of phenolic and flavonoid compounds (Figure 1).

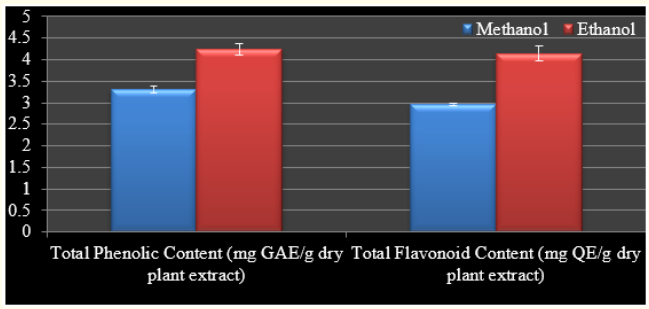


Figure 1: Total phenolic and flavonoid content in methanolic (M) and ethanolic (E) leaf extracts of *Fragaria vesca* L. Data are presented as mean ± SD of three independent experiments.

Antioxidant activity

The antioxidant potential of methanolic and ethanolic leaf extracts of *F. vesca* L. was evaluated using the DPPH radical

scavenging assay. Both extracts demonstrated concentration-dependent radical scavenging activity across the tested concentrations (0.125–1 mg/mL). The ethanolic extract exhibited stronger antioxidant activity, reflected by a lower IC_{50} value (0.100 ± 0.001 mg/mL) compared to the methanolic extract (0.243 ± 0.001 mg/mL). Standard antioxidants, including ascorbic acid, quercetin, vitamin E, and butylated hydroxytoluene, were used as positive controls (Figure 2).

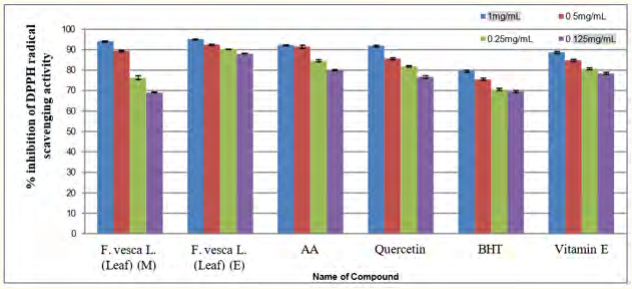


Figure 2: DPPH radical scavenging activity of methanolic (M) and ethanolic (E) leaf extracts of *Fragaria vesca* L. at different concentrations (0.125, 0.25, 0.5, 1 mg/mL). Ascorbic acid, quercetin, vitamin E, and BHT were used as positive controls. Data represent mean ± SD.

Effect of *Fragaria vesca* L. leaf extract on memory performance

Acute toxicity studies revealed no signs of mortality or behavioural abnormalities, indicating that all tested doses of *F. vesca* L. leaf extracts were safe. The nootropic potential was evaluated using the passive avoidance paradigm. Scopolamine-treated mice exhibited a significant reduction in step-down latency (113.17 ± 1.60), indicating memory impairment. In contrast, mice treated with *F. vesca* L. leaf extracts showed a significant increase in step-down latency (279.9 ± 1.31***###), demonstrating marked memory enhancement. The effect was comparable to that of the standard drug piracetam (Figure 3). Data is presented as Mean ± SEM (n = 6); One way ANOVA followed by Tukey Post Hoc test and Bonferroni test. *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ when compared to normal control; $^{###}$ $p < 0.001$, $^{##}$ $p < 0.01$ and $^{#}$ $p < 0.05$ when compared to negative control from Figure 3-7.

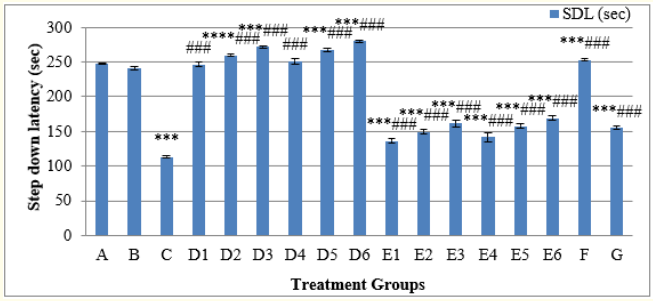


Figure 3: Effect of methanolic (M) and ethanolic (E) Fragaria vesca L. leaf extracts on step-down latency (SDL) in mice using the passive avoidance paradigm. Data represent mean ± SD.

Groups:

A: Control (distilled water, p.o., 10 mL)

B: Control (normal saline, i.p., 10 mL)

C: Scopolamine HBR (i.p., 0.4 mg/kg)

D1–D3: Methanolic extract (75, 150, 300 mg/kg)

D4–D6: Ethanolic extract (75, 150, 300 mg/kg)

E1–E3: Methanolic extract + Scopolamine (0.4 mg/kg)

E4–E6: Ethanolic extract + Scopolamine (0.4 mg/kg)

F: Piracetam (i.p., 200 mg/kg)

G: Piracetam + Scopolamine (0.4 mg/kg)

Effect on biochemical parameters

Biochemical analysis of brain homogenates revealed significant neuroprotective effects of *F. vesca* L. leaf extracts. Treatment with both methanolic and ethanolic extracts resulted in a significant increase in total protein and reduced glutathione (GSH) levels, along with a marked reduction in malondialdehyde (MDA) levels and acetylcholinesterase (AChE) activity. Among the two extracts, the ethanolic extract exhibited superior efficacy across all tested parameters. These effects were observed at both low and high dose levels when compared to scopolamine-treated and control groups (Figures 4–7).

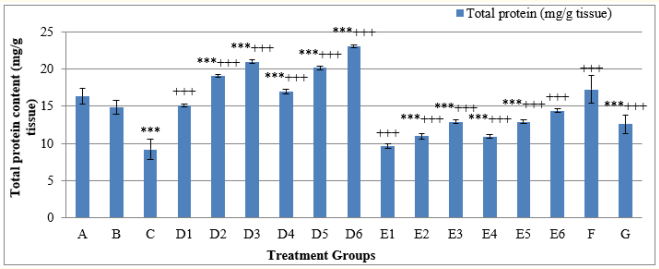


Figure 4: Neuroprotective effect of methanolic (M) and ethanolic (E) *Fragaria vesca* L. leaf extracts on total protein content (mg/g tissue) in mice brain homogenates. Data represent mean ± SD.

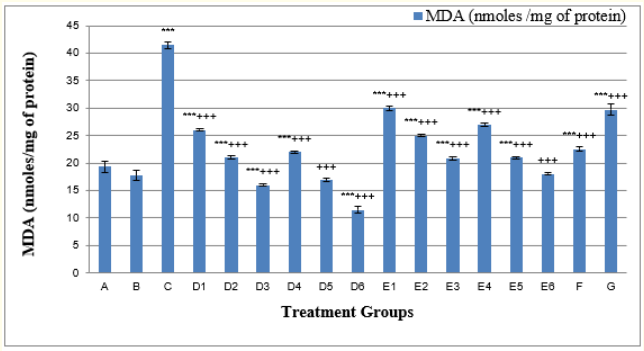


Figure 5: Neuroprotective effect of methanolic (M) and ethanolic (E) *Fragaria vesca* L. leaf extracts on malondialdehyde (MDA) levels (nmoles/mg protein) in mice brain homogenates. Data represent mean ± SD.

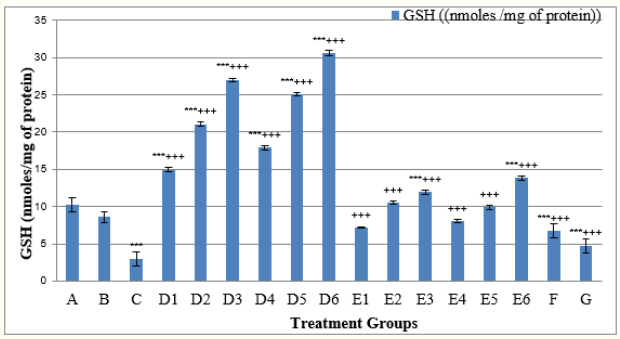


Figure 6: Neuroprotective effect of methanolic (M) and ethanolic (E) *Fragaria vesca* L. leaf extracts on reduced glutathione (GSH) levels (nmoles/mg protein) in mice brain homogenates. Data represent mean ± SD.

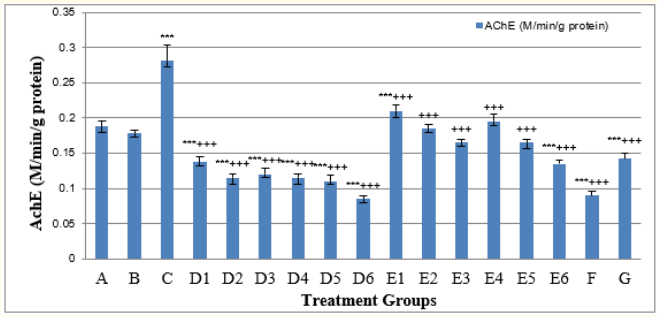
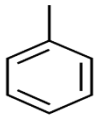
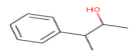
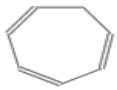
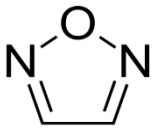
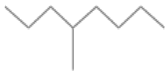
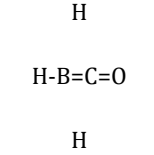
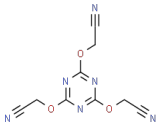


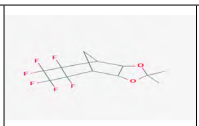
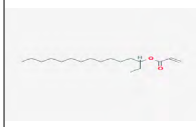
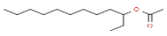
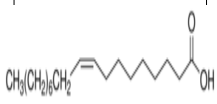
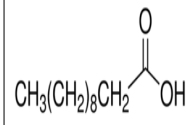
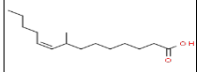
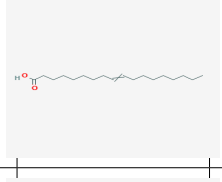
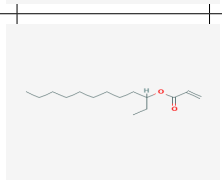
Figure 7: Neuroprotective effect of methanolic (M) and ethanolic (E) *Fragaria vesca* L. leaf extracts on acetylcholinesterase (AChE) activity (M/min/g protein) in mice brain homogenates. Data represent mean ± SD.

GC–MS analysis

GC–MS analysis of the ethanolic leaf extract of *Fragaria vesca* L. identified a total of 28 chemical compounds, of which 15 were reported for the first time in this plant species. Several identified compounds are known to possess antioxidant, anti-inflammatory, and neuroprotective properties. The presence of these novel bioactive constituents supports the observed memory-enhancing and anti-Alzheimer effects of *F. vesca* L. in the scopolamine-induced memory impairment model. Detailed compound identification and classification are presented in Table 1.

Sr. No.	Compound Name	Molecular Formula	Chemical Structure	Molecular Weight	RT	Therapeutic Activity
1)	Toulene	C_7H_8		92.141 g/mol	2.902	Anticonvulsant, cardiovascular, neurobehavioral and *therapeutic activity* against *toluene* induced dementia of LEP type in rats.

2)	Benzeneethanol, alpha-methyl-$$ alpha-Methylphenethyl alcohol $$ Benzyl methyl carbinol $$ Phenethyl alcohol	$C_9H_{12}O$		136 g/mol	2.902	Antimicrobial, antioxidant and antifertility activities
3)	1,3,5-Cycloheptatriene	C_7H_8		92 g/mol	2.902	Antibacterial, antioxidant and cardiovascular activity
4)	1,2,5 Oxadiazole	$C_2H_2N_2O$		70 g/mol	3.268	Anticancer and anti-trypanosomal activity anti-depressant, anti-inflammatory, anti-HIV and analgesic activities
5)	Octane, 4 methyl	C_9H_{20}		128 g/mol	3.268	Antifungal, antibacterial, anti-tumor, anti-angiogenic and anti-cancer activity
6)	Hexane, 3 ethyl-$$ 3-Ethylhexane $$	C_8H_{18}		114 g/mol	3.268	Antioxidant, antimicrobial, anticariogenic and neurological activity
7)	Octane	C_8H_{18}		114.23 g/mol	3.268	Antifungal, anti-parkinson, anti-tumor and anti-angiogenic activity
8)	Cyclopropene $$ 1-Cyclopropene #$$	C_3H_4		40 g/mol	3.268	Anthropogenic, antitumor and anti-alzheimer activity
9)	1,2, 5-Oxadiazole $$ Furazan $$ Azoxazole $$ 1- Oxa-2,5-diazacyclopentadiene $$	$C_2H_2N_2O$		70 g/mol	5.093	Antimicrobial, anti-HIV and anticancer activity
10)	Borane carbonyl $$ Boron, carbonyltrihydro-,(T-4)- Borane-carbon monoxide	CH_3BO		42 g/mol	5.093	Antitumor and anticancer activity
11)	1,3,5-Triazine, 2,4,6 tris (Cyanomethoxy)- $$ 2,2',2''-[1,3,5-Triazine 2,4,6 triyltris (oxy)] trisacetonitrile $$ 2,4,6 Tricyanomethoxy	$C_9H_6N_6O_3$		246 g/mol	5.093	Anticancer, antimalarial, antibacterial and anti-protozoal agents.

12)	8,9,9,10,10,11-Hexa-fluoro-4,4- dimeth-yk-3,5 dioxatetracy-clo[5.4.1.0(2,6).0(8,11)] dodecane$$	$C_{12}H_{12}F_6O_2$		302 g/mol	21.393	Antioxidant and antican-cer Activity
13)	5-(Prop-2-enoyloxy) pentadecane $$ 1- Butyl-undecyl acrylate #$$	$C_{18}H_{34}O_2$		282 g/mol	21.393	Antioxidant and gastro-protective Activity
14)	3- (Prop-2-enoyloxy) pentadecane $$ 1-Ethyl-tridecyl acrylate # $$	$C_{18}H34\ O_2$		282 g/mol	21.393	Antimicrofouling, antioxi-dant and gastroprotective activity
15)	3-Acetoxydodecane $$ 1-Ethyldecyl acetate # $$	$C_{14}H_{28}O_2$		228 g/mol	21.393	Antioxidant and anticho-linesterase activities
16)	2- Acetoxydodecane $$ 1- Methylundecyl acetate # $$	$C_{14}H_{28}O_2$		228 g/mol	21.393	Antioxidant and antimi-crobial properties
17)	Oleic Acid	$C_{18}H_{34}O_2$		282 g/mol	23.085	Anti-inflammatory, anti-bacterial and anticancer Activity
18)	Undecanoic acid, hydroxyl-, lactone $$ Oxacyclodecan-2-one # $$	$C_{11}H_{20}O_2$		184 g/mol	23.085	Antifungal, antioxidant and cyclooxygenase activi-ties
20)	Oxycyclotetradecan-2-one $$ Tridecanoic acid, 13-hydroxy-,mu-lactone $$ Tridecanolide $$ 1, 13- Tridecanolide $$-Ox	$C_{13}H_{24}O_2$		212 g/mol	23.085	NF
21)	9-Octadecenoic acid (Z)-, hexyl ester $$ Oleic acid, hexyl ester $$ Hexyl oleate $$ Hexyl (9Z)-9-octadecenoate # $$	$C_{24}H_{46}O_2$		366 g/mol	24.160	Antiallergic, antimicrobial, anti-proliferative and anti-diabetic aactivities
22)	3- (Prop-2-enoyloxy) pentadecane $$ 1- Ethyl-tridecyl acrylate # $$	$C_{17}H_{32}O_2$		268g/mol	24.160	NF

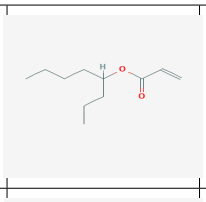
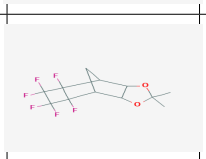
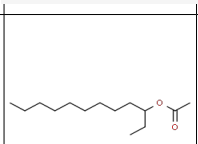
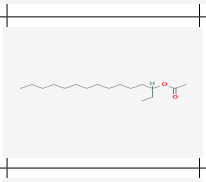
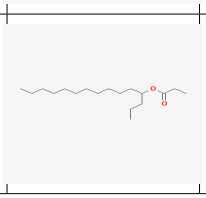
23)	4- (Prop-2-enoyloxy) pentadecane $$ 1- Propyldecyl acrylate # $$	$C_{18}H_{34}O_2$		282g/mol	24.160	Antimicrobial and alkylation activities
24)	8,9,9,10,10,11- Hexafluoro-4,4-dimethyl-3,5-dioxatetracyclo [5.4.1.0 (8,11)] dodecane $$	$C_{12}H_{12}F_6O_2$		302g/mol	24.160	Antioxidant, antibacterial and anticancer activity
25)	3- Acetoxydodecane $$ 1- Ethyldecyl acetate # $$	$C_{14}H_{28}O_2$		228g/mol	25.393	NF
26)	Octanoic acid, 2-tetrahydrofurylmethyl ester $$ Tetrahydro-2- furanylmethyl octanoate # $$	$C_{13}H_{24}O_3$		228g/mol	25.393	Antitumor, antifungal and anti-alzheimer activities
27)	3-Acetoxypentadecane $$ 1- Ethyltridecyl acetate # $$	$C_{17}H_{34}O_2$		270 g/mol	25.393	Antimicrobial, anti-anxiety and anticonvulsant, anti-hyperglycemic and anti-hyperlipidemic activities
28)	4-Propionyloxypentadecane $$ 1-Propyldodecyl propionate # $$	$C_{18}H_{36}O_2$		284 g/mol	25.393	NF

Table 1: Chemical Profiling of *Fragaria vesca* L. Ethanolic Leaf extract using GCMS Peak Report.

Discussion

Alzheimer's disease (AD) is a progressive neurological disorder characterized by cognitive deterioration, functional impairment, and behavioural disturbances [5,6,8]. A central pathological feature of AD is cholinergic dysfunction, which has driven the search for effective cholinesterase inhibitors, particularly from natural sources, to improve cognitive performance and delay disease progression. Animal models remain a reliable approach for evaluating nootropic, and scopolamine-induced memory impairment is widely used to mimic cholinergic deficits associated with AD [16].

In the present study, *Fragaria vesca* L. leaf extracts effectively reversed scopolamine-induced behavioural and biochemical alterations. Administration of the extracts significantly increased step-down latency in the passive avoidance test, indicating improved memory retention compared with scopolamine-treated and control groups. These behavioural improvements were further supported by biochemical findings, including increased total protein and reduced glutathione (GSH) levels, alongside decreased malondialdehyde (MDA) levels and acetylcholinesterase (AChE) activity in brain tissue. Collectively, these results suggest a protective effect against oxidative stress and cholinergic dysfunction.

Oxidative stress plays a critical role in neuronal damage and the progression of AD. The observed reduction in lipid peroxidation and enhancement of endogenous antioxidant defences indicate that *F. vesca* L. exerts a strong antioxidant effect, which may contribute to its neuroprotective properties. These findings are consistent with previous studies reporting that plants rich in phenolics and flavonoids possess significant antioxidant and neuroprotective potential [18,29].

Notably, the extract demonstrated significant efficacy even at lower doses, showing comparable or superior effects to standard therapeutic agents. The improvement in protein content, suppression of lipid peroxidation, elevation of GSH levels, and inhibition of AChE activity highlight the multifaceted mechanism through which *F. vesca* L. may mitigate memory impairment. Similar neuroprotective profiles have been reported for other plant-based interventions, further supporting the therapeutic relevance of phytochemicals in the management of neurodegenerative disorders [28].

Overall, the findings of this study provide compelling evidence that *Fragaria vesca* L. leaf extract possesses potent nootropic and anti-Alzheimer potential, likely mediated through antioxidant activity and modulation of the cholinergic system. These results support its further investigation as a cost-effective and eco-friendly candidate for the prevention and management of Alzheimer's disease.

Conclusion

Fragaria vesca L. leaf extract demonstrates significant potential in the prevention and management of neurological disorders such as Alzheimer's disease. This study highlights its neuroprotective and antioxidant properties, as well as its ability to modulate cholinergic activity, which collectively contribute to the improvement of memory and cognitive function. The findings are of considerable value, as they support the development of eco-friendly, cost-effective, and accessible therapeutic agents for patients experiencing cognitive decline, offering a promising approach for future interventions.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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