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# Antioxidant and anti-acetylcholinesterase properties of *Fragaria × ananassa* and *Actinidia deliciosa*: A comparative study towards neuroprotective applications

Mayank Shewale[1], Oshin Ambekar[1], Sanjana Patel[1], Angurbala Bafna[2], Rashmi Limaye[1] & Payal Puri[1]*

[1]Institute of Innovative Learning and Research Academy, Indore 452010, Madhya Pradesh, India

[2]Department of Biochemistry, Government Holkar Science College, Indore 452001, Madhya Pradesh, India

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Neurodegenerative diseases pose a significant global health challenge, with oxidative stress, inflammation, and reduced cholinergic neurotransmission being key pathological mechanisms. While *Fragaria × ananassa* (strawberry) and *Actinidia deliciosa* (kiwifruit) are recognised for their health benefits, a direct comparative evaluation of their specific anti-acetylcholinesterase (AChE) activity, a critical neuroprotective mechanism is currently lacking. This study comparatively evaluates the neuroprotective potential of these fruit extracts by examining their phytochemical profiles and their antioxidant, anti-inflammatory, and AChE activities. Qualitative analysis confirmed a richer phytochemical profile in strawberry extract. However, kiwifruit consistently demonstrated consistently superior performance across the functional assays, showing greater total antioxidant capacity (TAC) and more potent anti-inflammatory effects (83% inhibition of hemolysis vs. 73% for strawberry). Critically, a significant finding was the prominent anti-AChE activity in both extracts, with kiwifruit exhibiting a highly potent inhibitory effect (75% inhibition), substantially surpassing the inhibition demonstrated by the strawberry extract (25% inhibition). These comparative results underscore that both fruits are valuable sources of neuroprotective compounds, with kiwifruit extract showing a distinct advantage in both antioxidant strength and specific anti-AChE potential, suggesting a promising application in managing

**Keywords:** Neuroprotection, Anti-inflammatory activity, GC-MS profiling, Polyphenols, Cholinergic deficit

Strawberries (*Fragaria × ananassa - (Duchesne ex Weston) Duchesne ex Rozierand*) and kiwifruits (*Actinidia deliciosa- (A.Chev.) C.F.Liang & A.R.Ferguson*) are recognised for their nutritional value and health benefits, largely attributed to their abundant bioactive flavonoids and distinct phytochemical profiles[1]. Strawberries are rich in ellagic acid, anthocyanins, and flavonols, while kiwifruits are notable for vitamin C, carotenoids, and phenolic acids. This complementary composition is associated with a decreased risk of chronic diseases[2,3]. These health benefits are primarily mediated by flavonoids, which are critical in counteracting the two major systemic pathologies associated with chronic disease progression: oxidative stress (by scavenging free radicals) and chronic inflammation (by regulating inflammatory responses)[4]. Neurodegenerative disorders, with Alzheimer's disease (AD) representing a prominent example, constitute an escalating global health crisis[5,6]. AD, the most common form of dementia, is defined by a gradual deterioration of cognitive abilities, culminating in profound memory deficits, impaired decision-making, and alterations in personality[7,8]. AD pathology is marked by impaired cholinergic neurotransmission a system essential for memory and learning, primarily due to elevated acetylcholinesterase (AChE) activity[9,10]. Blocking this enzyme is a vital treatment strategy, as it increases the concentration of the essential neurotransmitter, acetylcholine (ACh), potentially improving cognitive function and slowing disease progression[11,12]. Flavonoids combat these key pathological mechanisms by modulating neuroinflammation and oxidative stress, and certain flavonoids may also inhibit AChE, preserving acetylcholine levels and potentially bolstering cognitive function[13-15]. Despite recognition for their phytochemical richness and health benefits, the direct neuroprotective mechanisms of strawberries and kiwifruits, specifically via acetylcholinesterase (AChE) inhibition, are not well understood. Current research primarily emphasises their general antioxidant and anti-inflammatory roles, leaving a gap in our knowledge regarding their comparative anti-AChE potential. Therefore, this study addresses this gap by

*Correspondence:
Phone: +91 93007 08700
E-mail: iilracademy@gmail.com

comparatively evaluating the antioxidant, anti-inflammatory, and anti-AChE activity of water and methanol extracts from both fruits in vitro. This research aims to advance natural, plant-derived strategies for neuroprotection by exploring the relationships among their unique flavonoid content, extraction methods, and inhibitory potency.

## Materials and Methods

### Sample preparation

Fresh samples of strawberry (*Fragaria* × *ananassa*) and kiwifruit (*Actinidia deliciosa*) were purchased from a local market in Indore, Madhya Pradesh, India. The fruit samples were botanically identified by morphology and authenticated at the Botany Department of Holkar Science College, Indore. To prepare the crude extracts, we individually mashed 4 gm portions of both strawberry and kiwi fruit pulp using a mortar and pestle. For each fruit, we used a 60:40 (v/v) mixture of ethanol and water as the liquid to pull out the desired substance[16].

### Phytochemical analysis

Post-extraction, crude strawberry and kiwi pulp extracts underwent qualitative analyses to identify key phytochemicals using established biochemical methods. The presence of Flavonoids was indicated by a deep yellow colour upon treatment with 40% NaOH. Polyphenols and Tannins were confirmed, respectively, by the blue-black colour resulting from the addition of 5% $FeCl_3$ and the Braymer's 10% $FeCl_3$ test. Saponins were revealed by the persistence of a stable froth in the Foam test upon vigorous shaking with distilled water. Terpenoids and Steroids were suggested by the reddish-brown interface produced in the Salkowski test (extract + chloroform + conc. $H_2SO_4$). Finally, the Keller-Killiani test indicated Glycosides by the presence of a reddish-brown junction and a bluish-green acetic acid layer[17].

### Thin Layer Chromatography for general phytochemical analysis

Thin Layer Chromatography (TLC) was employed for phytochemical analysis of crude extracts, using silica gel G as the stationary phase. Separation took place in a chamber, employing a mobile phase consisting of chloroform:methanol 15:1 (v/v). Once developed, components were visualised under UV light, allowing for the calculation of Retention factor (Rf) values for identification[17].

### Flavonoid extraction

For flavonoid extraction from strawberry and kiwi, a protocol of homogenization followed by acid hydrolysis was used[18]. Each fruit's pulp was separately homogenized in distilled water. Concentrated HCl was then added, and the mixtures were heated to dryness, producing a residue.

### Thin Layer Chromatography (TLC) for flavonoids in extracts

The analysis of flavonoids involved TLC on silica plates. Separation occurred using an ethyl acetate:butanol:water (2:1:3) mobile phase. Following development and air-drying, separated flavonoid spots were observed under UV light. Rf values were then calculated for each band to identify and quantify the flavonoid compounds[17].

### Determination of total flavonoid content (TFC)

The aluminum chloride ($AlCl_3$) method was employed to determine the total flavonoid content (TFC) present in the samples[18]. A quercetin (1 mg/mL) calibration curve served as the standard. The reaction mixture included 10% $AlCl_3$ and 1 M potassium acetate for colour development. Following a 30 minutes incubation at room temperature (25°C), the optical density (OD) was measured at 430 nm against a blank. The TFC was quantified in terms of milligrams of quercetin equivalents (QE) per gram of sample (mg/g).

### Estimation of total antioxidant capacity (TAC)

The phosphomolybdate method was employed to determine the total antioxidant capacity (TAC) through spectrophotometric analysis[19]. Ascorbic acid served as the standard, with a calibration curve established from varying concentrations of a 1 mg/mL stock solution. Samples and standards were treated with phosphomolybdate reagent and warmed to 95°C for 15 minutes. Optical density was then measured at 670 nm. The TAC values are reported in terms of milligrams of ascorbic acid equivalents per gram of sample (mg/g).

### Ferric Reducing Potential Assay (FRPA) for flavonoid antioxidant activity

The Ferric Reducing Potential Assay (FRPA) was used to quantify the antioxidant activity of flavonoids extracted from strawberry and kiwi[17]. We prepared a reaction mixture by combining the sample, 0.2 M phosphate buffer (pH 6.6), and 1% potassium ferricyanide. This mixture was then incubated at 50°C. Next, we introduced 10% trichloroacetic acid and 0.1% ferric chloride, followed by a final

incubation at room temperature (25°C). OD was measured at 670 nm. Using an ascorbic acid standard curve (1 mg/mL), the antioxidant activity was calculated and presented as mg of ascorbic acid equivalents per gram of sample.

### Human red blood cell (HRBC) membrane stabilization assay for anti-inflammatory activity

The anti-inflammatory activity of the extracts was determined using a modified human red blood cell membrane stabilization test[19]. A 10% erythrocyte suspension was prepared from fresh human whole blood (centrifugation and washing with isotonic saline) and diluted in pH 7.4 phosphate-buffered saline. Test extracts and the standard drug, aspirin (15 mg/mL), were incubated with the suspension, initially at 37°C and then at 54°C to induce hemolysis. Following centrifugation, the supernatant's optical density was measured at 540 nm to quantify hemolysis. The anti-inflammatory effect was calculated as the percentage inhibition of hemolysis using the following formula:

$$\text{Percentage inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

### Qualitative estimation of anti-acetylcholinesterase activity via TLC

The anti-acetylcholinesterase activity of flavonoids was qualitatively assessed using TLC[20]. Flavonoid samples were separated on a silica gel TLC plate with a trichloromethane:methanol (8:2 v/v) solvent system. Following separation, the plate was sequentially treated with 5 mM DTNB (5, 5-dithio-bis-2-nitrobenzoic acid) reagent and 5 mM acetylcholine iodide substrate. After air-drying, AChE enzyme was uniformly applied. Inhibition was observed as characteristic fading of flavonoid spots, indicating localised enzyme suppression.

### Anti-acetylcholinesterase activity detection of flavonoids

Flavonoid extracts were evaluated for their anti-acetylcholinesterase (AChE) activity using a colourimetric biochemical assay[21,22]. Reaction mixtures, containing flavonoid extracts, Tris buffer (pH 8.0), AChE enzyme, and DTNB reagent, were incubated at 37°C for 15 minutes. The enzymatic reaction was started by the subsequent addition of acetylcholine iodide. Donepezil hydrochloride served as a positive control; negative and blank controls were also included. Anti-AChE activity was determined by measuring the optical density at 405-420 nm against the formation of yellow thionitrobenzoate.

$$\%\ \text{inhibition} = 1 - \frac{\text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

### Flavonoid profiling via Gas Chromatography Mass Spectrometry (GC-MS)

Flavonoid profiles of purified kiwi and strawberry extracts were determined using a Shimadzu GCMS-QP2010 Ultra mass selective detector coupled with an integrated gas chromatograph (Shimadzu, Japan). Separations were performed on an Rtx-5MS capillary column (30 m×0.25 mm ID, 0.25 µm film thickness) with helium as the carrier gas at a constant flow rate of 1.69 mL/minute. Samples were prepared by dissolving purified flavonoid extracts in methanol, and a 1 µL aliquot was injected at 200°C. The oven temperature program commenced at 50°C, increased at 7°C/minute to 180°C, and then at 10°C/minute to a final temperature of 300°C, for a total run time of 28 minutes. The mass detector functioned at 250°C, utilising electron ionization (EI) set at 70 eV. We obtained mass spectra by scanning from 40 to 500 m/z. Compound identification was achieved by comparing retention times and mass fragmentation patterns with entries in the National Institute of Standards and Technology (NIST) library[23].

### Post GC-MS screening for flavonoids and glycosides

Initial GC-MS analysis and subsequent targeted screening confirmed the presence of specific compounds. Flavonoids were identified by the alkaline Reagent Test, indicated by a characteristic colour change. Glycosides, initially detected via mass spectrometry, were confirmed using the Keller-Kiliani Test, which also yielded specific colour reactions[17].

### Data handling and presentation

All quantitative functional assays (antioxidant, anti-inflammatory, and anti-acetylcholinesterase) were performed using a minimum of three independent replicates (n = 3). The data presented in the results section are the mean values derived from these replicates. Given the substantial comparative differences observed between the two extracts, no inferential statistical analysis was applied for direct comparison.

## Results

### Sample preparation

The method proved effective as crude extraction of kiwi and strawberry fruits produced coloured liquid extracts. The process exhibited high efficiency, with an 85% yield for kiwi and 83.75% for strawberry.

Consistent volume reduction in both extracts (Table 1) further indicates an effective solid-liquid separation, likely removing insoluble fruit matter.

**Phytochemical analysis of extracts**

This study qualitatively assessed the phytochemical profiles of crude kiwi and strawberry fruit extracts, identifying and comparing the relative abundance of various bioactive compounds. Both fruits consistently contained flavonoids, polyphenols, tannins, saponins, terpenoids, steroids, and glycosides. Notably, alkaloids were absent from both (Table 1). A consistent finding was the higher relative abundance of all detected phytochemicals in strawberry extract compared to kiwi. This indicates that strawberry generally possesses a richer profile of these compounds than kiwi.

**Thin Layer Chromatography for general phytochemical analysis**

Thin-layer chromatography (TLC) was utilized to characterize the phytochemical profiles of crude kiwi and strawberry extracts (Fig. 1). The separation results, detailed in Table 2, demonstrated distinct chemical compositions between the two fruits. The kiwifruit extract exhibited fewer, more distinct spots, primarily concentrated at high retention factors (Rf) (Table 2). This pattern suggests the presence of a less complex mixture, where the dominant compounds are relatively nonpolar and strongly interact with the nonpolar mobile phase. In contrast, the strawberry extract displayed a greater number of distinct spots distributed across a wider range of Rf values (Table 2). This broad distribution signifies a more complex and diverse mixture of compounds, covering a broader spectrum of polarities. This finding is consistent with the strawberry extract's generally richer qualitative phytochemical profile observed in preliminary tests. Overall, the TLC results confirmed the effective separation and distinct profiles of the phytochemical components within both fruit extracts.

**Flavonoid extraction**

This process yielded 0.6 gm of residue from strawberry and 1.2 gm from kiwi. The obtained residues were then reconstituted with methanol to a final volume of 5 mL.

**Thin Layer Chromatography (TLC) for flavonoids in extracts**

Thin-layer chromatography (TLC) was used to differentiate the flavonoid profiles of strawberry and kiwi extracts (Fig. 2). The complete chromatographic details, including spot count, colour, and retention factors (Rf), are summarised in Table 3. The strawberry extract exhibited a more complex profile, displaying a greater number of distinct spots with relatively similar high Rf values. This suggests the presence of multiple chromophoric compounds that share comparable structural characteristics and polarity, with a tendency toward nonpolar movement in the solvent system used. In contrast, the kiwifruit extract displayed a simpler profile with fewer distinct spots. These spots were characterised by significantly different Rf values, suggesting a greater diversity in compound polarity and chemical structure, even

Table 1 — Yield and qualitative phytochemical screening results for Kiwi and Strawberry extracts

| Extract feature | Kiwi extract | Strawberry extract |
|---|---|---|
| Extraction yield (%) | 85.00 | 83.75 |
| Volume reduction | Consistent | Consistent |
| Flavonoids | Present | Present (Higher abundance) |
| Polyphenols | Present | Present (Higher abundance) |
| Tannins | Present | Present (Higher abundance) |
| Saponins | Present | Present (Higher abundance) |
| Terpenoids | Present | Present (Higher abundance) |
| Steroids | Present | Present (Higher abundance) |
| Glycosides | Present | Present (Higher abundance) |
| Alkaloids | Absent | Absent |

Table 2 — Retention factors (Rf) of separated components in crude Kiwi and Strawberry extracts via thin layer chromatography

| Sample | No. of spots | Colour of spots | Rf value |
|---|---|---|---|
| Strawberry | 2 | Blue | 0.89 |
| | | Blue | 0.78 |
| Kiwi | 3 | Blue | 0.89 |
| | | Orange | 0.83 |
| | | Blue | 0.78 |

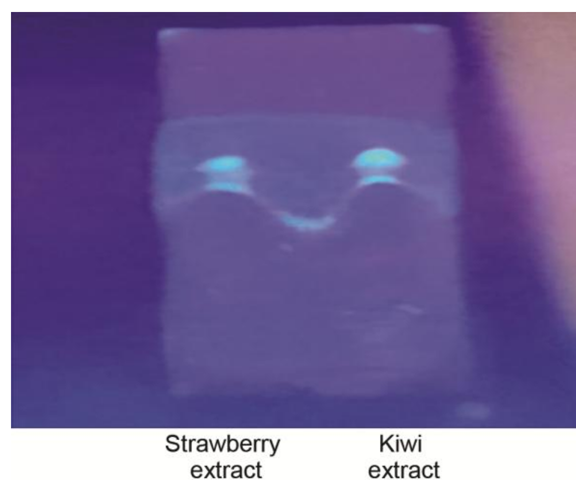


Fig. 1 — UV visualisation of a TLC plate demonstrating the separation of general phytochemicals present in crude extracts of Strawberry and Kiwi.

Fig. 2 — Thin-Layer Chromatography (TLC) analysis of flavonoid extracts from Strawberry(A) and Kiwi (B).

Table 3 — Chromatographic characteristics of flavonoid compounds in Strawberry and Kiwi extracts as determined by TLC

| Sample | No. of spots | Colour of spot | Rf value |
|---|---|---|---|
| Strawberry | 3 | Bluish-green | 0.62 |
| | | Orange | 0.68 |
| | | Yellow | 0.73 |
| Kiwi | 2 | Yellow | 0.45 |
| | | Orange | 0.87 |

within a simpler overall profile. Strawberry exhibited a greater number of detectable flavonoid compounds under the experimental conditions. It is noteworthy that while both fruits contained an orange pigment, their contrasting Rf values (Table 3) indicate that these are chemically distinct compounds. In conclusion, the TLC analysis successfully confirmed that strawberry and kiwifruit possess unique flavonoid compositions, distinguished by their chromatographic characteristics, highlighting their individual chemical profiles and properties.

**Determination of Total Flavonoid Content (TFC)**

The total flavonoid content (TFC) in strawberry and kiwi extracts was quantified using the $AlCl_3$ method. Both strawberry and kiwi extracts, at 10 µL aliquots, consistently showed an optical density of 0.04. This resulted in a calculated TFC of 20 mg/mL for both fruits, expressed as quercetin equivalents (QE) per gram of sample. These findings suggest that under the employed experimental conditions, strawberry and kiwi possess a comparable total flavonoid content.

**Total Antioxidant Capacity (TAC) assessment**

The total antioxidant capacity (TAC) was quantified using the phosphomolybdate assay, with ascorbic acid as the reference standard. Under the experimental conditions, kiwi demonstrated a higher TAC (0.118 mg/mL ascorbic acid equivalents) compared to strawberry (0.082 mg/mL ascorbic acid equivalents).

**Ferric Reducing Potential Assay (FRPA) assay**

The Ferric Reducing Potential assay (FRPA), using ascorbic acid as a standard, determined the total antioxidant capacity. Both strawberry and kiwi samples showed identical FRPA values of 4.6 mg ascorbic acid equivalents (AAE)/mL, indicating comparable ferric reducing antioxidant power.

**Human red blood cell (HRBC) membrane stabilization assay for anti-inflammatory activity**

This research investigated how well strawberry and kiwi extracts could reduce inflammation, using a test that examines their effect on red blood cell membranes. This method gauges the extracts' ability to protect red blood cells from heat-induced lysis, a process that simulates cellular membrane damage seen in inflammation. Both extracts significantly inhibited heat-induced hemolysis compared to the control, demonstrating anti-inflammatory activity. Kiwi extract proved more potent, achieving 83% inhibition of hemolysis, while strawberry extract showed substantial protection with 73% inhibition. These findings suggest that compounds in both fruits contribute to anti-inflammatory benefits, likely through membrane stabilization.

**Qualitative assessment of flavonoid anti-acetylcholinesterase activity via TLC**

Thin layer chromatography (TLC) qualitatively assessed the anti-acetylcholinesterase activity of flavonoids in strawberry and kiwi extracts. The strawberry sample exhibited one prominent flavonoid spot near the origin, while the kiwi sample displayed three distinct spots, with both also showing colored trails (Fig. 3A). Upon applying 3 U/mL acetylcholine enzyme, the flavonoid spots and trails from both samples rapidly faded and completely disappeared within one minute. Simultaneously, white spots emerged where flavonoids were initially present, indicating enzyme activity (Fig. 3B). The kiwi extract demonstrated more pronounced white spots and greater fading than the strawberry extract, suggesting its superior acetylcholinesterase inhibitory capacity.

**Acetylcholinesterase inhibitory activity of flavonoid extracts**

A colourimetric biochemical assay quantified the anti-acetylcholinesterase activity of flavonoid extracts

from strawberry and kiwi. AChE activity was inversely correlated with optical density (OD) at 405–420 nm, meaning a lower OD indicated greater enzyme inhibition. The control, representing no inhibition, had an OD of 0.04. The strawberry extract showed an OD of 0.03, translating to a modest 25% inhibition. However, both the kiwi extract and the Donepezil hydrochloride positive control exhibited an OD of 0.01, demonstrating a significant 75% inhibition of AChE. Remarkably, the kiwi extract achieved this superior inhibitory activity with less than half the volume of the strawberry extract, strongly suggesting its substantial neuroprotective potential.

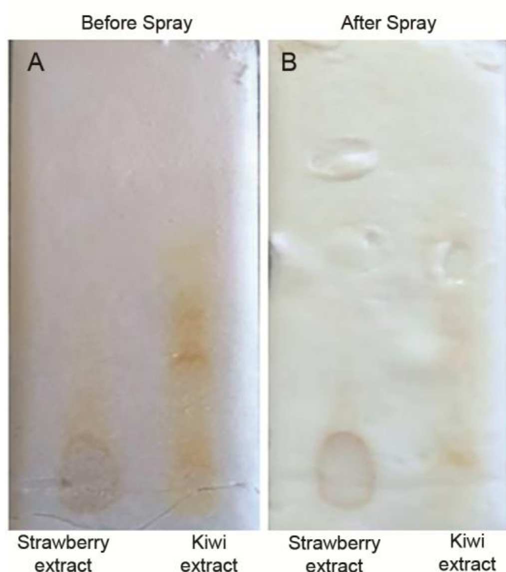


Fig. 3 — Qualitative assessment of anti-acetylcholinesterase activity of flavonoid extracts from Strawberry and Kiwi using Thin-Layer Chromatography.

**GC-MS analysis of flavonoids**

Gas Chromatography-Mass Spectrometry (GC-MS) was employed to analyse the volatile and semi-volatile compounds present in kiwi and strawberry samples.

The chromatogram for the kiwi sample revealed five main peaks, indicating the presence of several organic compounds (Fig. 4). A summary of the identified compounds, retention times and their relative abundances (area% and height %) is presented in Table 4.

The kiwi sample is dominated by the furan derivative, 5-(Hydroxymethyl)-2-(dimethoxymethyl) furan. It is the most abundant compound identified, constituting a substantial 60.28% of the total detected area (Fig. 4, peak 5). This compound is structurally related to 5-(hydroxymethyl) furfural, a known product of carbohydrate degradation. Another significant constituent was 3,3,3-Trifluoro-2-(4-hydroxy-2-methoxy-phenylsulfonate) (Fig. 4, Peak 3), a phenolic-like compound with a trifluoro-substitution, suggesting a complex plant metabolite. Other compounds detected in smaller proportions included n-Caproic acid vinyl ester (Fig. 4, Peak 1)

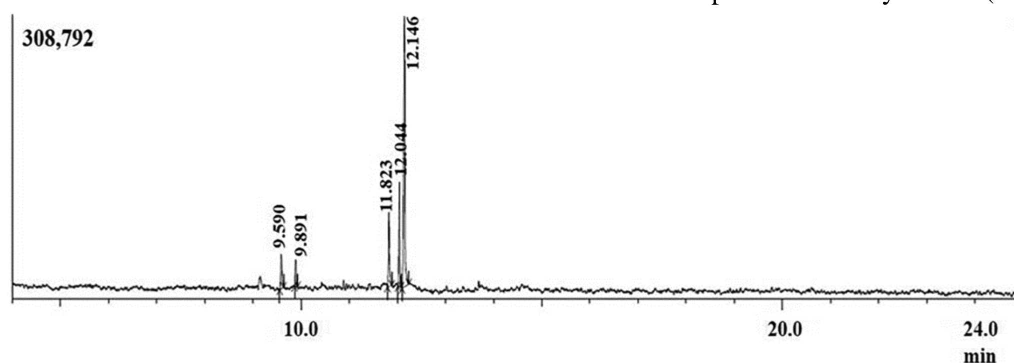


Fig. 4 — GC-MS chromatogram of the Kiwi sample.

Table 4 — GC/MS analyses of the chemical constituents in the kiwi sample indicating retention times, chemical constituents, and peak areas

| Retention time (Minute) | Chemical constituent | Formula | Peak area (%) | Height (%) |
|---|---|---|---|---|
| 9.59 | n-Caproic acid vinyl ester | C8H14O2 | 6.29 | 6.58 |
| 9.891 | Pyridine, 4-methoxy-1-oxide | C6H7NO2 | 4.06 | 5.38 |
| 11.823 | 3,3,3-Trifluoro-2-(4-hydroxy-2 methoxy-phenylsulfonate) | C9H8F3O5S | 13.31 | 14.46 |
| 12.044 | Unidentified Compound | - | 16.06 | 20.72 |
| 12.146 | 5-(Hydroxymethyl)-2-(dimethoxymethyl)furan | C8H12O4 | 60.28 | 52.86 |

and Pyridine, 4-methoxy-1-oxide- (Fig. 4, Peak 2). A notable unidentified compound accounted for 16.06% of the total area (Fig. 4, Peak 4), warranting further investigation for its characterisation.

The GC-MS analysis of the strawberry sample identified three main peaks (Fig. 5). The identified compounds and their relative abundances are summarised in Table 5.

The strawberry sample's chemical profile was also largely dominated by the same furan derivative observed in kiwi. 5-(Hydroxymethyl)-2-(dimethoxymethyl) furan was again the most abundant compound, representing 52.02% of the total detected area (Fig. 5, Peak 3). The phenolic-like compound, 3, 3, 3-Trifluoro-2-(4-hydroxy-2-methoxy-phenylsulfonate, was also a major component, contributing 36.53% of the total area (Fig. 5, Peak 1). Similar to the kiwi sample, an unidentified compound was present, accounting for 11.45% of the total area (Fig. 5, Peak 2).

GC-MS analysis of kiwi and strawberry flavonoid extracts primarily identified 5-(Hydroxymethyl)-2-(dimethoxymethyl) furan, comprising 60.28% (Table 4) and 52.02% (Table 5) of total detected compounds, respectively. This compound is likely a carbohydrate degradation product, potentially formed during fruit maturation, storage, or sample preparation, suggesting either high sugar content or its natural prevalence. Additionally, 3,3,3-Trifluoro-2-(4-hydroxy-2-methoxy-phenylsulfonate), a phenolic compound unusual for natural plant sources due to its trifluoro group, was also detected in both samples, warranting further investigation into its origin.

Despite prior phytochemical screening indicating the presence of flavonoids, intact flavonoid structures (both aglycan and glycan parts) were not identified. The detected compounds, such as 5-(Hydroxymethyl)-2-(dimethoxymethyl) furan, are typically degradation products or small molecules from carbohydrate metabolism. This suggests that the high temperatures of GC-MS likely caused thermal degradation of the labile aglycan portions and cleavage of glycosidic bonds, leading to the detection of stable glycan fragments or other breakdown products rather than the intact flavonoid molecules.

**Post GC-MS screening for flavonoids and glycoside**

Both kiwi and strawberry samples yielded positive results in the Alkaline Reagent test (Fig. 6A), indicating successful flavonoid isolation. Later Keller-Kiliani tests also confirmed these extracts contained glycosides with 2-deoxy sugars, as depicted in Fig. 6B, reinforcing the glycosidic nature of the identified compounds.

## Discussion

Neurodegenerative disorders pose a significant global health challenge, necessitating the development of effective and well-tolerated therapeutic strategies[19]. Natural compounds, particularly those found in dietary fruits, offer promising avenues due to their diverse bioactive properties[24]. Motivated by the neuroprotective potential of *Fragaria* × *ananassa*

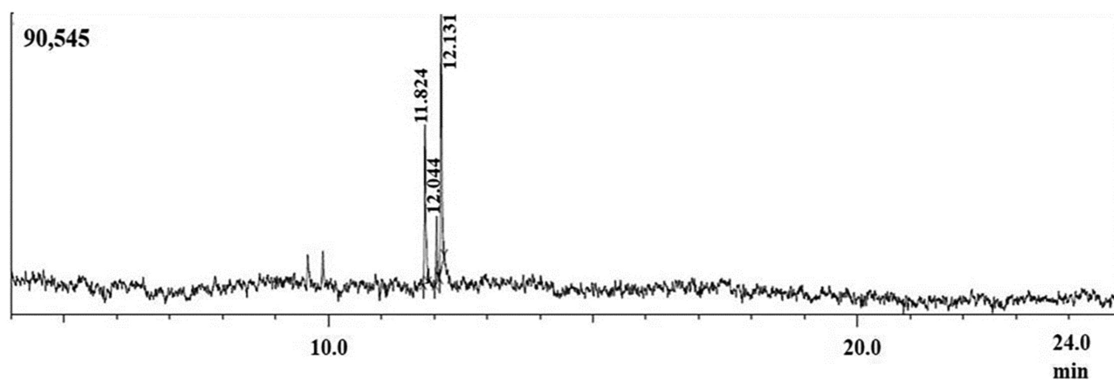


Fig. 5 — GC-MS chromatogram of the Strawberry sample.

Table 5 — GC/MS analyses of the chemical constituents in the strawberry sample indicating retention times, chemical constituents, and peak areas

| Retention time (Minute) | Chemical constituent | Formula | Peak area (%) | Height (%) |
|---|---|---|---|---|
| 11.824 | 3,3,3-Trifluoro-2-(4-hydroxy-2 methoxy-phenylsulphonate) | C9H7F3O6S | 36.53 | 33.08 |
| 12.044 | Unidentified Compound | - | 11.45 | 13.24 |
| 12.131 | 5-(Hydroxymethyl)-2-(dimethoxymethyl) furan | C8H12O4 | 52.02 | 53.68 |

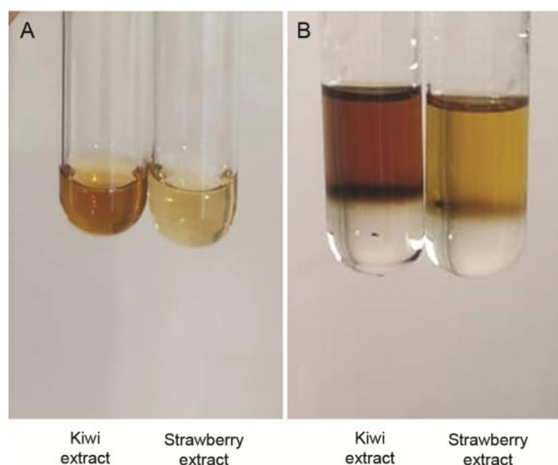


Fig. 6 — (A) Alkaline reagent test for flavonoids and (B) Keller-Kiliani test for glycosides in Kiwi and Strawberry extracts, post GC-MS.

(strawberry) and *Actinidia deliciosa* (kiwifruit), this study integrated chemical characterisation including qualitative phytochemical screening, total flavonoid content determination, and GC-MS analysis with biological evaluations. The latter encompassed assessments of their antioxidant, anti-inflammatory, and crucially, anti-acetylcholinesterase activities. This multidisciplinary approach facilitated direct correlation between the chemical profiles of these fruit extracts and their observed bioactivities, thereby providing mechanistic insights into their potential therapeutic roles.

The present study comprehensively investigated the phytochemical composition, antioxidant, anti-inflammatory, and anti-acetylcholinesterase activities of strawberry and kiwi extracts, with a particular emphasis on their neuroprotective potential, thereby contributing specific comparative data to existing literature. It systematically characterised the phytochemical composition and antioxidant capacity of strawberry and kiwi extracts. The phytochemical characterisation confirmed the presence of diverse compounds, including flavonoids, polyphenols, and terpenoids, with strawberry generally exhibiting a richer qualitative profile. While both fruits showed comparable total flavonoid content, kiwi extract consistently demonstrated a higher total antioxidant capacity (TAC) and ferric reducing potential (FRPA). These quantitative findings align with existing literature linking the inherent phytochemical composition of these fruits to their antioxidant properties[1] and support the foundational principles of oxidative stress and antioxidant mechanisms[4,5]. Critically, this study provides direct comparative data on the phytochemical profiles and antioxidant activities of strawberry and kiwi under controlled experimental conditions, which was previously lacking.

Furthermore, it demonstrates significant anti-inflammatory properties for both strawberry and kiwi extracts, as evidenced by their ability to stabilise the human red blood cell membrane. Kiwi extract, however, showed a superior ability to inhibit hemolysis compared to strawberry. This supports the broad role of flavonoids as potential anti-inflammatory molecules[10] and aligns with mechanisms of mitigating cellular damage during inflammatory responses[8]. Our data offers specific, comparative experimental evidence for the anti-inflammatory efficacy of these two fruit extracts, contributing to the knowledge base on natural anti-inflammatory agents.

A novel and significant contribution of the present study is the comprehensive assessment of anti-acetylcholinesterase (AChE) activity in strawberry and kiwi extracts. Both qualitative TLC and quantitative colourimetric assays consistently demonstrated that kiwi extract possesses a more pronounced inhibitory capacity against AChE than strawberry, strongly suggesting its potent neuroprotective potential. These findings are highly relevant given the established global burden of neurodegenerative disorders, particularly Alzheimer's disease, and the recognised therapeutic importance of targeting cholinergic neurotransmission through AChE inhibition[11-14]. They further support the concept that polyphenols can act as novel therapeutic agents in central nervous system disorders[15]. By providing direct comparative experimental evidence, this study uniquely identifies kiwi as a particularly promising natural source for potent neuroprotective compounds via AChE inhibition.

GC-MS has been utilised for compound identification, but notably, intact flavonoid structures were not detected, a phenomenon attributed to thermal degradation during the analytical process. This methodological challenge necessitated subsequent post-GC-MS chemical tests, specifically the Alkaline Reagent test and Keller-Kiliani test, to confirm the presence of flavonoids and glycosides, respectively. This observation regarding thermal degradation is strongly supported by recent scientific findings on flavonoid stability under heat. For instance, Lin *et al.*[25] directly demonstrated that

Polyhydroxy flavonols readily degraded during thermal processing, even in boiling water, via the opening of their heterocyclic ring C. Further evidence comes from studies on roasting, where Deng *et al.*[26] and Rohn *et al.*[27] consistently reported the breakdown of flavonoid glycosides under elevated temperatures. This inherent thermal instability underscores the importance of careful analytical method selection and the necessity of using complementary confirmatory tests for heat-sensitive plant compounds.

The current study offers valuable comparative data on the neuroprotective potential of strawberry and kiwi extracts but also highlights several limitations that can guide future research. A key methodological challenge was the thermal degradation of intact flavonoid structures during GC-MS analysis, a process where high temperatures likely destroyed heat-sensitive compounds. This issue underscores the importance of using complementary analytical techniques, such as the Alkaline Reagent and Keller-Kiliani tests, to confirm the presence of these compounds. This finding provides a crucial insight for future studies on heat-sensitive plant compounds, emphasising the need for methods that preserve molecular integrity. The superior anti-acetylcholinesterase (AChE) activity of kiwi extract observed in this *in vitro* study warrants further investigation. Future research should focus on isolating and identifying the specific compounds responsible for this effect. It is hypothesised that kiwifruit contains unique, heat-labile glycosylated flavonoids that are more potent AChE inhibitors than those found in strawberries, a proposition supported by the quantitative results showing a more potent inhibitory effect for kiwi extract despite comparable total flavonoid content. Subsequent research should also validate these promising neuroprotective effects through in vivo studies to assess their efficacy in a biological system. From a clinical perspective, the findings suggest that incorporating kiwifruit into one's diet could be a natural, preventative strategy against neurodegenerative disorders. The potent antioxidant, anti-inflammatory, and anti-AChE properties of kiwi extracts reinforce the broader notion that a diet rich in fruits and natural compounds can play a beneficial role in disease prevention and health maintenance.

## Conclusion

This study conducted a comprehensive comparative analysis of the phytochemical composition, antioxidant, anti-inflammatory, and anti-acetylcholinesterase activities of *Fragaria × ananassa* (strawberry) and *Actinidia deliciosa* (kiwifruit) extracts. While both fruits possess rich phytochemical profiles, the key comparative conclusion is the kiwifruit extract's superior functional potential: it consistently demonstrated significantly greater total antioxidant capacity, more potent anti-inflammatory effects, and a pronounced inhibitory effect against anti-acetylcholinesterase activity. This strong anti-AChE potential is particularly relevant for managing neurodegenerative disorders like Alzheimer's disease. Methodologically, the study highlighted challenges in analysing complex plant extracts, noting the thermal degradation of intact flavonoid structures during GC-MS and thus necessitating confirmatory chemical tests. In conclusion, this research reinforces the health benefits of both fruits and provides novel, direct comparative data on their specific bioactivities. The superior anti-AChE potential of kiwifruit extract warrants further investigation to isolate and characterise its active compounds and validate these neuroprotective effects through *in vivo* studies.

## Ethical statement

Approval to conduct hemolysis on human blood cells were approved by Institutional Ethical Committee of IILR (IEC No-19/07/21) Academy, Indore where work was done. The volunteer signed the informed consent for this study.


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## Conflicts of interest

The authors declare that they have no conflicts of interest.

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