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# Biochemical characterization of chamomile essential oil: Antioxidant, antibacterial, anticancer and neuroprotective activity and potential treatment for Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) causes dementia among older adults, increasing the global burden of dementia. Therefore, this study investigates the potential neuroprotective, antioxidant, and anticancer effects of chamomile essential oil (CCO) in Alzheimer's disease. CCO's main volatile compounds (VOCs) were α-bisabolol, camazulene, and bisabolol oxide A, representing 81 % of all VOCs. CCO scavenged 93 % of DPPH free radicals and inhibited the pathogenic bacteria, i.e., *Staphylococcus aureus* and *Salmonella typhi*, besides reducing 89 % of brain cancer cell lines (U87). Eighty albino rats were randomized into four groups: standard control, Alzheimer's disease group caused by AlCl3, and treated groups. The results indicated that the mean value of tumor necrosis factor α (TNF-α), amyloid precursor protein (APP), amyloid beta (Aβ), caspase-3, & B-cell lymphoma 2 (Bcl-2) was significantly elevated due to the harmful effect of AlCl3; however, CCO downregulated these values, and this effect was attributed to the considerable volatile compounds and phenolic compounds content. Additionally, CCO rats showed a significant increment in noradrenergic (NE), dopaminergic (DO), and serotoninergic systems with relative increases of 50, 50, and 14 % compared to diseased rats. The brain histology of CCO-treated rats showed a significant reduction in neuronal degeneration and improved brain changes, and its histology was close to that of the control brain. The results indicated that CCO offers a new strategy that could be used as an antioxidant and neuroprotective agent for AD due to its considerable contents of antioxidants and anti-inflammatory compounds.

## 1. Introduction

Alzheimer's disease (AD) is recognized as a frequent progressive neurodegenerative disease in older persons and a leading cause of dementia in the world's aging people (Sandupama et al., 2022). AD is connected with various hazard factors, including the history of the family, depression, age, skull trauma, oxidative stress, ecological metals exposure, gastrointestinal microbiome imbalance, neuroinflammation, & cognitive activity (Weng et al., 2020).

Several hypotheses have been planned to clarify this complicated disease. The first hypothesis depends on oxidative stress, which is generally associated with Aβ42 oligomers, which promote tau hyperphosphorylation, leading to toxification of the synapses and mitochondria and rigidity and damage to the cell membranes (Kurz and Perneczky, 2011). The second hypothesis is attributed to the amyloid cascade, which established that the abnormal buildup of A in the cranium is the primary etiology of Alzheimer's disease (Shallie et al., 2020).

In addition to neuroinflammation, where amyloid 42 (A42) plaques initiate microglia that in turn stimulate the release of proinflammatory cytokines like tumor necrosis factor (TNF-α) and interleukin 1 (IL-1),

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which sequentially promotes the production of A42 oligomers, there are several other important factors (Rather et al., 2018). According to the cholinergic hypothesis, the primary cause of AD is decreased acetylcholine production (ACh) (Piccarducci et al., 2023). Acetylcholinesterase (AChE) may play a role in β-amyloid fibrillogenesis, impairing cholinergic functions (Auti and Kulkarni, 2019). TNF- α is one of the most well-defined cytokines during AD pathogenesis (Kalliolias et al., 2016).

Dopamine (DO) and norepinephrine (NE) are important neuromodulators that control brain states, alertness, activity, reward, learning, and memory (Ranjbar-Slamloo and Fazlali, 2020). Neurotransmitter disturbances characterize Alzheimer's disease. However, norepinephrinergic system abnormalities are also included. It was reported that $AlCl_3$-induced AD in rats with a decline in the serum level of DO compared with their control (Elshamy et al., 2021). Accordingly, an essential and urgent need is to improve novel agents to manage this considerable problem. Natural products are popular because herbs are safer than synthetic drugs (Deng et al., 2023).

Chamomile essential oil is a deep blue liquid extracted from the flowers of the *Matricaria chamomila* plant. It is rich in azulenes, giving it its characteristic color and many beneficial properties. Chamomile essential oil is widely used in skincare products, oral hygiene products, and cosmetics to soothe inflammation, promote relaxation, and improve skin health (Srivastava et al., 2010).

Essential oils and medicinal plants are becoming increasingly popular in modern medicine because they are very active biologically and have a unique chemical composition (El-Tarabily et al., 2021; Hegazy et al., 2023; Al-Quwaie et al., 2023).

Essential oils are made from plants and are a mixture of aromatic and volatile compounds that are very light (usually below 500 Da) and can easily dissolve in organic solvents and lipids. Essential oils can easily cross cell membranes because they are lipid-soluble and have low molecular weight (Napoli and Di Vito, 2021; Mueed et al., 2023). Once inside a cell, essential oils can have a variety of biological effects, including antimicrobial activity, killing or inhibiting the growth of microbes by disrupting their cell membranes or interfering with their enzyme systems; Inhibition of cell membrane synthesis, preventing cells from constructing new cell membranes, which can lead to cell death; disruption of the structure of specific enzyme systems, which can alter the composition of certain enzyme systems in cells and interfere with their function; lowering the concentration of enzyme systems, which can also interfere with their process (El-Tarabily et al., 2021; Almuhayawi et al., 2023).

For example, essential oils can reduce the content of the enzyme HMG-CoA reductase, which is involved in cholesterol synthesis. This can lower cholesterol concentrations in the blood (Chung et al., 2007). In addition to destroying microorganisms, chamomile essential oils can cure and assist in managing several ailments, including heart disease, diabetes, Alzheimer's, and cancer (Li et al., 2022). Additionally, it reduces the inflammation (Chandrakanthan et al., 2020).

Because they contain various bioactive components, essential oils have a wide range of biological actions. Bisabolol & its oxides, A & B, chamazulene, & farnesene, are the primary components of chamomile essential oil. These compounds are responsible for many of the beneficial effects of chamomile essential oil, including its anti-inflammatory, antimicrobial, and sedative properties (Petronilho et al., 2012); they also have an inhibiting effect on the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities (Auti and Kulkarni, 2019). So, the target here was to determine the role of chamomile essential oil as a neuroprotective agent in rats suffering from AD.

## 2. Materials and methods

### 2.1. Materials

Fresh chamomile was purchased from the local market; all chemicals and media in this study were of analytical grade and purchased from (Sigma, USA). The bacterial strains *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhi*, and *Campylobacter jejuni* were used in this study to estimate the antibacterial activity of chamomile essential oils.

### 2.2. Extraction of essential oil from chamomile

Fresh chamomile plants were obtained from the local market, the flowers were manually separated to acquire a 14–15 % yield (w/w), and the plants were processed immediately. While it is customary to extract essential oils from dried samples, the flowers were utilized in this investigation due to the extraction method's specifications and the superior quality of the end product. The essential oil extraction process was conducted in the autumn at an average temperature of 20 °C and 80 % HR (Araujo et al., 2021). Clevenger's equipment used hydrodistillation to isolate the essential oil from chamomile. The amount of volatile oil produced was determined and calculated in a 100 g dry plant (Kalliolias et al., 2016).

### 2.3. Essential oils analysis

The Aligant GC (Agilent Technologies, USA) was outfitted with a 60 × 0.25 mm × 0.25 μm separation column and 10 μL of volatile organic compounds that had been separated (VOCs). The temperature of the column was adjusted at 250 °C to desorb volatile compounds, then the temperature was down by 40 °C for 3 min and then raised by 5 °C every minute to reach 235 °C for 10 min. The mobile phase consisted of helium flowing with a 1.8 mL/min degree. The detector voltage and temperature were adjusted to 230 °C and 72 eV, respectively.

The detected compounds ranging in mass from 40 to 450 milli absorbance unit (mAu) were identified using the mass spectrum. A comparison between the obtained volatile compounds' mass spectra and the NIST database is needed to identify these compounds (Pino and Barzola-Miranda, 2020).

### 2.4. Phenolic and flavonoids assessments

#### 2.4.1. Total polyphenols (TPC)

TPC was estimated by Saad et al., (2021c). The total phenols content in all extracts was determined using a colorimetric oxidation/reduction process by Jenway-UV–VIS Spectrophotometer. Folin–Ciocalteu reagent (AOAC, 2012) as an oxidizing reagent. 1 mL of each extract was combined with 2 mL of Folin–Ciocalteu reagent and 1 mL of $Na_2CO_3$ 7.5 %. The mixture was kept at 50 degrees Celsius for five minutes before being cooled. The control consisted of 0.5 mL of water. The absorbance was assessed at 760 nm. Based on the calibration curve, TPC was determined as gallic acid equivalent (GAE) according to the linear equation that follows:

$Y = 0.1751X + 0.3498.$

$R2 = 0.9734.$

The absorbance (Y); the concentration (X) (mg GAE $g - 1$ extract); R2 = Correlation Coefficient.

#### 2.4.2. Total flavonoids (TF)

With some modifications, TF was assessed, as per Saad et al., (2021a). 0.5 mL of each extract was combined with 1.5 mL of an ethanolic solution containing 20 mg/L $AlCl_3$. After one hour of addition in a dark environment, when the temperature is ambient, the absorption of the yellow color produced was assessed to be 450 nm compared to the control. TF was converted to an equal amount of quercetin (QE) via the following equation on the base of the calibration curve:

$y = 0.0025x - 0.1271.$

$R2 = 0.9885.$

X is the absorbance, and y is the concentration (μg QE/g extract).

### 2.5. Evaluation of antioxidant efficacy

#### 2.5.1. DPPH test

The antioxidant of CCO was estimated by Saad et al. (2021). After incubation for thirty minutes Using 0.1 mL, the generated color was calculated to be 517 nm in the dark of CCO (1600 μg/mL) and DPPH solution (3 mL). The percentage of antioxidant activity is calculated as follows

$$\%\ \text{Scavenging activity} = \frac{AC - AS}{AC} \text{x } 100 \quad (1)$$

#### 2.5.2. Anticancer activity

The sulforhodamine B (SRB) procedure was carried out per Vichai & Kirtikara (2006) in the U87 cell line. Brain cancerous cells (U87) were treated with varying doses of CCO (50–160 μg/mL) in a microplate reader and incubated at 37 °C for two days. After that, cancerous cells were subjected to fixation with 50 % trichloroacetic acid (TCA, 50 μL) at a cold temperature of 4 °C for one hour to stabilize their cellular components. The plate was thoroughly washed five times with tap water to remove extra TCA. The fixed cells were stained for 30 min using a 0.057 % SRB dye solution prepared in 1 % acetic acid to label the cellular proteins specifically, imparting a pink color to the protein-bound dye. After the staining step, the plate was thoroughly rinsed with 1 % acetic acid to eliminate any unbound SRB dye molecules, ensuring that only the dye bound to cellular proteins remained. The air-dried plate was then treated with 100 μL of 10 mM Tris base to solubilize the bound protein stain. Finally, the absorbance was recorded at 540 nm via a microplate reader.

#### 2.5.3. Antibacterial activity

The antibacterial of CCO was estimated by disc assay. The bacterial strains were grown overnight in a Muller Hinton broth (MHB). The cultured bacteria (1.5 x $10^8$ CFU/mL) were exposed to different CCO concentrations. A 0.1 mL of bacterial suspension was spread on the surface of Petri plates. Paper discs (6 mm) saturated with different concentrations of CCO were placed on the plate. The diameters of the inhibition zones were estimated (mm) after incubating the plates at 37 °C for 24 h using a ruler as the measuring tool. The positive control was levofloxacin to compare the antibacterial results.

### 2.6. Animals and ethical approval

Animals were housed in a light/dark cycle at a suitable temperature and 40–60 % humidity before the experimentation; the animals were acclimatized for one week. All animals were supplied with a standard pellet feed & water. After a week of acclimatization, 80 rats were distributed into four groups (each containing ten animals). The ZU-IACUC committee examined and approved the animal study.

### 2.7. Induction of Alzheimer's disease

Aluminum chloride solution was prepared and injected intraperitoneally into rats with a 100 mg/kg concentration bw/ day for 42 days (Gomaa et al., 2019). The CCO was provided on five groups of rats (n = 5); groups orally received 4, 6, 8, 10, and 12 g extract /kg b.w., respectively.

### 2.8. Experimental layout

Randomly, rats were assigned into four groups of ten rats each. Group I: control rats supplied with a vehicle, group II: AD group, group III: AD + donepezil hydrochloride (DP) group in which rats were injected intraperitoneally with aluminum chloride solution, then treated with donepezil hydrochloride (trade name; Arecipt) (1 mg/kgb.w./day) by oral route for 42 days, one-hour post administration of aluminum chloride, group IV: AD + CCO group in which rats were injected intraperitoneally. Fig. 1 shows the Experimental layout of CCO in treating AD disease in rats exposed to $AlCl_3$.

#### 2.8.1. Collection of samples

After an overnight fast, all animals were slaughtered with 2 % ether anesthesia, blood was taken, and the serum was stored at − 80 °C. The hippocampi of isolated rat brains were homogenized in PBS (pH 7.4) and then centrifuged for 10 min. The supernatants were kept at −20 °C until the biochemical analysis could be conducted.

### 2.9. Biochemical measurements

The Avi-Bion ELISA Kit was applied to assess degrees of TNF-α, acetylcholine (ACh), amyloid beta (Aβ), & amyloid precursor protein (APP), & apoptosis markers (Cas-3 and Bcl2) in rats (Orgenium Laboratories, Finland).

#### 2.9.1. Estimation of neurotransmitters

Dopamine, norepinephrine, and serotonin levels in the brain were assessed via high-performance liquid chromatography (HPLC a Shimadzu LC-10AS) with a quaternary pump, solvent degasser, an autosampler, and a C18 column. The mobile stage was a gradient solvent solution comprising water and acetonitrile with 5–60 % formic acid at a 1 mL/min flow rate. The samples were injected automatically through the use of a SIL-40Cxs autosampler. Utilizing an ESI-connected triple-quadruple spectrometer, the metabolites were identified. In the MS/MS step, fragmentation of the precursor ions was induced using 35 % of the collision energy while the instrument was operating in negative ion mode. The ions were distinguished by their mass-to-charge ratio ($m/z$) using a full-scan mass spectrometry technique, which contains the complete mass spectrum between 100 and 1500 $m/z$ (Saad et al., 2021b).

#### 2.9.2. Histological studies

At the terminal of the trial, three rats from each group were randomly selected for brain histopathology. Samples were collected from the brain. Tissue samples were fixed in formalin, prepared, embedded in paraffin, sectioned, & stained with hematoxylin & eosin (H&E). Microscopic evaluation was performed at two magnification powers, 200X and 400X.

### 2.10. Statistical analysis

SPSS version 25 was utilized for all data analysis (IBM, USA). A p-value of 0.05 was judged for the significant differences. The results were presented by applying the mean (SD) standard deviation; if the $p$-value from a one-way analysis of variance (ANOVA) was less than 0.05, the least significant differences (LSD) post hoc test was employed.

## 3. Results

### 3.1. Volatile compounds of chamomile flower essential oil

Table 1 shows the VOCs in chamomile flower essential oil. The data indicated that the main compounds in the GC–MS profile were α-Bisabolol, Camazulene, Bisabolol oxide A, 1,6-Dioxaspiro [4.4] non-3-ene, 2-(2,4-hexadiyn-1-ylidene) with contents of 26.1, 25.3, 27.8, and 25.2 % in CCO, respectively with a relative increase of 6–9 % than the whole plant. The content in entire plant essential oils was 22.3, 21.3, 24.6, and 20.5 %, with relative decreases of 12–25 % compared to flower CCO; these main compounds represent about 77 % of volatile compounds in chamomile callus. Medium contents of VOCs detected in β-Phellandrene, β-Farnesen, o-Menth-8-ene, 4-isopropylidene-1-vinyl-, α-Bisabolol oxide B, and *cis*-Geranylacetone (1.5–6 %). Also, we found that α-Pinene and Limonene were more distinct in plants than in the flower (See Table 2).

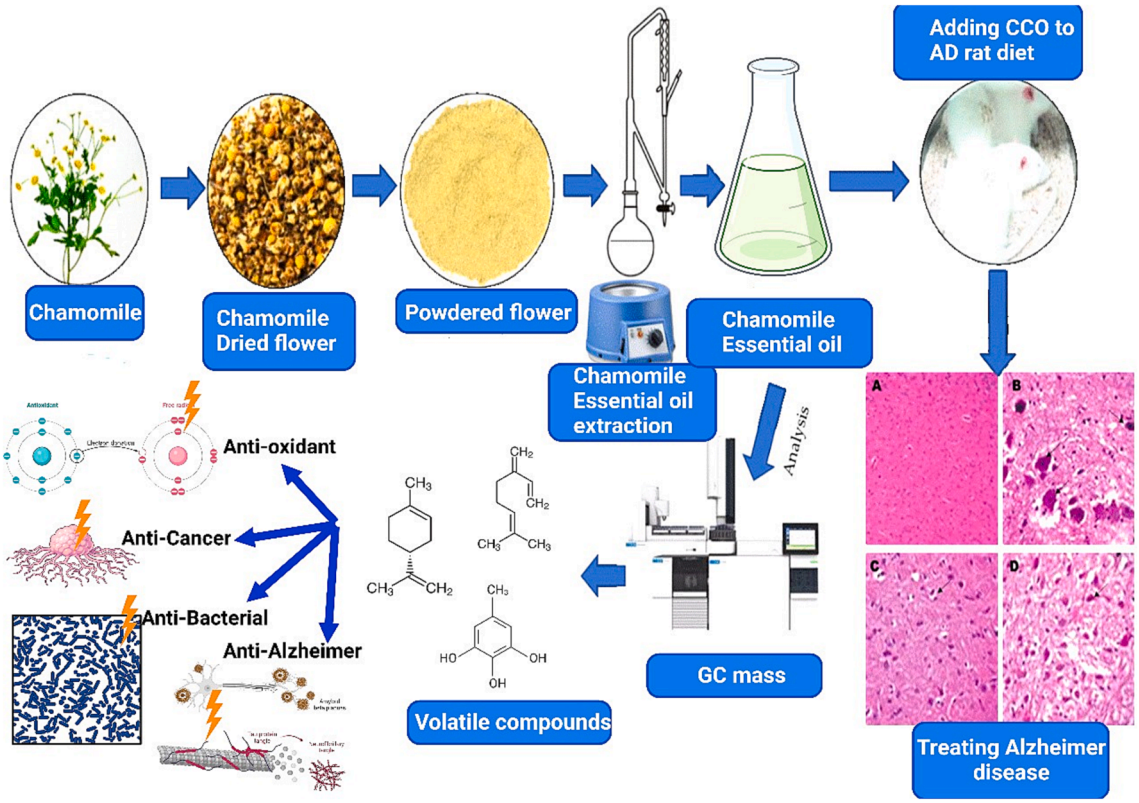


**Fig. 1.** Experimental layout of CCO in treating AD disease in rats exposed to $AlCl_3$.

### 3.2. Total phenolic & flavonoid content

As presented in Fig. 2 A & B, the total phenolic content in flower CCO enhanced with a relative increase of 15 % compared to all the essential oils in the plant. Fig. 2A showed that TPC in the CCO flower was 510 mg/g, and in plant essential oil was 444 mg/g. Concerning total flavonoids in Fig. 2B, flower CCO increased the flavonoid content by 37 % compared to plant, while the flavonoid content increased by 88 % over control. The TFC contents were 160 and 116 mg/g in flower and plant essential oils, respectively.

### 3.3. Biological activities of chamomile callus and flowers' essential oil

#### 3.3.1. Antioxidant activities

Fig. 3 displays that the radical scavenging activities of flower essential oil were better than plant essential oil, where the antioxidant activity of extract represented 75 % in scavenging DPPH free radicals, where plant CCO increased scavenging activity to 82 %; further enhancement in antioxidant activity of flower CCO were reached 93 %. The SC50 was calculated and found that 100 μg/mL of CCO scavenged 50 % of DPPH free radicals, 56 μg/mL in plant, and 35 μg/mL in flower (data not shown).

#### 3.3.2. In vitro anticancer activity against brain cancer cell lines

Fig. 4 shows the cytotoxicity potential of CCO levels (50–1600 μg/mL) compared to standard Cisplatin. The results indicated that the % inhibition activity of brain cancer cell lines increased with CCO concentration, where the highest concentration (1600 μg/mL) significantly reduced 89 % of U87 cancer cells against 85 % for Ciptalin. The IC50 of CCO was 200 μg/mL compared to 400 μg/mL for Ciptalin. The optical microscopy images in Fig. 3 A, B, and C correlated with histograms (Fig. 3D).

#### 3.3.3. Antibacterial activity

The antibacterial activity against harmful bacteria is displayed in Table 3, such as *Listeria monocytogenes* (LM), *Staphylococcus aureus* (SA), *Campylobacter jejuni* (CJ), and *Salmonella typhi* (ST). The inhibition zones increased ($p < 0.05$) with concentrations. The most vulnerable bacteria to CCO 1600 μg/mL concentration was SA (IZ, 5.1 cm), followed by LM at 4.2 cm, and ST was the most resistant bacteria with IZ of 3.1, followed by CJ at 3.5 cm. The lowest concentration that inhibits the tested bacteria ranged from 25 to 50 μg/mL.

### 3.4. Proinflammatory and apoptosis markers

Table 3 revealed a considerable increase in the serum Aβ, TNF-α, and APP contents with a corresponding reduction in ACh (650.2 ± 2.1, 77.0 ± 0.8, 97.5 ± 0.8, and 3.6 ± 0.5 in rats with AD compared to their control. However, the treatment with CCO attenuated these parameters close to control (380.4 ± 1.9, 48.2 ± 0.2, 59.8 ± 0.8, and 5.56 ± 0.8 with relative decreases of 71, 60, and 64 % in Aβ, TNF-α, and APP parameters compared to AD groups, followed by an enhancement of 52 % in Ach. In rats with Alzheimer's disease (AD), apoptosis markers Cas-3 and Bcl-2 expression levels were raised ($p = 0.001$) compared to the control. However, CCO administration effectively reduced these elevated levels, bringing them closer to those observed in the control group.

#### 3.4.1. Neurotransmitter's content

Table 4 displays a considerable drop in the amount of NE in the cranium tissues of rats with AD (G1) compared to their control counterparts. Also, AD rats treated with DP showed an increase in NE compared with (G2). Furthermore, AD rats, when treated with CCO (G4), showed a significant increment in the brain levels of noradrenergic (NE), dopaminergic (DO), and serotoninergic systems (6.2 ± 0.8, 4.8 ± 0.9, and 3.2 ± 0.4 as compared to rats with AD in G2 (3.3 ± 0.03, 3.2 ± 0.04, and 2.8 ± 0.13) with relative increases of 50, 50, and 14 %.

**Table 1**
Volatile compounds profile in CCO by detected GCMS.

| No | Compound name (MS identification) | Chamomile | |
|---|---|---|---|
| | | Plant | Flower |
| 1 | α-*cis*-Ocimene | 0.3 ± 0.0b | 0.5 ± 0.02a |
| 2 | β-*cis*-Ocimen | 0.8 ± 0.02b | 1.1 ± 0.1a |
| 3 | α-Bisabolol | 24.6 ± 1.5b | 26.1 ± 0.9a |
| 4 | Bisabolol oxide A | 26.1 ± 0.9b | 27.8 ± 1.6a |
| 5 | α-Bisabolol oxide B | 2.9 ± 0.2b | 3.2 ± 0.2a |
| 6 | *cis*-Z-α-Bisabolene epoxide | 0.6 ± 0.01b | 0.9 ± 0.01a |
| 7 | Borneol | 0.7 ± 0.002 | 0.9 ± 0.0 |
| 8 | 3-Cyclohexen-1-ol | 0.5 ± 0.01 | 0.8 ± 0.0 |
| 9 | β-Farnesen | 6.0 ± 0.2 | 6.8 ± 0.8 |
| 10 | 1,6-Dioxaspiro [4.4] non-3-ene, 2-(2,4-hexadiyn-1-ylidene) | 22.3 ± 0.9b | 25.2 ± 0.9a |
| 11 | o-Menth-8-ene, 4-isopropylidene-1-vinyl- | 3.5 ± 0.5 | 4 ± 0.5 |
| 12 | (-)-Spathulenol | 0.4 ± 0.01 | 0.6 ± 0.01 |
| 13 | α-Pinene | 1.0 ± 0.2a | 0.5 ± 0.04b |
| 14 | β-Phellandrene | 1.5 ± 0.3 | 1.5 ± 0.5 |
| 15 | γ-Terpinen | 0.6 ± 0.01b | 0.9 ± 0.01a |
| 16 | m-Cymene | 0.4 ± 0.02 | 0.5 ± 0.06 |
| 17 | Artemisia ketone | 0.6 ± 0.02 | 0.8 ± 0.05 |
| 18 | Coumarin, 7-methoxy | 0.8 ± 0.04b | 1.1 ± 0.4a |
| 19 | Camazulene | 23.5 ± 0.5b | 25.3 ± 1.3a |
| 20 | *cis*-Geranylacetone | 3.2 ± 0.9b | 4.2 ± 0.2a |
| 21 | Caryophyllene oxide | 0.8 ± 0.01 | 0.9 ± 0.05 |
| 22 | Limonene | 1.1 ± 0.6b | 1.6 ± 0.6a |
| 23 | 7-Isopropyl-1,4-dimethyl-2-azulenol | 0.9 ± 0.01 | 1 ± 0.4 |

Data are presented mean ± SE, lowercase letters in rows indicating significant differences.

**Table 2**
Antibacterial activity of chamomile essential oil against pathogenic bacteria.

| Tested bacteria | Concentrations μg/mL/IZ (cm) | | | | | | MIC |
|---|---|---|---|---|---|---|---|
| | 50 | 100 | 200 | 400 | 800 | 1600 | |
| SA | 1.5 ± 0.01$^{a}$ | 2.1 ± 0.1$^{a}$ | 2.9 ± 0.2$^{a}$ | 3.5 ± 0.2$^{a}$ | 4.3 ± 0.3$^{a}$ | 5.1 ± 0.5$^{a}$ | 25 ± 0.9$^{d}$ |
| LM | 0.9 ± 0.02$^{b}$ | 1.8 ± 0.2$^{b}$ | 2.7 ± 0.4$^{ab}$ | 3.0 ± 0.6$^{b}$ | 3.8 ± 0.1$^{b}$ | 4.1 ± 0.2$^{b}$ | 30 ± 1.2$^{c}$ |
| CJ | 0.8 ± 0.05$^{b}$ | 1.6 ± 0.2$^{b}$ | 1.9 ± 0.6$^{b}$ | 2.4 ± 0.4$^{c}$ | 2.9 ± 0.4$^{c}$ | 3.5 ± 0.4$^{c}$ | 40 ± 0.3$^{b}$ |
| SA | – | 0.8 ± 0.01$^{c}$ | 1.7 ± 0.1$^{c}$ | 2.2 ± 0.5$^{c}$ | 2.8 ± 0.8$^{c}$ | 3.1 ± 0.3$^{c}$ | 50 ± 0.9$^{a}$ |

*Listeria monocytogenes* (LM), *Staphylococcus aureus* (SA), *Campylobacter jejuni* (CJ), and *Salmonella typhi* (ST). Data are presented mean ± SE. Lowercase letters in the same column indicate significant differences.

### 3.5. Brain histology

Fig. 5 shows the brain tissues stained by H&E of rats treated with aluminum chloride to induce Alzheimer-like disease compared to the control group & groups treated by CCO and AP. The X200 magnification photomicrograph of the brain revealed the typical structure of brain cells & neurons (Fig. 3 A), while in $AlCl_3$-treated rats, the microscopic image displayed degeneration & shrinkage of neurons, with the formation of clumps of amorphous pink material in the cerebral cortex, necrosis of neurons, creation of localized region of malacia in the cerebrum, neuritic plaques, and neurofibrillary tangles (Fig. 3B), Fig. 3C that CCO-treated rats after AD induction, showed a considerable reduction in neuronal degeneration and improvement in brain changes, and its histology close to control brain. In the Ap-treated group, the photomicrograph of the brain at a magnification of X400 revealed modest to moderate neuronal degeneration (Fig. 3D).

## 4. Discussion

Due to hereditary and environmental factors, neurodegenerative diseases are becoming increasingly prevalent among those over 65 in affluent nations. AD is projected to impact 1 in 85 individuals by 2050 (Uddin et al., 2016). AD, an irreversible neurodegenerative disorder, is defined by the gradual loss of neurons in the brain, resulting in a progressive decline in short-term memory, cognitive function, and overall mental well-being. The initial clinical symptom is short-term memory loss, followed by mental and learning issues like forgetting names & words when speaking, mood swings, difficulty computing, & inability to use common items & tools (Lakshmi et al., 2015).

Pathological indicators involve apoptotic neuronal death, overexpression of highly phosphorylated tau proteins (Nampoothiri et al., 2015), amyloid plaques, neurofibrillary tangles (beta-amyloid proteins accumulate due to degeneration of neuronal processes), oxidative stress, and cholinergic dysfunction (Majdi et al., 2020). Amyloid plaques consist of amyloid- (A), a fragment of the amyloid-protein precursor (APP). β-secretase (BACE 1), subsequent-secretase, cleaves APP to generate A (Song et al., 2021).

The buildup of A monomers culminates in the formation of oligomers, fibrils, & insoluble amyloid plaques, which interfere with synaptic and neuronal function. Creating intracellular circumstances favorable to the existence of neurofibrillary tangles, leading to the death of neurons and consequent impairment of neurotransmitter function (Selkoe and Hardy, 2016). Intracellular neurofibrillary tangles, composed of hyper- and incorrectly phosphorylated tau protein, are the other characteristic protein aggregation in AD (Srivastava et al., 2021). The neuroprotective mechanism of donepezil reduces the damage induced by A. Additionally, by reducing IL-1 and cyclooxygenase-2 synthesis, donepezil can lower brain and spleen systemic inflammation (Kim et al., 2017). As possible therapeutic agents, natural goods are gaining favor. As demonstrated by neuroprotective therapies, animal- and plant-derived compounds, like omega-3, fatty acids, & plant-derived chemicals, lower cellular toxicity and have anti-inflammatory characteristics (Rehman et al., 2019).

The course of Alzheimer's disease is hastened by inflammation, which leads to neurodegeneration. Thus, early prevention & control of inflammation may aid in treating or reducing Alzheimer's disease symptoms. It was demonstrated that phytochemicals with anti-inflammatory, antioxidant, & neuroprotective properties can promote and prevent neurodegeneration in AD. The course of Alzheimer's disease is hastened by inflammation, which leads to neurodegeneration. Therefore, early prevention and control of inflammation may aid in treating or reducing AD symptoms. It has been demonstrated that phytochemicals with anti-inflammatory, antioxidant, & neuroprotective properties can promote & hinder neurodegeneration in AD (Cooper & Ma, 2017).

Alzheimer's is an atypical neurodegenerative disorder characterized by neuron loss in the hippocampus and cerebral cortex. Mental dysfunction, aberrant behaviors, and memory loss characterize this illness. Several alternative theories were postulated to explain the pathophysiology of AD; considerable evidence suggests that memory impairment in AD is mediated by synapse failure & loss. Therefore, medicines are critically needed to repair or sustain synapse function and

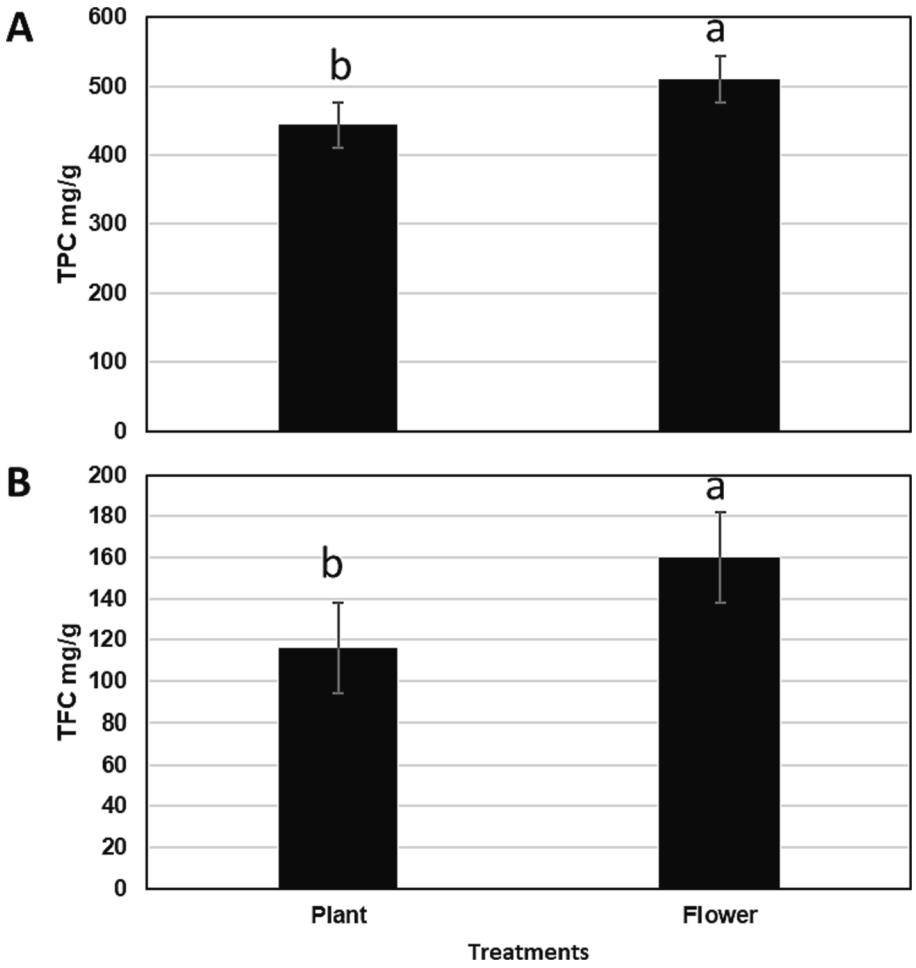


**Fig. 2.** (A) Total phenolic content, (B) Total flavonoid content of chamomile essential oil. Data are presented mean ± SE. Lowercase letters in the above column indicate significant differences.

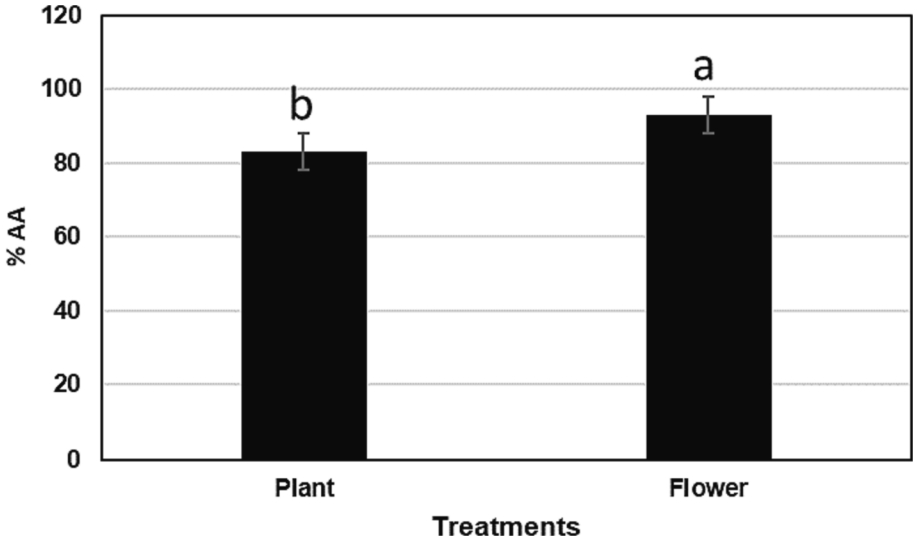


**Fig. 3.** DPPH scavenging activity of chamomile essential oil. Data are presented mean ± SE. Lowercase letters in the above column indicate significant differences.

cognition (Lourenço et al., 2019; Gutiérrez et al., 2022). Disruption in cholinergic neurotransmission corresponds dramatically with the degree of Alzheimer's disease-related neuropathological abnormalities (Haider et al., 2020). Aluminum disrupts cholinergic neurotransmission, which stimulates the activity of AChE & promotes ACh breakdown in the cranium, according to reports (Xie et al., 2015; Justin Thenmozhi et al., 2017).

The aggregation of senile plaques in the brain was discovered to be one of the explanations for the development of AD. A, the primary component of senile plaques, is formed by plated sheet fibrils, neuritis of

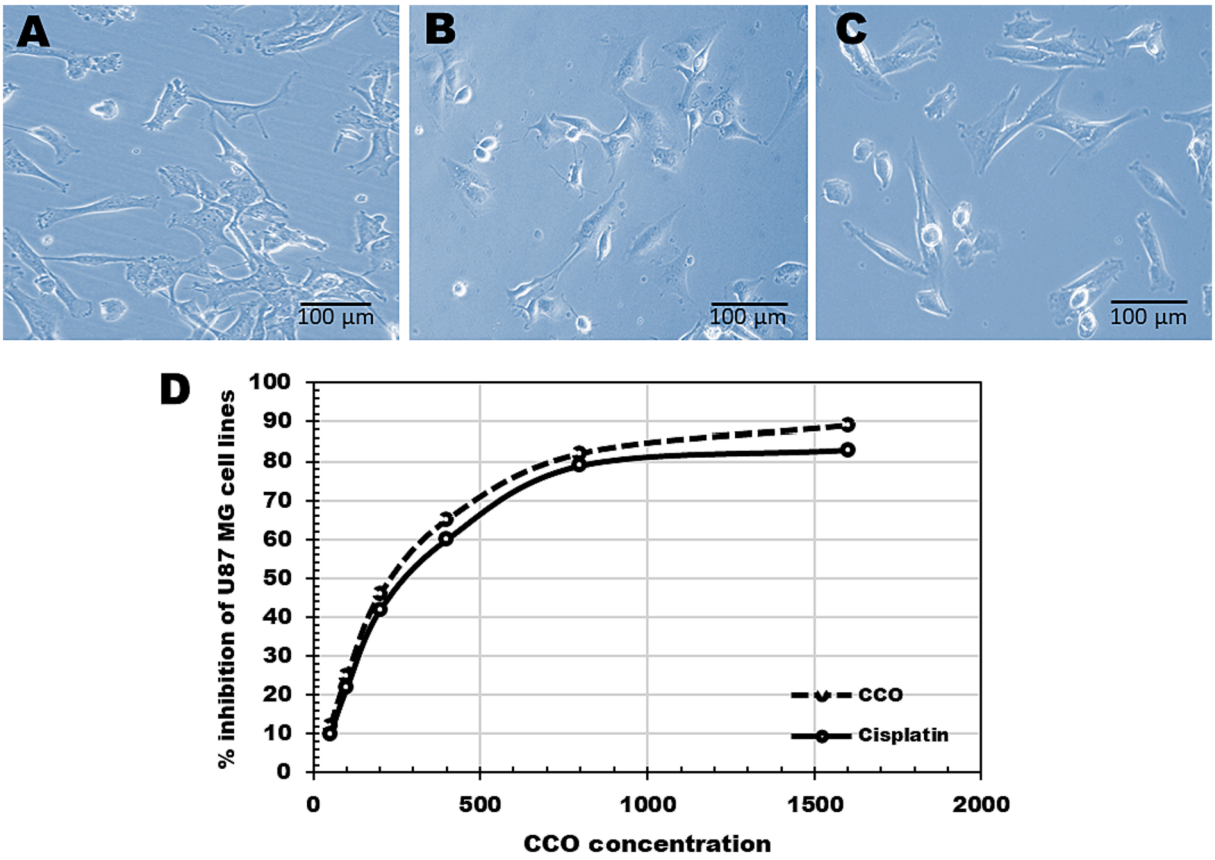


**Fig. 4.** (A-C) Optical microscope image of CCO and Cisplatin cytotoxic effects on U87 cancer cells. (D) the histogram shows the % inhibition of T cell lines vitality.

**Table 3**
Cytokines levels and acetylcholine in AD and CCO treated rats.

| Treatments | APP (Pg/ml) | Aβ (Pg/ml) | TNF-α (ng/L) | ACh (U/ml) | Cas-3 | Bcl2 |
|---|---|---|---|---|---|---|
| T1 | 165.0 ± 1.2 $^{d}$ | 34.0 ± 2.9$^{c}$ | 62.96 ± 10.0$^{c}$ | 8.21 ± 0.3 $^{a}$ | 1.0 ± 0.01$^{b}$ | 1.2 ± 0.0$^{c}$ |
| T2 | 650.2 ± 2.1 $^{a}$ | 77.0 ± 0.8 $^{a}$ | 97.5 ± 0.8 $^{a}$ | 3.6 ± 0.5$^{c}$ | 3.5 ± 0.02$^{a}$ | 7.5 ± 0.2$^{a}$ |
| T3 | 461 ± 2.3$^{b}$ | 43.0 ± 3.6$^{b}$ | 77.5 ± 6.1$^{b}$ | 3.9 ± 0.7$^{c}$ | 1.3 ± 0.01$^{b}$ | 2.1 ± 0.1$^{b}$ |
| T4 | 380.0 ± 1.9$^{c}$ | 48.2 ± 0.2$^{b}$ | 59.8 ± 0.8 $^{d}$ | 5.56 ± 0.8$^{b}$ | 1.1 ± 0.02$^{b}$ | 1.5 ± 0.2$^{c}$ |

Data sharing the same superscript = not significant (NS); sharing different superscript = significant where $p < 0.05$ or $p < 0.001$ Amyloid precursor protein (APP), Amyloid beta (Aβ), Tumour necrosis factor (TNF-α), Acetylcholine (ACh). Data are presented mean ± SE. Lowercase letters in the same column indicate significant differences.

**Table 4**
Levels of dopamine, norepinephrine, and serotonin in different groups.

| Treatments | Brain | | |
|---|---|---|---|
| | Norepinephrine µg/ g. tissue | Dopamine µg/ g. tissue | Serotonin µg/ g. tissue |
| T1 | 4.5 ± 0.9$^{b}$ | 5.1 ± 0.3 $^{a}$ | 3.4 ± 0.2 $^{a}$ |
| T2 | 3.3 ± 0.3$^{c}$ | 3.2 ± 0.4$^{c}$ | 2.8 ± 0.1$^{c}$ |
| T3 | 3.8 ± 0.2$^{c}$ | 4.4 ± 0.8$^{b}$ | 2.8 ± 0.4$^{c}$ |
| T4 | 6.2 ± 0.1$^{c}$ | 4.8 ± 0.1$^{b}$ | 3.2 ± 0.4$^{b}$ |

Data are presented mean ± SE. Lowercase letters in the same column indicate significant differences.

degraded synapses and dendrites, and astrocytes under assault and inflammation. In addition, it was determined that A is the initial step in the pathophysiology of AD. The disparity between A production & A clearance supports the elevation of toxic A oligomers and, hence, the amelioration of ACh deficiency (Sharma, 2019). As found in this work, $AlCl_3$ exposure increased A production and decreased its breakdown (Praticò et al., 2002; Medhat et al., 2019).

In Alzheimer's disease, A42 plaques play a crucial role by stimulating microglia; these excited microglia increase the formation of proinflammatory cytokines like TNF- & interleukin 1 (IL-1), which in turn drive the creation of new A42 oligomers (Brosseron et al., 2014). This cycle of inflammatory and oxidative stress changes neurons' ionic homeostasis. Consequently, neuronal and synaptic dysfunction and selective damage to ACh neurons lead to ACh deficiency and dementia (Prema et al., 2017).

The elevated level of TNF-α in the AD group was consistent with earlier research (Decourt et al., 2017; Shata et al., 2020); the experiment revealed that $AlCl_3$ produced neuroinflammation via an increase in the formation of inflammatory cytokines like IL-1 and IL-6, & TNF; this was connected to the activation of microglia in a feed-forward circuit during a process known as reactive microgliosis (Tjalkens et al., 2017). In contrast, CCO ameliorated this impact in the treated group due to its high levels of active chemicals, as shown in Fig. 1 and Table 1, which are known for their anti-inflammatory characteristics. In this investigation, the anti-inflammatory activities of CCO were demonstrated by a decrease in TNF-, APP, and A levels in the treated group.

TNF-α was shown to boost the production of APP in astrocytes and neurons; these findings suggest a link between neuroinflammation and the amyloid precursor system in advance of AD (Chen et al., 2020), according to the present study. Neurotransmitters are the subject of much research because they are critical for learning & memory; besides the cognitive impairment, approximately half of Alzheimer's patients

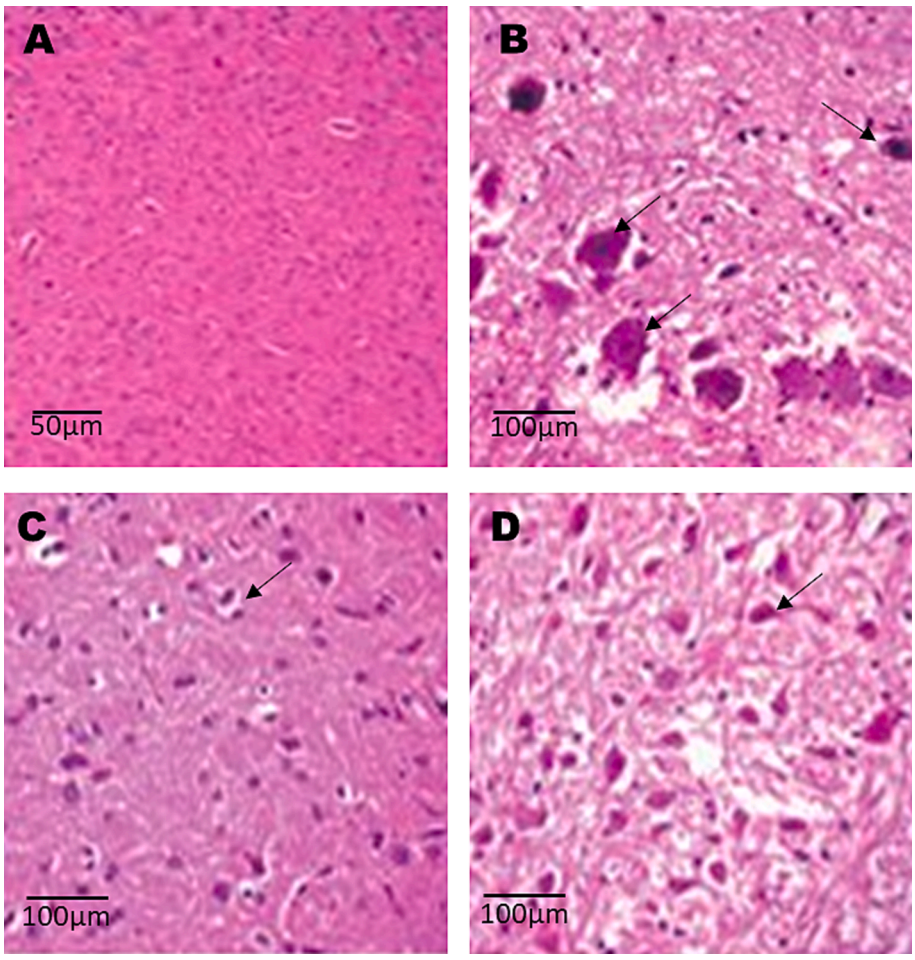


**Fig. 5.** Brain sections stained by hematoxylin and eosin of rats treated by aluminum chloride; A) control brain; B) rats treated with $AlCl_3$-induced Alzheimer's' disease, C) rats treated with chamomile essential oil after inducing AD, D) donepezil hydrochloride-treated group after inducing AD.

display dysfunctions of the noradrenergic, dopaminergic, and serotoninergic systems (Ceyzériat et al., 2021).

It was hypothesized that NE and DO may collaborate to facilitate learning and maintain the environment required for ordinary cognitive activities (Ranjbar-Slamloo and Fazlali, 2020). A remarkable interconnectedness exists between dopamine and noradrenaline signaling pathways, with the cortex and hippocampus serving as prominent interaction regions.

In Alzheimer's disease, dopamine pathway changes and neuronal death have been described, decreasing DO content. This suggests that DO is implicated in the pathogenesis of cognitive decline and non-cognitive symptoms associated with this disease (Burns and VanDer-Heyden, 2006). In this investigation, serotonin levels were seen to decrease. Interactions between the serotonin and dopamine (DO) systems are intriguing. Indeed, it has been revealed that DO neurons express the serotonin receptor (5HT2AR) (Nocjar et al., 2002).

Various investigations have provided evidence of the varied chemical compositions and pharmacological capabilities of essential oils, which include, but are not limited to antinociceptive, anxiolytic-like, and anticonvulsant effects. The supplementary use of essential oils, whether applied topically, inhaled, orally eaten, or consumed orally, has been a prevalent practice for treating conditions such as anxiety, sleeplessness, convulsions, pain, and cognitive impairment. The application of synthetic pharmaceuticals to address a variety of conditions and symptoms is linked to an extensive array of adverse effects; however, applying natural products is a global trend for enhancing health and the environment (El-Saadony et al., 2022). As a result, an extensive array of research organizations worldwide have been motivated to investigate the effectiveness of natural substitutes, including essential oils (Quinibi et al., 2023).

Additionally, CCO affects the release of DO the 5HT2AR activity (Albizu et al., 2011). Studies have revealed that bisabolol possesses the potential to improve cognitive performance and alleviate neuroinflammation in neurodegenerative illnesses like AD. Bisabolol, Camazulene, Bisabolol oxide A, 1,6-Dioxaspiro[4.4]non-3-ene, 2-(2,4-hexadiyn-1-ylidene) with concentrations of 26.1, 25.4, 27.8, and 25.2 % in CCO, as well as flavonoids and total phenolic compounds, which are distinguished by their antioxidant & anti-inflammatory activity (Morshedloo et al., 2018; Abd El-Hack et al., 2022). Another study agrees with our results, describing for the first time that chamomile essential oils exerted their anti-inflammatory and antioxidant activity by modulating macrophages and $CD4^+$ T cells-mediate immune response (Decicco et al. 2023), and that may contribute to mitigating the Alzahimar.

## 5. Conclusion

Discovering greener and more sustainable extraction techniques that adhere to green chemistry principles is essential to promote eco-friendly analytical chemistry. The findings indicated that the extraction method significantly altered essential oils' chemical profiles and biological activities. Oxygenated sesquiterpenes bisabolol oxides A and B were the most abundant compounds in CCO. In rats with aluminum chloride-induced cognitive impairment, the current study demonstrated a neuroprotective impact of chamomile essential oil on the cholinergic system via up-regulation of ACh and anti-inflammatory activity via reduced inflammatory cytokines and apoptosis markers. Therefore, it can be a

therapeutic, cost-effective, and safe alternative to conventional Alzheimer's therapy.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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