


 Cite this: *RSC Adv.*, 2026, 16, 30355

# Unveiling the antioxidant and anti-epileptic potential of *Castanea sativa* against pentylenetetrazole in mice: a comparative LC-MS analysis of two ethanol-based fractions

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Epilepsy is a chronic neurological disorder in which oxidative stress and neuroinflammation play key pathogenic roles. There is growing interest in natural antioxidants with neuroprotective properties as complementary therapies. *Castanea sativa* (*C. sativa*) fruits, rich in phenolic acids and flavonoids, may offer such potential. The current study aimed to assess the effect of *Castanea sativa* fractions on pentylenetetrazol (PTZ)-induced epilepsy in mice *via* modulation of brain Gamma-Aminobutyric acid (GABA), Excitatory amino acid transporter 2 (EAAT2), Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), and Malondialdehyde (MDA). Fruits were sequentially extracted using 70% and 100% ethanol to yield two fractions, which were phytochemically profiled *via* liquid chromatography-mass spectrometry (LC-MS). *In vitro* antioxidant capacity was assessed using DPPH, ABTS, and FRAP assays. *In vivo* efficacy was evaluated using a PTZ-induced seizure mouse model, with animals receiving vehicle, diazepam (1 mg kg<sup>-1</sup>), or *C. sativa* extracts (400 mg kg<sup>-1</sup>) orally, 30 minutes before PTZ injection (60 mg kg<sup>-1</sup>). LC-MS profiling revealed over 70 metabolites, primarily phenolic acids (ellagic and gallic derivatives), flavonoid glycosides (quercetin and kaempferol), and several novel lignans and iridoid glycosides. The 100% ethanol fraction exhibited greater chemical diversity and peak intensities, alongside markedly stronger antioxidant activity, as reflected by lower IC<sub>50</sub> values (193.65  $\mu$ g mL<sup>-1</sup> for DPPH and 128.87  $\mu$ g mL<sup>-1</sup> for ABTS) and nearly fivefold higher FRAP capacity (26.59  $\mu$ g TE mg<sup>-1</sup> fraction) compared to the 70% ethanol fraction. *In vivo*, the 100% extract delayed seizure onset 2.8-fold and reduced seizure duration and mortality, showing efficacy close to diazepam. Additionally, both extracts enhanced brain GABA, EAAT2, and reduced Glutathione (GSH) levels, while decreasing MDA, TNF- $\alpha$ , and Nuclear Factor kappa-B (NF- $\kappa$ B) compared to the PTZ group. These findings support the antioxidant and neuroprotective potential of *C. sativa* fruit extracts, especially the 100% ethanol fraction, highlighting their promise as functional food ingredients or nutraceutical adjuncts in epilepsy management.

 Received 31st March 2026  
 Accepted 27th May 2026

DOI: 10.1039/d6ra02693a

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## 1 Introduction

Epilepsy is a chronic and prevalent neurological disorder, primarily caused by abnormal electrical activity in the brain, which manifests as recurrent seizures. These seizures result in

disruptions of normal brain function and may appear as altered consciousness, behavioral changes, or dramatic convulsions. Globally, epilepsy affects approximately 52 million individuals, making it a significant public health concern. Its prevalence varies according to factors such as age, geographical region, and underlying conditions, including hypoxia, genetic predisposition, neuronal abnormalities, and congenital disorders.<sup>1,2</sup>

Gamma-aminobutyric acid (GABA) plays a crucial role in the central nervous system as the principal inhibitory neurotransmitter, maintaining the balance between neural excitation and inhibition.<sup>3,4</sup> Pentylenetetrazol (PTZ)-induced seizures occur through its interaction with GABA receptors, altering receptor acylation and disrupting neuronal stability.<sup>5</sup> This imbalance between excitatory and inhibitory signals contributes to seizure activity. Additionally, Excitatory Amino Acid Transporter 2 (EAAT2) plays a vital role in regulating extracellular glutamate

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levels and preventing excitotoxicity. As the predominant glutamate transporter, EAAT2 is considered a promising target for achieving neuroprotection in epilepsy therapy.<sup>6</sup> Current epilepsy treatment strategies aim to improve the quality of life, minimize side effects, and achieve complete seizure control.<sup>1</sup> While monotherapy can effectively prevent seizures in many patients, approximately 35% remain refractory to standard treatment even with polytherapy.<sup>7</sup> This highlights the urgent need for alternative approaches, particularly natural compounds that may alleviate symptoms and reduce the frequency or severity of seizures. Epileptic seizures are often associated with secondary complications, including brain injury, cerebrovascular dysfunction, hypoxia, and tumors.<sup>8</sup> Although the exact mechanisms remain unclear, factors such as oxidative stress, inflammation, neuronal cell loss, and disrupted neurotransmission have been implicated in seizure pathogenesis.<sup>9</sup>

*C. sativa* Mill. (sweet chestnut), a deciduous tree belonging to the Fagaceae family, is widely cultivated across Europe, North Africa, and parts of Asia Minor.<sup>10</sup> The tree has long been valued for its edible nuts, which are rich in carbohydrates, unsaturated fatty acids, high-quality proteins, vitamins (notably C and E), and essential minerals such as potassium, magnesium, and iron.<sup>11</sup> These nutritional properties make chestnuts a significant food resource with both dietary and economic importance, particularly in rural areas. Beyond its nutritional value, *C. sativa* is gaining attention for its pharmacological properties. It contains a wide array of bioactive compounds, including polyphenols, flavonoids, tannins, ellagic acid, and gallic acid derivatives, which contribute to its antioxidant, anti-inflammatory, antimicrobial, and potential neuroprotective activities.<sup>12,13</sup> Such properties support its use in the development of functional foods and natural therapeutic agents. Traditionally, different parts of the plant such as the leaves, bark, and fruits have been used in folk medicine to treat common ailments including coughs, diarrhea,

and inflammation.<sup>14,15</sup> These traditional applications align with current pharmacological research and highlight the plant's ethnopharmacological relevance. This study aims to investigate the antioxidant and neuroprotective effects of ethanol-derived fractions from *C. sativa* fruits. It explores their protective potential against oxidative stress-related neurological disorders, with a focus on epilepsy. Comprehensive LC-MS analysis will be used to characterize fraction-specific phytochemicals. Both *in vitro* and *in vivo* models will assess their functional efficacy and biomedical relevance.

## 2 Results and discussion

### 2.1 Metabolome profiling of the studied

For a comprehensive overview of the secondary metabolome of the leaves of the studied *C. sativa* extracts, UPLC-HRMS/MS was employed in both negative and positive ionization modes. The base peak chromatogram (BPC) revealed both qualitative and quantitative differences in the constitutive metabolome of the studied extracts (Fig. 1). Initial inspection of the BPC revealed the much higher abundance of the detected metabolites in the 100% ethanol extract (CS 100) (Fig. 1).

Subsequently, feature-based molecular networks (FBMNs) were constructed using the GNPS platform to enable visual inspection of the chemical space and to analyze the distribution of metabolites across the studied species.<sup>16</sup> In the constructed networks, node attributes were assigned such that the color corresponds to the specific *C. sativa* ethanol extracts, and the nodes were depicted as pie charts to reflect the distribution of each ion across the extracts (Fig. 2 a and b).

The recorded metabolome comprised diverse metabolite classes such as phenolic acids, apocarotenoids, alkaloids, flavonoids, terpenes, stilbenes, lignans, iridoids, and others. More than 70% of the annotated features are reported for the first time in the *C. sativa* extracts (SI Table S1).

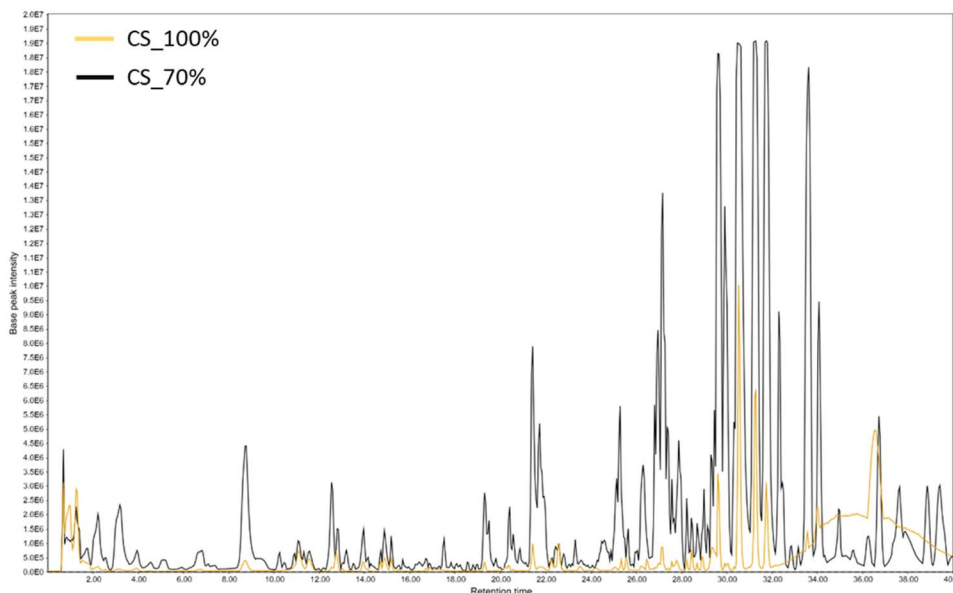


Fig. 1 BPC of the studied *C. sativa* extracts in the negative ionization mode. Black color: 100% extract, and yellow color: 70% extract.



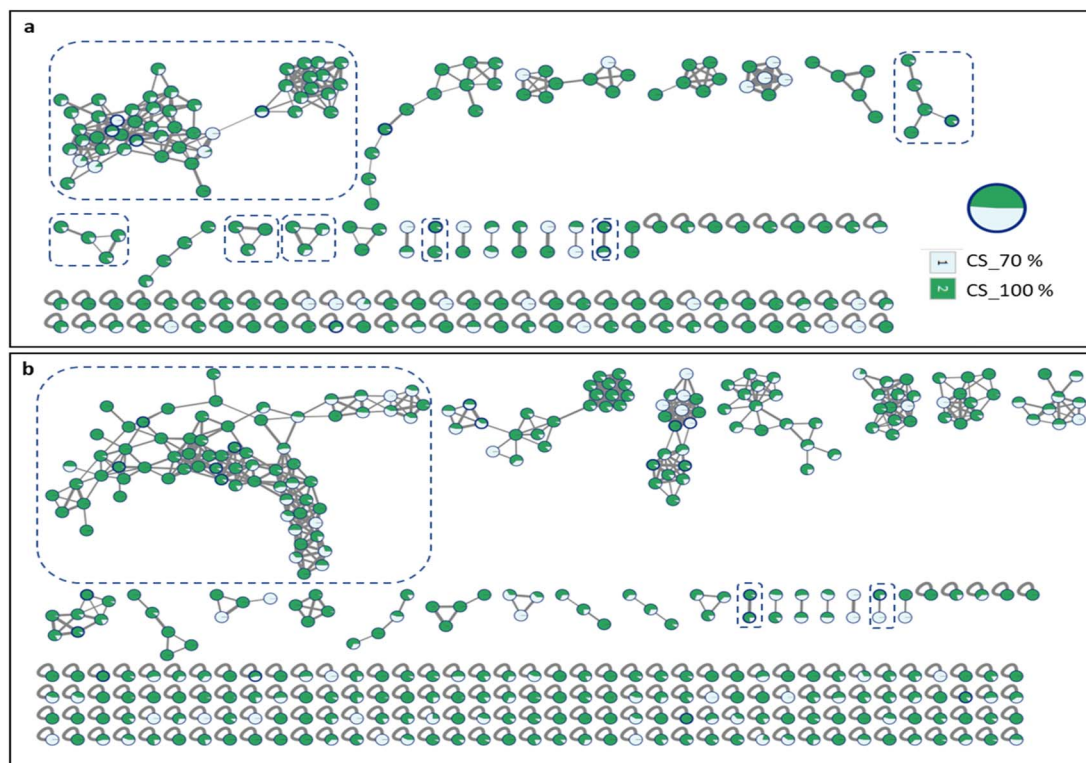


Fig. 2 (a and b): Constructed feature-based molecular networks (FBMN) from the acquired UPLC-HRMS/MS data of *C. sativa* extracts in the negative and positive ionization modes, respectively. Annotated clusters are displayed in boxes.

A total of 73 metabolites were annotated, primarily based on their chromatographic behavior, molecular composition, and fragmentation patterns, with further support from the feature-based molecular networks (FBMNs) and *in silico* fragmentation trees generated by Sirius. Detailed LC and MS data of the annotated metabolites are tabulated in SI Table S1.

Following is a discussion of the annotated metabolites in their elution order.

**2.1.1 Phenolic compounds.** Phenolic constituents were among the first detected in the order of elution, reflecting their relatively high polarity. Identified simple phenolics included gallic acid (**1**,  $m/z$  169.0144,  $[M - H]^-$ ,  $C_7H_6O_5$ ) and ethyl gallate (**27**,  $m/z$  197.0452,  $[M - H]^-$ ,  $C_9H_{10}O_5$ ), both known for their potent antioxidant activities.<sup>17,18</sup> Cinnamic acid (**4**,  $m/z$  166.0854,  $[M + NH_3]^+$ ,  $C_9H_8O_2$ ) was also detected, highlighting the presence of hydroxycinnamic derivatives. Other phenolic acids such as dihydroxybenzoic acid (**6**,  $m/z$  153.0191,  $[M - H]^-$ ) and homogentisic acid (**16**,  $m/z$  167.0346  $[M - H]^-$ ,  $C_8H_8O_4$ ) reinforce the antioxidant potential of the extract.<sup>19</sup> Additionally, glycosylated phenolic acid was observed as *O*-galloyl salidroside (**36**,  $m/z$  451.1224  $[M - H]^-$ ,  $C_{21}H_{24}O_{11}$ ). The identification of complex glycosides like phenylethyl-*O*-primeveroside (**34**,  $m/z$  439.1565  $[M + Na]^+$ ,  $C_{19}H_{28}O_{11}$ ), emphasizes the functional diversity in glycosylated phenolics, which can modulate solubility and biological activities.<sup>14,20,21</sup>

Further, phenolic alcohols and aldehydes, including phenylacetic acid (**12**,  $m/z$  137.0596  $[M + H]^+$ ,  $C_8H_8O_2$ ) and dihydroxybenzaldehyde (**14**,  $m/z$  137.0239  $[M - H]^-$ ,  $C_7H_6O_3$ ),

were observed, alongside conjugated derivatives such as (hydroxyphenyl)ethyl hexose (**13**,  $m/z$  299.1123  $[M - H]^-$ ,  $C_{14}H_{20}O_7$ ), suggesting enzymatic transformations typical in plant secondary metabolism.<sup>14,22</sup>

**2.1.2 Alkaloids.** Among alkaloids, hypoxanthine (**2**,  $m/z$  137.045  $[M + H]^+$ ,  $C_5H_4N_4O$ ), xanthine (**3**,  $m/z$  151.0258  $[M - H]^-$ ,  $C_5H_4N_4O_2$ ), and quinolinediol (**11**,  $m/z$  162.0541  $[M + H]^+$ ,  $C_9H_7NO_2$ ) were detected. These nitrogen-containing compounds play key roles in plant defense and offer promising pharmacological activities, including neuroprotection and anti-inflammatory effects.<sup>23–25</sup>

**2.1.3 Flavonoids.** Flavonoids were abundant, with key representatives like taxifolin (**38**,  $m/z$  303.05  $[M - H]^-$ ,  $C_{15}H_{12}O_7$ ), quercetin (**67**,  $m/z$  301.0339  $[M - H]^-$ ,  $C_{15}H_{10}O_7$ ), and luteolin (**68**,  $m/z$  285.0395  $[M - H]^-$ ,  $C_{15}H_{10}O_6$ ), highlighting the species' richness in flavonols and flavanones. Modified forms, including quercetin dimethyl ether sulphate (**71**,  $m/z$  409.0216  $[M - H]^-$ ,  $C_{17}H_{14}O_{10}S$ ) and di-*O*-methyl aromadendrin (**66**,  $m/z$  315.086  $[M - H]^-$ ,  $C_{17}H_{16}O_6$ ), were also identified, suggesting extensive methylation and sulfation processes which may enhance the stability and bioavailability of these molecules.<sup>26–28</sup>

Several flavonoid glycosides were detected, starting with (epi) catechin-*O*-hexoside (**7**,  $m/z$  443.1885  $[M - H]^-$ ,  $C_{21}H_{32}O_{10}$ ) and extending to compounds such as quercetin-*O*-hexoside (**50**,  $m/z$  463.086  $[M - H]^-$ ,  $C_{21}H_{20}O_{12}$ ) and syringetin-*O*-glucoside (**58**,  $m/z$  507.1119  $[M - H]^-$ ,  $C_{23}H_{24}O_{13}$ ).



**2.1.4 Stilbenes.** Stilbenoid compounds, such as trihydroxy-stilbene-*O*-hexoside (**53**,  $m/z$  389.1227  $[M - H]^-$ ,  $C_{20}H_{22}O_8$ ) and piceid-2''-*O*-coumarate (**65**,  $m/z$  535.1583  $[M - H]^-$ ,  $C_{29}H_{28}O_{10}$ ), were also annotated. Stilbenes are known for their strong antioxidant, anti-inflammatory, and anti-cancer properties, suggesting that *C. sativa* may possess untapped therapeutic applications.<sup>29,30</sup>

**2.1.5 Lignans.** Interestingly, several lignans were reported for the first time in *C. sativa* extracts. These included isomers of isolariciresinol-*O*-hexoside in both negative and positive ionization modes (**28** & **44**  $m/z$  521.1996  $[M - H]^-$ , 547.2136  $[M + Na]^+$ ,  $C_{26}H_{34}O_{11}$ ), lyoniresinol-*O*-hexoside (**46**,  $m/z$  581.2773  $[M - H]^-$ ,  $C_{28}H_{38}O_{13}$ ), and matairesinol (**60**,  $m/z$  359.1483  $[M + H]^+$ ,  $C_{20}H_{22}O_6$ ). Lignans are well known for their antioxidant, estrogenic, and potential anticancer effects, expanding the phytochemical profile and medicinal potential of chestnut.<sup>31–33</sup>

**2.1.6 Iridoids and iridoid glycosides.** The occurrence of iridoids, such as kanokoside A (**40**,  $m/z$  475.1801  $[M - H]^-$ ,  $C_{21}H_{32}O_{12}$ ) and agnucastoside B (**43**,  $m/z$  567.2393  $[M + H]^+$ ,  $C_{26}H_{40}O_{12}$ ), alongside iridoid glycosides like hydroxygeniposide (**19**,  $m/z$  403.1232  $[M - H]^-$ ,  $C_{17}H_{24}O_{11}$ ), adds another dimension to the bioactive landscape of *C. sativa*. These compounds are commonly associated with anti-inflammatory, hepatoprotective, and antimicrobial activities.<sup>34–36</sup>

**2.1.7 Terpenoids.** The LC-MS profile also highlighted a significant number of terpenoid compounds. Among sesquiterpenes and apocarotenoids, molecules such as dihydrophaseic acid-*O*-hexoside (**8**,  $m/z$  443.1885  $[M - H]^-$ ,  $C_{21}H_{32}O_{10}$ ) and blumenol C hexoside (**57**,  $m/z$  395.2027  $[M + Na]^+$ ,  $C_{19}H_{32}O_7$ ) were annotated. Dehydro-vomifoliol (**31**,  $m/z$  223.1319  $[M + H]^+$ ,  $C_{13}H_{18}O_3$ ) and damascone (**63**,  $m/z$  193.1579  $[M + H]^+$ ,  $C_{13}H_{20}O$ ) highlight the carotenoid degradation pathway activity in chestnut tissues. Diterpenoids such as canavalioid (**44**,  $m/z$  545.2566  $[M - H]^-$ ,  $C_{26}H_{42}O_{12}$ ) and cinnacaside (**55**,  $m/z$  543.242  $[M - H]^-$ ,  $C_{26}H_{40}O_{12}$ ) further emphasize the presence of larger, bioactive terpenoid molecules.<sup>14,37</sup>

**2.1.8 Miscellaneous compounds.** The dataset also revealed amino acid derivatives like tryptophan (**9**,  $m/z$  203.0825  $[M - H]^-$ ,  $C_{11}H_{12}N_2O_2$ ), which plays a crucial role in plant metabolism and signaling.<sup>38–40</sup> A variety of coumarins were detected, including hydroxycoumarin (**25**,  $m/z$  177.0186  $[M - H]^-$ ,  $C_9H_6O_4$ ) and trimethoxycoumarin (**51**,  $m/z$  237.0753  $[M + H]^+$ ,  $C_{12}H_{12}O_5$ ), compounds typically associated with anti-leishmanial, antitrypanosomal, antimicrobial, and antioxidant properties and have great cytotoxic potential.<sup>41–43</sup>

The LC-MS profiling of *C. sativa* revealed an exceptionally rich and varied secondary metabolome, with several newly annotated compounds, particularly among lignans and iridoids. The dominance of phenolic compounds, including flavonoids and phenolic acids, aligns with the traditionally known antioxidant and medicinal properties of chestnut. Meanwhile, the identification of stilbenes, alkaloids, and terpenoids provides promising leads for future pharmacological investigations. Collectively, these findings enhance the understanding of *C. sativa* as a reservoir of bioactive compounds with potential nutraceutical and therapeutic applications.

## 2.2 Biological activities

**2.2.1 In vitro antioxidant assays.** An important quality criterion for medicinal plants is their antioxidant activity, which is commonly evaluated using a range of *in vitro* antioxidant assays. However, antioxidant capacity cannot be precisely measured by a single method, as each assay operates *via* different mechanisms and possesses its own advantages and limitations.<sup>44,45</sup> Therefore, the use of multiple complementary assays is essential for a reliable and comprehensive evaluation.

Oxidative stress is increasingly recognized as a major contributor to the development and progression of epilepsy. During seizures, excessive production of reactive oxygen species (ROS) may lead to neuronal damage, disruption of cellular signalling pathways, and exacerbation of epileptic conditions.<sup>44,45</sup>

Natural compounds with antioxidant properties are therefore gaining increasing attention as potential therapeutic agents to counteract oxidative damage.<sup>46,47</sup> *C. sativa* (sweet chestnut) is a rich source of bioactive phytochemicals, including phenolic acids, flavonoids, and terpenoids, several of which have been reported to exhibit antioxidant properties.<sup>46</sup>

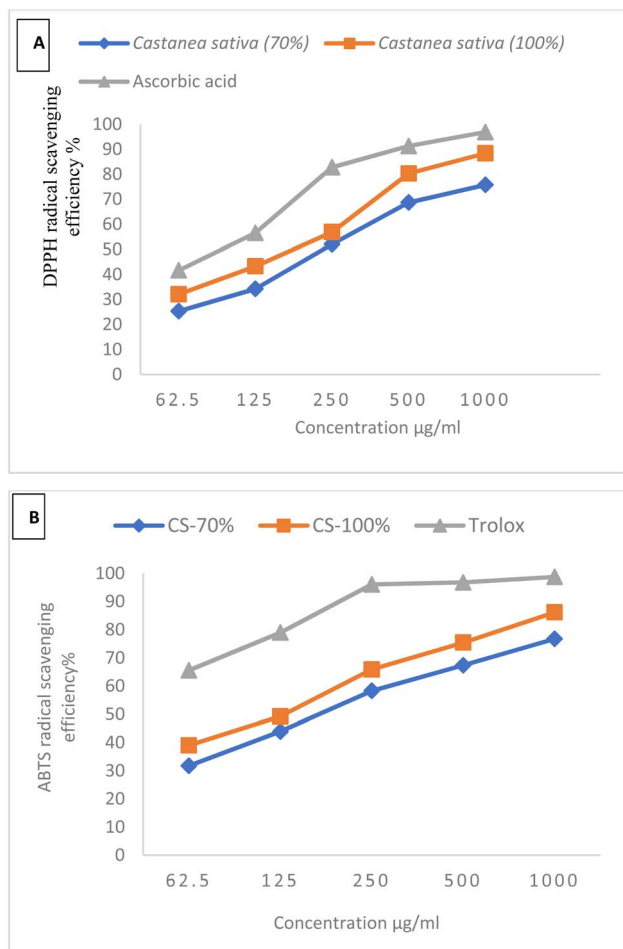
This study evaluated two solvent fractions of *C. sativa* (70% and 100% extracts) for their antioxidant capacity using multiple *in vitro* assays: DPPH radical scavenging, ABTS radical cation decolorization, and FRAP (ferric reducing antioxidant power). The results, presented in Fig. 3 and Table 1, demonstrated significant antioxidant activities, especially in the 100% fraction.

In the DPPH assay (Fig. 3A), both *C. sativa* fractions displayed dose-dependent radical scavenging activity, with the 100% fraction significantly outperforming the 70% fraction. The  $IC_{50}$  values (Table 1) were  $228.77 \pm 1.99 \mu\text{g mL}^{-1}$  for the 70% fraction and  $193.65 \pm 0.50 \mu\text{g mL}^{-1}$  for the 100% fraction, compared to  $94.47 \pm 0.21 \mu\text{g mL}^{-1}$  for ascorbic acid. This indicates that the 100% fraction is approximately 1.18-fold more potent than the 70% fraction. Although ascorbic acid remained the most effective antioxidant, with an  $IC_{50}$  nearly half that of the 100% fraction, the remarkable activity of the 100% *C. sativa* fraction highlights its potential as a powerful natural antioxidant.

Similarly, in the ABTS assay (Fig. 3B), both fractions showed strong, concentration-dependent radical scavenging. The  $IC_{50}$  values (Table 1) were  $178.97 \pm 1.79 \mu\text{g mL}^{-1}$  for the 70% fraction and  $128.87 \pm 0.40 \mu\text{g mL}^{-1}$  for the 100% fraction, while Trolox exhibited superior potency at  $47.67 \pm 1.26 \mu\text{g mL}^{-1}$ . This demonstrates that the 100% fraction is about 1.39-fold more effective than the 70% fraction, underscoring its enhanced antioxidant capacity. Although Trolox remains the benchmark antioxidant, the 100% *C. sativa* fraction shows strong promise as a natural radical scavenger.

The FRAP assay (Table 1) further confirmed the superior antioxidant power of the 100% fraction, which exhibited a ferric reducing antioxidant power of  $26.59 \pm 1.77 \mu\text{g TE mg}^{-1}$  sample, compared to just  $5.17 \pm 0.35 \mu\text{g TE mg}^{-1}$  for the 70% fraction. This indicates that the 100% fraction has over five times greater electron-donating ability, highlighting the crucial role of





**Fig. 3** Antioxidant activities of *C. sativa* fractions using *in vitro* assays (A) DPPH radical scavenging activity; (B) ABTS radical cation decolorization assay. Results are expressed as mean  $\pm$  SD ( $n = 3$ ). Both 70% and 100% ethanol fractions exhibited dose-dependent antioxidant activity.

solvent polarity in extracting potent antioxidant compounds from *C. sativa*.

Overall, Fig. 3 and Table 1 clearly show that the 100% *C. sativa* fraction exhibited superior antioxidant activity across all methods. Although both fractions performed well, neither matched the antioxidant potency of pure standards (ascorbic acid and Trolox). Nevertheless, the results highlight the potential of *C. sativa* fractions as promising natural antioxidant candidates. The phytochemical analysis of *C. sativa* fractions (70% and 100%), performed using LC-MS, revealed a rich composition of bioactive

compounds. Both fractions contained significant amounts of phenolic acids, alkaloids, flavonoids, coumarins, lignans, iridoids, amino acids, and aromatic compounds, all of which are recognized for their potent antioxidant properties. The 100% ethanol fraction of *C. sativa* revealed a more diverse and richer chemical profile compared to the 70% fraction. Notably, it contained additional classes such as sesquiterpenes (apocarotenoids), iridoid glycosides, and a greater variety of phenolic acids and flavonoids. This enhanced phytochemical diversity likely accounts for its superior antioxidant performance, as evidenced by its lower  $IC_{50}$  values in DPPH and ABTS assays, as well as higher FRAP values relative to the 70% fraction. The presence of apocarotenoids and terpenoid-related compounds in the 100% fraction is especially significant since these molecules are well-known for boosting electron-donating capacity and free radical scavenging activity.<sup>47</sup> Although the 70% fraction demonstrated considerable antioxidant potential, it lacked several highly bioactive compounds found in the 100% fraction. This difference highlights the critical influence of solvent polarity and extraction efficiency on recovering potent antioxidant phytochemicals.<sup>48</sup> Both fractions consistently contained multiple phenolic acids and flavonoids, which are key contributors to antioxidant activity. These compounds exert their effects through several mechanisms, including hydrogen atom donation, stabilization of free radicals, and metal ion chelation.<sup>49</sup> The antioxidant capacities observed in the *C. sativa* fractions align well with previous research identifying chestnuts as a rich source of natural antioxidants. However, reported antioxidant values often vary widely due to differences in chestnut varieties, extraction methods, and the specific assays used for evaluation.<sup>50</sup> For instance, Neri *et al.* (2010)<sup>51</sup> reported ABTS antioxidant capacity values ranging from 302 to 311  $\mu\text{mol}$  Trolox equivalents per 100 g in Italian chestnut varieties, reflecting strong radical scavenging activity.<sup>51</sup> Similarly, Blomhoff *et al.* (2006)<sup>52</sup> documented FRAP values as high as 755  $\mu\text{mol}$  Fe(II) per 100 g in comparable chestnut samples, indicating significant ferric reducing power. The bioactive composition of chestnut fruits is influenced by several factors, including climatic conditions, cultivars, geographic location, soil nutrients, and water availability.<sup>52</sup> Barros *et al.* (2010)<sup>53</sup> reported TEAC (Trolox Equivalent Antioxidant Capacity) values between 0.564 and 1.046 mmol Trolox/kg across different chestnut varieties, emphasizing that antioxidant activity can vary considerably depending on the specific crop and growing conditions. Our findings for the *C. sativa* fractions demonstrate promising antioxidant activity that corresponds well with these earlier reports, reinforcing the role of chestnuts as a valuable source of bioactive antioxidant compounds.<sup>53</sup> Additionally, de Melo *et al.* (2023)<sup>54</sup>

**Table 1** *In vitro* antioxidant potency of *C. sativa* fractions assessed by DPPH, ABTS, and FRAP assays.  $IC_{50}$  values ( $\mu\text{g mL}^{-1}$ ) for DPPH and ABTS scavenging activities and FRAP values ( $\mu\text{g}$  Trolox equivalents per mg extract) are expressed as mean  $\pm$  SD ( $n = 3$ )

Sample	$IC_{50}$ $\mu\text{g mL}^{-1}$ (DPPH)	$IC_{50}$ $\mu\text{g mL}^{-1}$ (ABTS)	FRAP ( $\mu\text{g TE mg}^{-1}$ fraction) $\pm$ SD
<i>C. sativa</i> fraction (70%)	228.77 $\pm$ 1.99	178.97 $\pm$ 1.79	5.17 $\pm$ 0.35
<i>C. sativa</i> fraction (100%)	193.65 $\pm$ 0.50	128.87 $\pm$ 0.40	26.59 $\pm$ 1.77
Ascorbic acid (DPPH standard)	94.47 $\pm$ 0.21	—	—
Trolox (ABTS standard)	—	47.67 $\pm$ 1.26	—



demonstrated that antioxidant therapy can reverse several parameters altered by seizure or epilepsy induction, including reducing seizure frequency and mortality. Their study suggests that antioxidants exert neuroprotective effects and help mitigate the detrimental impacts of epilepsy. Given that *C. sativa* is rich in potent antioxidant compounds, its extracts may offer promising neuroprotective benefits in epilepsy management.<sup>54</sup>

### 2.3 Effect of *C. sativa* extracts on seizure scores and latency

Mice injected with PTZ caused an increase in mouse ears and facial twitching, convulsive waves noticed through the mice bodies, myoclonic jerks, and both turning over onto their side position and turning over onto their back position. Treatment with *C. sativa* ethanol extract (70%) delayed mice ear and facial twitching by 56% and plant ethanol extract (100%) delayed them by 100% when compared with PTZ group. The convulsive waves noticed through mice bodies are delayed significantly by *C. sativa* ethanol extract treatment (70%) and (100%) by 46% and 88%, respectively, when compared with modeled animals. Myoclonic jerks with rearing were significantly delayed through treatment with two ethanolic plant extracts (70% and 100%) by 20% and 93%, respectively, when compared with epileptic mice. Treatment of mice with *C. sativa* ethanol extract (70% and 100%) delayed both turning over onto side position by 41% and 76% while turning over onto their back position associated with generalized tonic-clonic seizures delayed with *C. sativa* ethanol extract (70% and 100%) by 67% and 89%, when compared with positive control group. In addition, the treatment with 100% ethanolic extract reduced ear and facial twitching, convulsive waves noticed through the mice bodies, myoclonic jerks, and both turning over onto side position and turning over onto their back position by 100%, 83%, 63%, 177% and 26% more than diazepam (Fig. 4). Previously, *C. sativa* ethanol extract has efficacy in prolonging latency periods. The neuroprotective effect is attributed to *C. sativa* ethanol extracts represented in their rich content of bioactive compounds such as flavonoids, alkaloids, tannins, terpenes, and phenolic acids,<sup>14,37,55,56</sup> which act as potent antioxidants and anti-inflammatory agents.<sup>14,37</sup>

### 2.4 Effect of *C. sativa* extracts on oxidative stress

Our findings confirm that pentylenetetrazol (PTZ) induces oxidative stress and epileptic seizures through increased production of reactive oxygen species (ROS), leading to elevated brain malondialdehyde (MDA) and depleted glutathione (GSH) levels by 116% and 35% as compared to normal control (Fig. 5a and b). This aligns with previous studies,<sup>57–59</sup> which identified PTZ-induced oxidative damage as a key factor in seizure onset. The significant increase in MDA and decrease in GSH observed in the PTZ group reflect enhanced lipid peroxidation and compromised antioxidant defense mechanisms. Importantly, treatment with *C. sativa* ethanol extracts (70% and 100%) significantly reduced the brain content of MDA by 24% and 42% and elevated GSH by 30% and 48% as compared to PTZ group. The 100% ethanolic extract effectively scavenged ROS, resulting in reduced MDA levels and restoration of GSH content more than diazepam by 11% and 5% respectively (Fig. 5a and b). The antioxidant effect

of *C. sativa* ethanol extracts is owing to their active constituents, especially flavonoids as quercetin, which has a high antioxidant effect, counteracts oxidative stress, decreases lipid oxidation (MDA), and increases antioxidants as GSH.<sup>14,37,59–62</sup>

### 2.5 Effect of *C. sativa* extracts on inflammation and neurotransmitters

ROS overproduction and antioxidant depletion are directly linked to neuronal hyperexcitability, mitochondrial dysfunction, and endoplasmic reticulum stress, which in turn lead to GABA depletion, lipid peroxidation, and seizure activity.<sup>63–65</sup> In the current study, PTZ-induced seizures exhibited a significant reduction in brain TNF- $\alpha$ , GABA, EAAT2 levels by 50%, 57% and 25% as compared to normal control, consistent with hyperexcitability and impaired glutamate clearance.<sup>66</sup> However, administration of *C. sativa* ethanol extracts (70% and 100%) significantly restored the levels of TNF- $\alpha$  by 27% and 33%, GABA by 22% and 64% and EAAT2 by 13% and 32% as compared to PTZ group. In addition, the treatment with 100% ethanolic extract reduced TNF- $\alpha$  levels by 7% and restored EAAT2 content by 9% more than diazepam (Fig. 5c–e). *C. sativa* ethanol extracts exert neuroprotective effects not only through antioxidant mechanisms but also by suppressing inflammation and modulating cytokine activity.<sup>57,67</sup>

The ability of *C. sativa* to inhibit NF- $\kappa$ B activation and reduce TNF- $\alpha$  expression further highlights its anti-inflammatory action. This effect is likely due to the synergistic activity of its polyphenolic compounds, particularly tannins, lignans, and gallic acid derivatives, which are known to modulate immune responses and counteract oxidative stress.<sup>68</sup> Several studies emphasized the role of terpenes and phenols in protecting neural tissues from oxidative damage.<sup>69–72</sup> The observed increase in EAAT2 and GABA levels, along with reduced MDA and TNF- $\alpha$ , collectively point to a strong neuroprotective potential of *C. sativa* against PTZ-induced brain injury. Moreover, *C. sativa* extract helped maintain cellular homeostasis, prevented DNA damage, and reduced apoptosis in neuronal cells effects that are crucial in managing neurodegeneration.<sup>73</sup> The broad pharmacological activity of the extract underscores its potential as a functional food or nutraceutical agent in the prevention and management of epilepsy and other oxidative stress-related neurological disorders.<sup>12,74</sup>

*C. sativa* extracts are well known with their biological safety without *in vivo* toxicity previous studies had proved that chestnut (*C. sativa*) extracts have high biological safety. Both oral and dietary administration of *C. sativa* extracts in animal models has been shown to stimulate healthy enterocyte proliferation without causing any genotoxicity or disrupting cellular metabolism, in addition, in human models is safe didn't cause irritants or allergic contact dermatitis. *C. sativa* extracts are widely reported to possess low *in vivo* toxicity and lack genotoxic or irritant effects under physiological conditions. Crucially, the extract's bioactive constituents, particularly polyphenols and tannins, have been shown to inherently prime cellular defense mechanisms. Under basal conditions, these fractions actively upmodulate endogenous antioxidant enzymes *via* the Nrf2 signaling pathway and directly inhibit cholinesterase activity



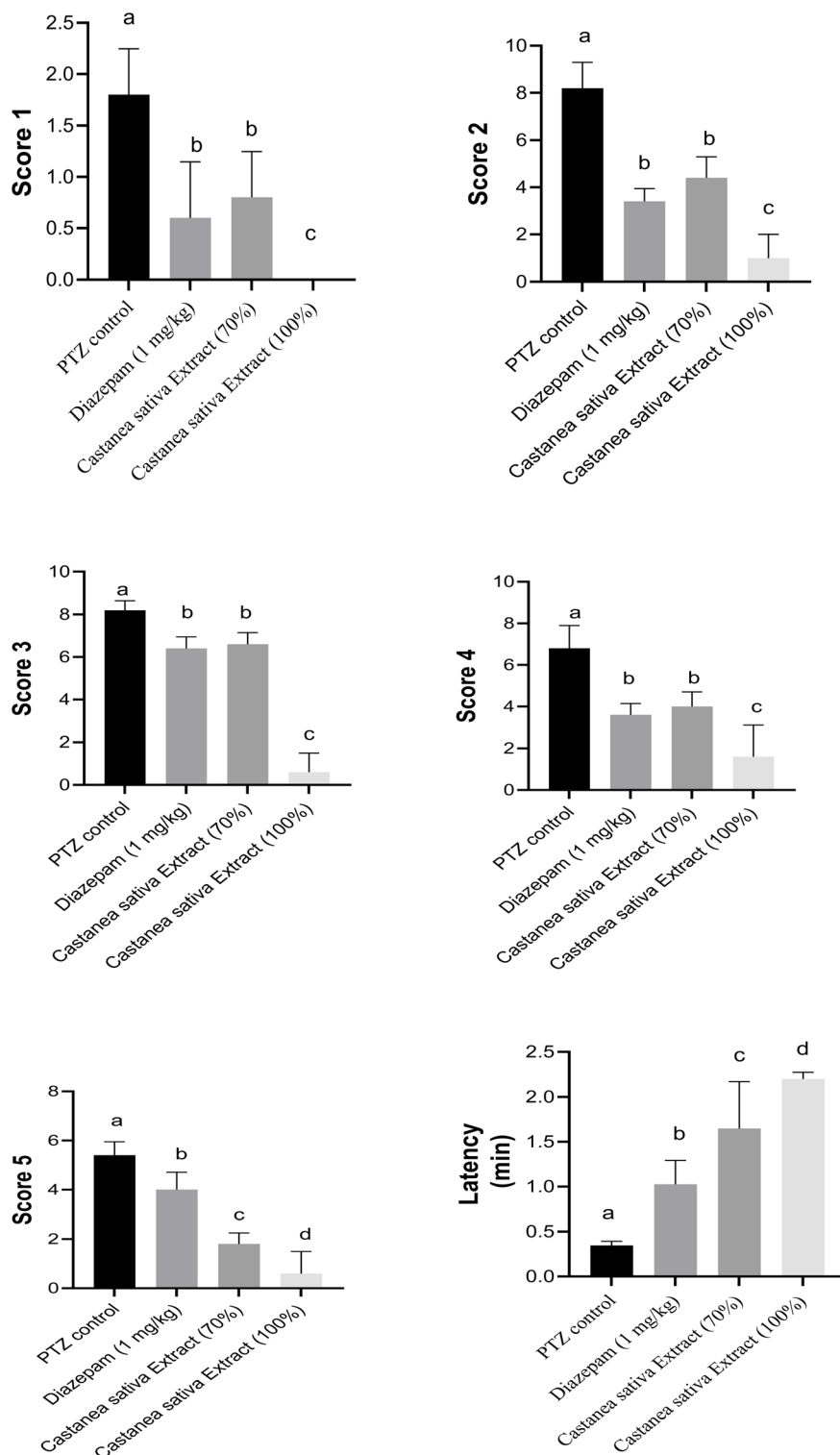


Fig. 4 Effect of *C. sativa* extracts on seizure scores and latency. The results are presented as mean  $\pm$  standard deviation ( $n = 6$ ) using one-way analysis of variance (ANOVA), followed by Fisher's LSD test. Same letter means non-significant difference, while different letter means significant difference at  $p < 0.05$ .

independent of pathological insults. Furthermore, prophylactic models indicate that *C. sativa* actively fortifies neuronal resilience prior to oxidative stress. Therefore, while we acknowledge that the observed changes in GABA levels and antioxidant

markers in our PTZ model likely represent a combination of both direct protective responses and basal pharmacological modulation.<sup>75</sup> In addition, the bioactive compounds identified in the *C. sativa* extract must be capable of crossing the blood-

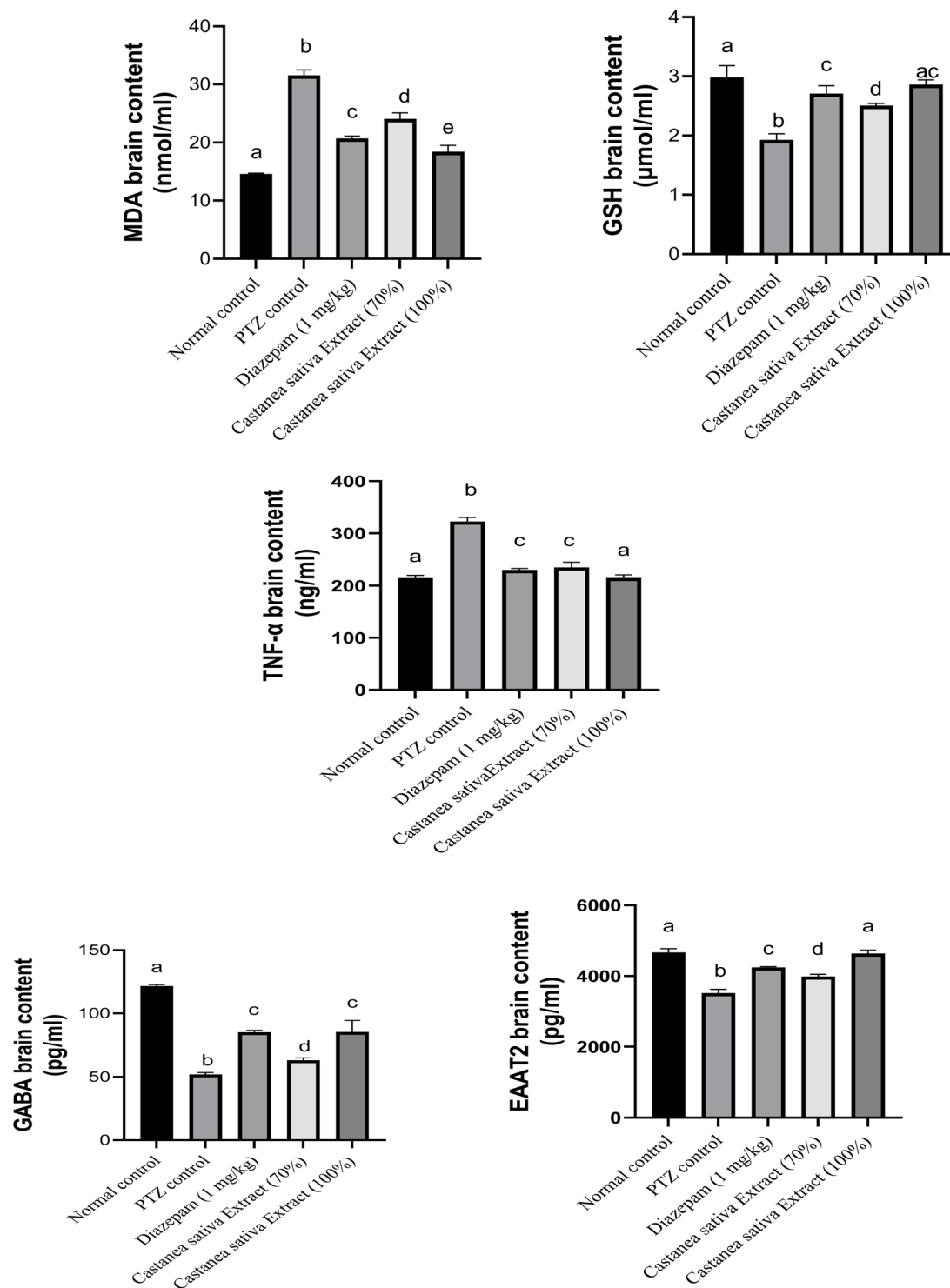


Fig. 5 Effect of *C. sativa* extracts on MDA, GSH, TNF- $\alpha$ , GABA, EAAT. The results are presented as mean  $\pm$  standard deviation ( $n = 6$ ) using one-way analysis of variance (ANOVA), followed by Fisher's LSD test. Same letter means non-significant difference, while different letter means significant difference at  $p < 0.05$ .

brain barrier (BBB). Previous studies have demonstrated that several of the major flavonoids identified in our LC-MS profiling, particularly quercetin and luteolin, possess adequate lipophilicity to permeate the BBB and achieve therapeutic concentrations within brain tissues to modulate synaptic transmission.<sup>76,77</sup>

## 2.6 Histopathological results

**2.6.1 Cerebral cortex.** The normal group showed a normal cerebral cortex (Fig. 6A). The neuropil stained pink. The granule cells exhibited prominent nucleoli and open face nuclei (Fig. 6B). The PTZ group showed neuron degeneration appeared



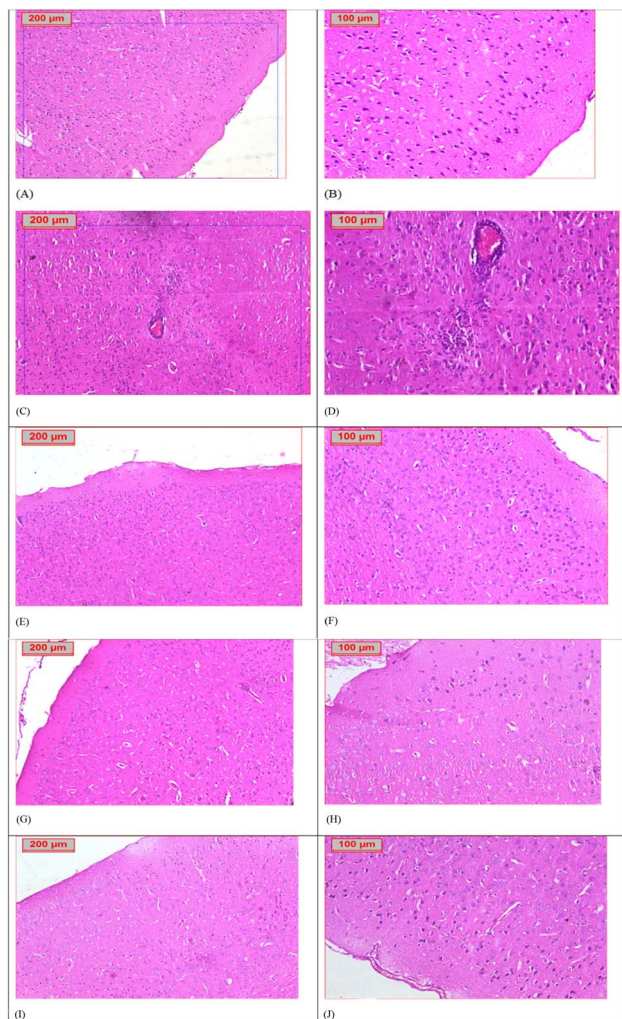


Fig. 6 Photomicrographs of the cerebral cortex from rat brain: (A and B) normal group, (C and D) PTZ group, (E and F) diazepam, (G and H) *C. sativa* ethanol extract (70%), (I and J) *C. sativa* ethanol extract (100%) (H&E (A)  $\times 100$ , (B)  $\times 200$ ).

as structural changes. Some neurons were faintly stained; shrunken and their nuclei were dark. Both had perineuronal spaces and the surrounding neuropil showed vacuolation (Fig. 6C). Focal accumulation of neuroglial cells around dilated congested blood capillaries was observed (Fig. 6D). The Diazepam treatment group showed preservation of the cerebral cortex structure (Fig. 6E). The most neurons were normal (Fig. 6F). *C. sativa* ethanol extract (70%) treatment group revealed partial preservation of the cerebral cortex structure (Fig. 6G). Some of neurons appeared to be normal, while others were shrunken and had darkly stained nuclei (Fig. 6H). *C. sativa* ethanol extract (100%) treatment group showed normal architecture of the cerebral cortex (Fig. 6I). The neurons appeared to be normal. The pyramidal cells had rounded open face nuclei, basophilic cytoplasm. The granule cells showed open face nuclei and prominent nucleoli (Fig. 6J).

**2.6.2 Hippocampus.** The normal groups demonstrated the architecture of the hippocampus with its C-shape structure; the cornu ammonis (CA) and the dentate gyrus. The three territories

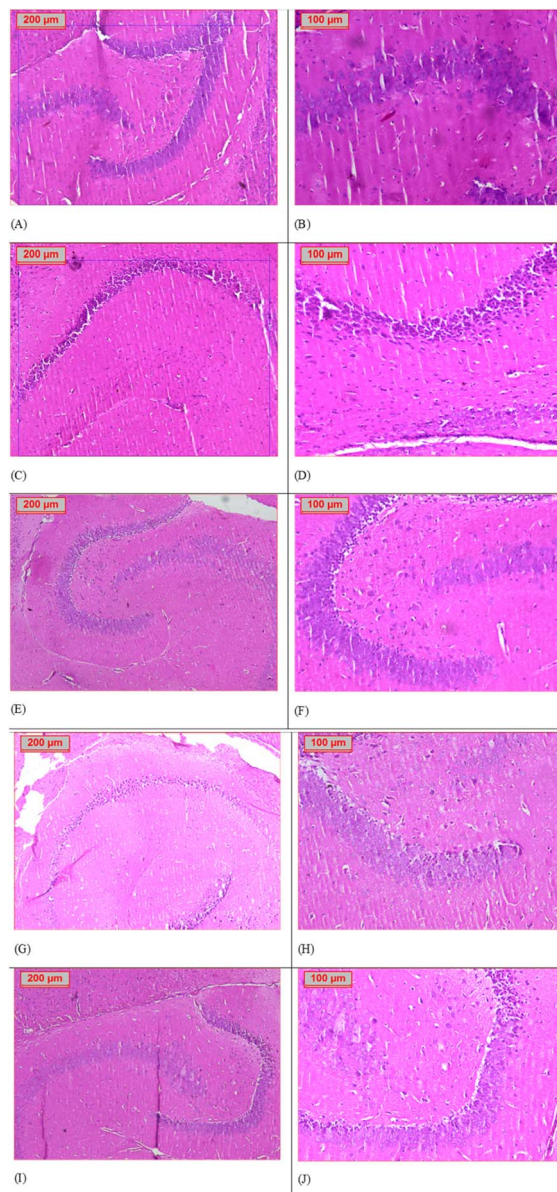


Fig. 7 Photomicrographs of the hippocampus from rat brain: (A and B) normal group, (C and D) PTZ group, (E and F) diazepam, (G and H) *C. sativa* ethanol extract (70%), (I and J) *C. sativa* ethanol extract (100%) (H&E (A)  $\times 100$ , (B)  $\times 200$ ).

of the cornu ammonis (CA) that form part of the hippocampus were identified (Fig. 6A). The polymorphic and molecular and polymorphic layers had sparse neuroglial cells with small blood vessels and small nuclei scattered among the neuropil (Fig. 6B). The dentate gyrus (DG) was observed as a V-shaped territory, it is formed of, granule cell layer (G), the molecular (M) layer and polymorphic layers (P2) (Fig. 7A). The granule cell layer (G) of the dentate gyrus formed mainly of uniformly sized closely packed granule cells with rounded cell bodies and vesicular nuclei (Fig. 7B). The PTZ group showed neuronal atrophy appeared as a decrease in pyramidal cell layer thickness (Fig. 7C). The DG granule cell layer decreased in thickness with basophilic cytoplasm and pyknotic nuclei and halo appearance



and karyolitic nuclei (Fig. 7D). Pyramidal cell axonal edema and degeneration appeared as pale vacuolated regions in the molecular area (Fig. 7D). Diazepam treatment group showed that the darkly stained pyramidal cells were rarely seen in CA. In contrast to the positive group, the granule cell layer was hypertrophied (Fig. 7E). Some Granule cells size was reduced with vestigial heavily staining pyknotic nuclei (Fig. 7F). *C. sativa* ethanol extract (70%) treatment group showed that the histological integrity of the CA territories was noted (Fig. 7G). Partial preservation of the central vesicular nuclei and basophilic cytoplasm in the pyramidal cells. Only a small number of atrophic and darkly staining neurons and perineuronal spaces were seen. The granule cells were dense in population; cell bodies were rounded with vesicular nuclei (Fig. 7H). *C. sativa* ethanol extract (100%) treatment group revealed nearly regular microscopic appearances of hippocampus. The typical three strata of CA areas were preserved (Fig. 7I). Basophilic cytoplasm and central vesicular nuclei were appeared in the pyramidal cells. The DG granule cells also retained their structure. In the polymorphic and molecular levels, the glial cells and interneurons that are heavily stained were identified (Fig. 7J).

Our histopathological results showed that *C. sativa* ethanol extracts have a neuroprotective effect against PTZ-induced epilepsy and able to ameliorate epilepsy due to their antioxidant and anti-inflammatory properties of the active constituents specially flavonoids.<sup>78,79</sup>

Histopathological examination of the CA regions of the cerebral cortex and hippocampus demonstrated that convulsions produced a marked reduction in the pyramidal layer thickness and this was evidenced by the significant decrease in the viable pyramidal cells. Neuronal injury and loss had been widely established in epilepsy research. Moreover, the current research depicted a reduction in the number of nerve cells in the cerebral cortex and hippocampus. The nerve cells with darkly stained nuclei were considered dark neurons and were thought to be a group of cells with early cytoskeleton damage and had small dense nuclei inside them. It was stated that seizures caused morphological changes in the brain tissue, including the development of dark neurons. It had been proposed that brain tissue destruction and the formation of dark neurons were aided by excitatory neurotransmitter release inside neurons during convulsions.

Reactive gliosis has also been identified as a factor in epileptogenesis in epilepsy. The astrocytes and microglia became activated to enhance network hyperexcitability. Reactive astroglia harms the brain through the release of inflammatory cytokines causing cytotoxicity and seizure induction, as well as the production of large quantities of ROS in the brain. Microglia are activated by circulating cytokines and neurotransmitters generated by active or injured neurons, or substances migrating through the blood–brain barrier. The results of this study demonstrate the accumulation of neuroglial cells in sections of the cerebral cortex sections from positive group.

The histopathological findings revealed that treatment in group 4 has a neuroprotective and neuroregeneration effect and that the neuronal loss in the hippocampus and cerebral cortex was reduced.

## 3 Experimental

### 3.1 Plant material

*C. sativa* fruits (Chestnut, Family: Fagaceae) were collected from the Horticulture Research Institute, Agricultural Research Centre, Giza, Egypt, in January 2023. The collection and experimental research conducted in this study complied with relevant institutional, national, and international guidelines and legislation. The plant material was authenticated by Dr Ayman Elkafrawy, expert taxonomist at Horticulture Research Institute. Voucher specimens were deposited in the herbarium of the National Research Centre (CAIRC) under voucher number M254, a public herbarium providing access to deposited materials. We declare that the collection of plant material is in agreement with relevant institutional, national, and international guidelines and legislation.

### 3.2 Plant material preparation

After authentication, the chestnut fruits were prepared for extraction. The fruits were washed thoroughly with distilled water and dried in an oven at 40 °C until a constant weight was achieved. The dried fruits were manually cracked to separate the kernels, while the peels and pulp were discarded. The kernels were further subjected to hot air-drying at 40 °C until a constant weight was attained, then packed in airtight containers and stored at –20 °C for subsequent analysis.

### 3.3 Extraction procedure

A total of 500 g of powdered chestnut kernels was extracted with 100% ethanol (500 g in 5000 mL). The extraction was performed either by continuous stirring at room temperature (25 °C) for 24–48 hours or using ultrasonication at 40 kHz for 30 minutes. After extraction, the mixture was filtered through Whatman No. 1 filter paper, and the solvent was evaporated under reduced pressure using a rotary evaporator at 40 °C. The resulting dried extract weighed approximately 30 g and was stored at –20 °C for further analysis. Following the 100% ethanol extraction, the residual plant material was air-dried and subsequently re-extracted using 70% ethanol (ethanol: water, 70:30 v/v) at the same 1:10 (w/v) ratio, under identical extraction conditions. The obtained extract was filtered and concentrated using a rotary evaporator at 40 °C to remove ethanol. The final aqueous fraction was freeze-dried, yielding approximately 55 g of 70% ethanol extract, which was stored at –20 °C until further analysis.

### 3.4 Samples preparation of UPLC-HRMS/MS measurements

The dried fractions were redissolved in MeOH (HPLC grade) to a final concentration of 2 µg mL<sup>-1</sup>. Chromatographic separation and tandem mass spectrometry measurements was performed as described before.<sup>80</sup>

### 3.5 Data preprocessing, molecular networking, and compound dereplication

Feature-based molecular networks (FBMN) were constructed through the Global Natural Products Social Molecular Networking (GNPS) platform (<https://gnps.ucsd.edu/>) to analyze



the acquired UPLC-HRMS/MS data in both positive and negative ionization modes. Raw data files were first converted to 32 bit mzXML format using MSConvert and subsequently processed in MZmine 4.3.0 for feature detection, deconvolution, alignment, and export. The resulting.mgf file (containing MS/MS spectra) and the corresponding feature quantification table (.csv) were uploaded to GNPS for FBMN analysis following the standard online workflow.<sup>81</sup> Molecular networks were constructed using the following parameters: precursor ion mass tolerance of 0.05 Da, fragment ion mass tolerance of 0.05 Da, cosine similarity score threshold of 0.7, and a minimum of 4 matched fragment ions. Additional network parameters included a maximum of 10 connections per node (topK = 10) and a minimum cluster size of 1. No custom scripts or modifications to the GNPS workflow were applied.

In the resulting networks, nodes corresponded to precursor ions (features), while edges presented spectral similarity between MS/MS fragmentation patterns.

Putative annotations were assigned through spectral library matching within GNPS and further supported by molecular formula and structure predictions obtained using Sirius: CSI (version 6.1.0), applying a mass accuracy threshold of 10 ppm and considering elemental compositions (C, H, N, O, S, P).<sup>82</sup> Annotation confidence was evaluated based on agreement between accurate mass, fragmentation patterns, and database matches. Propagation of annotations within molecular families was performed cautiously and used only as supportive evidence rather than definitive identification.

The resulting network files (.graphML), cluster information, and spectral match outputs were used for downstream analysis and visualization.

### 3.6 Antioxidant capacity evaluation

The antioxidant capacity of the ethanolic fractions of *C. sativa* was assessed using multiple biochemical assays, including: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity assay, ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)) radical cation decolorization assay, and FRAP (Ferric Reducing Antioxidant Power) assay. These assays were performed to evaluate the free radical scavenging ability and reducing power of the fractions.

### 3.7 DPPH radical scavenging assay

The potential antioxidant activity of *C. sativa* fractions was evaluated using the method described by Blois (1958), with slight modifications. Different concentrations of the extract (62.5–1000  $\mu\text{g mL}^{-1}$ ) were prepared for testing. The DPPH radical scavenging activity was measured by mixing 1 mL of the extract with 1 mL of a 0.1 mM DPPH solution in methanol. The reaction mixture was then incubated in the dark for 30 minutes at room temperature. The absorbance of the resulting solution was measured at 517 nm using a spectrophotometer. The antioxidant activity was calculated using the following formula:

$$I\% = A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}} \times 100$$

where  $A_{\text{sample}}$  and  $A_{\text{blank}}$  indicate the absorption of extract sample and blank sample, respectively.

The  $\text{IC}_{50}$  value, which represents the concentration required to scavenge 50% of the DPPH radicals, was determined from the dose–response curve, plotted as radical scavenging activity (RSA) versus the concentration of the extract.

### 3.8 ABTS radical scavenging assay

The potential antioxidant activity of *C. sativa* fractions was evaluated using the method described by Re *et al.* (1999),<sup>83</sup> with slight modifications. Different concentrations of the extract (62.5–1000  $\mu\text{g mL}^{-1}$ ) were prepared. The ABTS<sup>+</sup> solution was mixed with the extract, and the absorbance was measured at 734 nm after 6 minutes of incubation at room temperature.

The antioxidant activity was calculated using the formula:

$$I\% = A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}} \times 100$$

where  $A_{\text{sample}}$  and  $A_{\text{blank}}$  indicate the absorption of extract sample and blank sample, respectively. The  $\text{IC}_{50}$  value was determined from the dose–response curve.

### 3.9 FRAP assay

The ferric reducing antioxidant power (FRAP) of *C. sativa* fractions was determined according to the method described by Benzie and Strain (1996), with slight modifications. Different concentrations of the extracts were prepared and tested. The reaction mixture was incubated at room temperature for 30 min, after which the absorbance was measured at 593 nm using a spectrophotometer.

Results were expressed as micromoles of Trolox equivalents per mg of dry extract ( $\mu\text{mol TE mg}^{-1}$ ). A standard calibration curve was constructed using Trolox solutions at concentrations ranging from 25 to 400  $\mu\text{g mL}^{-1}$ , prepared in methanol. The *C. sativa* fractions were dissolved in ethanol at appropriate concentrations prior to analysis.

## 4 Animals

Male Swiss mice (20–35 g) were chosen for the study. The mice were housed in plastic cages having filter tops under controlled conditions of 12 h light and 12 h dark cycles as well as 50% humidity at 28 °C. The mice were fed with standard pellet diet throughout the period of experiment and water was provided ad libitum. The study protocol took place in accordance with the ethical guidelines for care and use of experimental animals approved by the Medical Research Ethics Committee at the National Research Centre, Egypt (Reg. No. 01420625).

### 4.1 Chemicals and kits

MDA and GSH were purchased from biodiagnostic kits. TNF- $\beta$ , GABA and EAAT2 were obtained from SunLong Biotec Co., Ltd, China and NOVA, Beijing, China.



## 4.2 Experimental design

Status epilepticus was induced in mice by injecting pentylene-tetrazol (PTZ) i.p. at an initial dose of 30 mg kg<sup>-1</sup>, followed by 10 mg kg<sup>-1</sup> every 10 min till 60 min.<sup>84</sup> Mice were assigned to five groups as follows: negative control: distilled water (5 mL kg; oral), PTZ control: mice received only pentylene-tetrazol (PTZ, convulsant agent), diazepam: mice received diazepam (1 mg kg; orally) 30 min prior to PTZ injection, *C. sativa* extract (70% and 100%); mice received *C. sativa* extract (70% and 100%) at dose of 400 mg kg; orally 30 min prior to PTZ injection.

## 4.3 Assessment of seizure

Seizure behaviors throughout the experiment were categorized into five stages with different scores as follows: stage 0: displayed zero response; stage 1: detection of ear and facial twitching; stage 2: convulsive waves are noted through rats' bodies; stage 3: characterized by myoclonic jerks with rearing; stage 4: turning over onto side position; while stage 5: turning over onto their back position associated with generalized tonic-clonic seizures.<sup>85</sup> Seizure scores, the latency time, and the PTZ dose for each studied group required to achieve status epilepticus were recorded. Mice will then be sacrificed by decapitation. Brain samples will be collected and either kept in -80° for further biochemical analyses or in formalin for histopathological studies.

## 4.4 Tissue biochemical analysis

Brain from each mouse was immediately separated, and rinsed with phosphate-buffered saline (PBS) to get rid of excess blood. Weighed parts were homogenized (MPW-120 homogenizer, Med instruments, Poland) in PBS to get 20% homogenate stored overnight at -20 °C. The homogenates were centrifuged at 5000×g for 5 minutes, using a cooling centrifuge (Sigma and laborzentrifugen, 2k15, Germany).<sup>86</sup> The supernatant was removed immediately and stored at -80 °C. Brain contents of MDA, GSH, TNF-β, GABA and EAAT2 were determined using ELISA kits.<sup>87</sup>

# 5 Histopathological examination

Brain tissue was fixed in 10% formolsaline overnight, dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin. 4 μm-thick sections were stained with Hematoxylin and eosin (H&E) to demonstrate the histological structures of the cerebral cortex and hippocampus. Using a Leica Qwin 500 image analysis system, the images were acquired to assess the pathological changes.

## 5.1 Statistical analysis

The *in vitro* results are presented as mean ± standard deviation (SD). To assess the significance of differences between groups, one-way analysis of variance (ANOVA) was performed, followed by Tukey's multiple comparison test. A *p*-value of less than 0.05 was considered statistically significant. All statistical analyses were conducted using GraphPad Prism software (version 9.00, GraphPad Software, LLC, San Diego, USA).

All the *in vivo* numerical values are expressed as means ± standard deviation of the means (SD). Comparisons among different groups took place, adopting one-way analysis of variance (ANOVA) followed by Fisher's LSD test for multiple comparisons. Statistical tests were performed, utilizing Graphpad Prism software, version 5 (Inc., USA). The difference at *p* < 0.05 was considered to be significant.

# 6 Conclusions

The results of this study demonstrate that *C. sativa* ethanol extracts, especially the 100% fraction, possess remarkable antioxidant and neuroprotective properties. This fraction exhibited the highest chemical complexity and biological activity, supported by the presence of diverse bioactive compounds such as flavonoids, phenolic acids, lignans, and iridoids, as identified through LC-MS profiling. It showed superior free radical scavenging capacity and reducing power compared to the 70% fraction. In an epilepsy model, the 100% fraction effectively alleviated oxidative stress, reduced inflammation, and modulated neurotransmitter levels. These outcomes highlight the therapeutic promise of this fraction in addressing oxidative stress-related neurological disorders. Moreover, the findings support the potential of *C. sativa* not only in pharmaceutical development but also as a functional ingredient in nutraceuticals and brain-supportive foods.

## Author contributions

Abeer Salama: conceptualization, methodology, resources, investigation, formal analysis, visualization, supervision, and writing – original draft; writing, review and editing; Rasha M. M. Mohasib: conceptualization (original idea of plant selection), methodology (sample preparation and fractionation), investigation (*in vitro* antioxidant assays), and writing – original draft; Hagar H. Mourad: methodology, investigation, and writing – original draft; Nesrine M. Hegazi: conceptualization, methodology, investigation, software and writing – original draft; Tarik A. Mohamed: conceptualization, methodology, investigation, software, supervision, and writing – original draft; Mohamed-Elamir F. Hegazy: conceptualization, validation, resources, supervision, and writing – review and editing.

## Conflicts of interest

The authors declare no conflict of interest.

## Data availability

The author confirms that the data supporting the findings of this study are available within the article [and/or] its supplementary information (SI). Supplementary information: Table 1: Chromatographic and spectral data of the annotated metabolites from *Castanea sativa*. See DOI: <https://doi.org/10.1039/d6ra02693a>.



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