



Article

Neuroprotective Effects of *Glycyrrhiza glabra* Total Extract and Isolated Compounds

Ali O. E. Eltahir ^{1,†}, Sylvester I. Omoruyi ², Tanya N. Augustine ², Robert C. Luckay ³
and Ahmed A. Hussein ^{1,*}

¹ Chemistry Department, Cape Peninsula University of Technology, Symphony Rd. Bellville, Cape Town 7535, South Africa; aliomers250@gmail.com

² School of Anatomical Sciences, Faculty of Health Sciences, University of the Witwatersrand, Parktown, Johannesburg 2193, South Africa; sylvester.omoruyi@wits.ac.za (S.I.O.); tanya.augustine@wits.ac.za (T.N.A.)

³ Department of Chemistry and Polymer Science, Stellenbosch University, Matieland, Stellenbosch 7602, South Africa; rcluckay@sun.ac.za

* Correspondence: mohammedam@cput.ac.za; Tel.: +27-21-959-6193; Fax: +27-21-959-3055

† Current address: Department of Chemistry, Faculty of Education, Omdurman Islamic University, Omdurman 14415, Sudan.

Abstract: *Glycyrrhiza glabra* L. is a plant commonly utilized in herbal medicine and stands out as one of the more extensively researched medicinal plants globally. It has been documented with respect to several pharmacological activities, notably, neuroprotective effects, among others. However, the neuroprotective activity of pure phenolic compounds has not been reported yet. The chromatographic of a methanolic extract yielded twenty-two compounds, viz.: naringenin 4'-O-glucoside (1), 3',4',7-trihydroxyflavanone (butin) (2), liquiritin (3), liquiritin apioside (4), abyssinone (5), glabrol (6), isoliquiritin (7), neoisoliquiritin (8), isoliquiritin apioside (9), licuraside (10), 3'[O], 4'-(2,2-dimethylpyrano)-3,7-dihydroxyflavanone (11), glabrocoumarin (12), glabrene (13), isomedicarpin (14), 7-hydroxy-4'-methoxyflavone (formononetin) (15), ononin (16), glycyroside (17), (3S)-7,4'-dihydroxy-2'-methoxyisoflavan (18), glabridin (19), neoliquiritin (20), 3,11-dioxooleana-1,12-dien-29-oic acid (21), and 3-oxo-18 β -glycyrrhetinic acid (22). The results of the neuroprotection evaluation showed that *G. glabra* total extract (TE) and compounds 1, 7, 11, 16, and 20 protected SH-SY5Y cells by inhibiting the depletion of ATP and elevated caspase 3/7 activities induced by MPP+. Indeed, this study reports for the first time the structure and activity of compound 11 and the neuroprotective activity of some phenolic constituents from *G. glabra*.

Keywords: *Glycyrrhiza glabra*; licorice; phenolic compounds; neuroprotection



Citation: Eltahir, A.O.E.; Omoruyi, S.I.; Augustine, T.N.; Luckay, R.C.; Hussein, A.A. Neuroprotective Effects of *Glycyrrhiza glabra* Total Extract and Isolated Compounds. *Pharmaceuticals* **2024**, *17*, 852. <https://doi.org/10.3390/ph17070852>

Academic Editors: Vincenzo Musolino and Rosario Mare

Received: 5 May 2024

Revised: 16 June 2024

Accepted: 21 June 2024

Published: 28 June 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Neurodegenerative diseases are a group of diseases characterized by the loss of neurons in the brain [1]. Notable amongst them are Parkinson's disease and Alzheimer's disease. Specifically, Parkinson's disease (PD) involves the loss of dopaminergic neurons in the substantia nigra pars compacta of the midbrain [2]. Classical symptoms of PD include tremors, bradykinesia, akinesia, and postural imbalance [3]. At the molecular level, the progressive loss of dopaminergic neurons is triggered by a cascade of events, including the alteration of the mitochondrial electron transport chain following the accumulation of reactive oxygen species and the loss of adenosine triphosphate (ATP) in the cells [4]. This cascade of events will eventually lead to the death of neurons [5]. Although the actual cause of PD is yet to be fully understood, the onset is believed to result from an interplay between the environment and genetic factors [2]. For instance, exposure to certain neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a heroin analogue, has been implicated in the onset of the disease [6]. Age is also a major contributing factor, as PD mainly affects people aged 60 and above [7]. To date, treatment of PD

remains daunting, as existing treatments only treat symptoms, and levodopa in particular, a dopamine replacement agent which is the current standard care, can nevertheless also lead to Parkinsonism symptoms after prolonged usage [8]. In this regard, medicinal and natural products have continued to serve as a resource base for exploiting biochemical entities that could offer neuroprotective activities [9–11].

Licorice is a name given to different species of the *Glycyrrhiza* (Licorice) genus. Notable amongst them is *G. glabra*, one of the most valuable medicinal plants. It consists of about 30 taxa, of which only 15 taxa have been studied so far [12], mainly including the species *G. glabra* L., *G. uralensis* Fisch., *G. inflata* Bat., *G. echinata* L., *G. lepidota*, *G. pallidiflora* Maxim., and *G. macedonica*, [13–15]. The plants, renowned for their medicinal properties, are widely used in herbal medicine and are among the most extensively studied medicinal plants globally. They have a notable pharmaceutical history in regions such as China, India, Iran, Spain, Italy, Russia, and North Africa, and remain crucial in the exploration of new pharmaceuticals. Several modern medical research studies have concentrated on their chemical constituents and biological effects, aiming to understand the underlying mechanisms. Numerous pharmacological studies, including clinical investigations, have explored the effects of licorice root extract or its isolated compounds on the nervous system. These plants exhibit various pharmacological activities, including neuroprotective, anti-inflammatory, antimicrobial, anticancer, gastroprotective, hepatoprotective, and cardioprotective effects. Additionally, they have been found to be effective treatments for influenza, cough, lung diseases, pneumonia, bronchitis, skin diseases, and hormone replacement therapy [16–23]. Recently, the plant was used for the treatment of immunostimulating effects in the contexts of coronavirus disease 2019 (COVID-19) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus [24,25]. *G. glabra* and *G. uralensis* are the most studied species of this genus. Furthermore, the literature has reported up to 400 compounds from the *Glycyrrhiza* genus, which have been categorized into various classes such as phenolics (flavanones, flavones, flavanonols, chalcones and dihydrochalcones, isoflavones, and coumestans/phenyldihydrocoumarins, and their methoxylated, prenylated, and glycosolated derivatives) and triterpenoids, including their glycosides [26].

Although licorice species have been extensively studied for their biological activities, including their neuroprotective activities, the present study investigates the neuroprotective activities of the TE of *G. glabra* and its isolated compounds in an in vitro model of PD in the neuroblastoma cell line, SH-SY5Y, using the neurotoxin 1-methyl-4-phenylpyridinium (MPP⁺). Indeed, several studies have demonstrated that MPP⁺, which is a byproduct of MPTP, a toxin known to induce parkinsonism, induces neurotoxicity in the SH-SY5Y cells [27,28]. We also report for the first time the activities of novel compounds isolated and identified from licorice species and show for the first time the neuroprotective activities of these compounds in a PD model.

2. Results

2.1. Chemical Characterization of the Isolated Compounds

Chromatographic purification of the total extract using different techniques, including semi-prep HPLC, resulted in the isolation of twenty-two (22) pure compounds, which were identified as naringenin 4'-O-glucoside (1) [29,30], 3', 4', 7-trihydroxyflavanone (butin) (2) [31,32], liquiritin (3) [33,34], liquiritin apioside (4) [33], abyssinone (5) [35], glabrol (6) [33,36,37], isoliquiritin (7) [38,39], neoisoliquiritin (8) [38,39], isoliquiritin apioside (9) [40–42], licuraside (10) [40–43], 3'[O],4'-(2,2-dimethylpyrano)-3,7-dihydroxyflavanone (11), glabrocoumarin (12) [44], glabrene (13) [37,45], isomedicarpin (14) [33,46], 7-hydroxy-4'-methoxyflavone (formononetin) (15) [33], ononin (16) [30,33], glycyroside (17) [30,33], (3S)-7,4'-dihydroxy-2'-methoxyisoflavan (18) [47], glabridin (19) [33,34,37], neoliquiritin (20) [48], 3,11-dioxooleana-1,12-die-29-oic acid (21) [49], and 3-oxo-18 β -glycyrrhetic acid (22) [50]. The isolated compounds were identified based on detailed spectroscopic analyses and comparison with those reported in the literature (Figure 1).

Please note that certain pages of this article have been removed in order to reduce the file size so that the PDF can be uploaded on the system (the system has a limit of 1MB for files and several PDF files are larger than this).

The first and last pages of each paper (with full bibliographic details and affiliations) are included.

If the entire unredacted paper is required, this can be emailed directly to whomever requires them by contacting Dr. Busisiwe Maseko on Busisiwe.Maseko@wits.ac.za

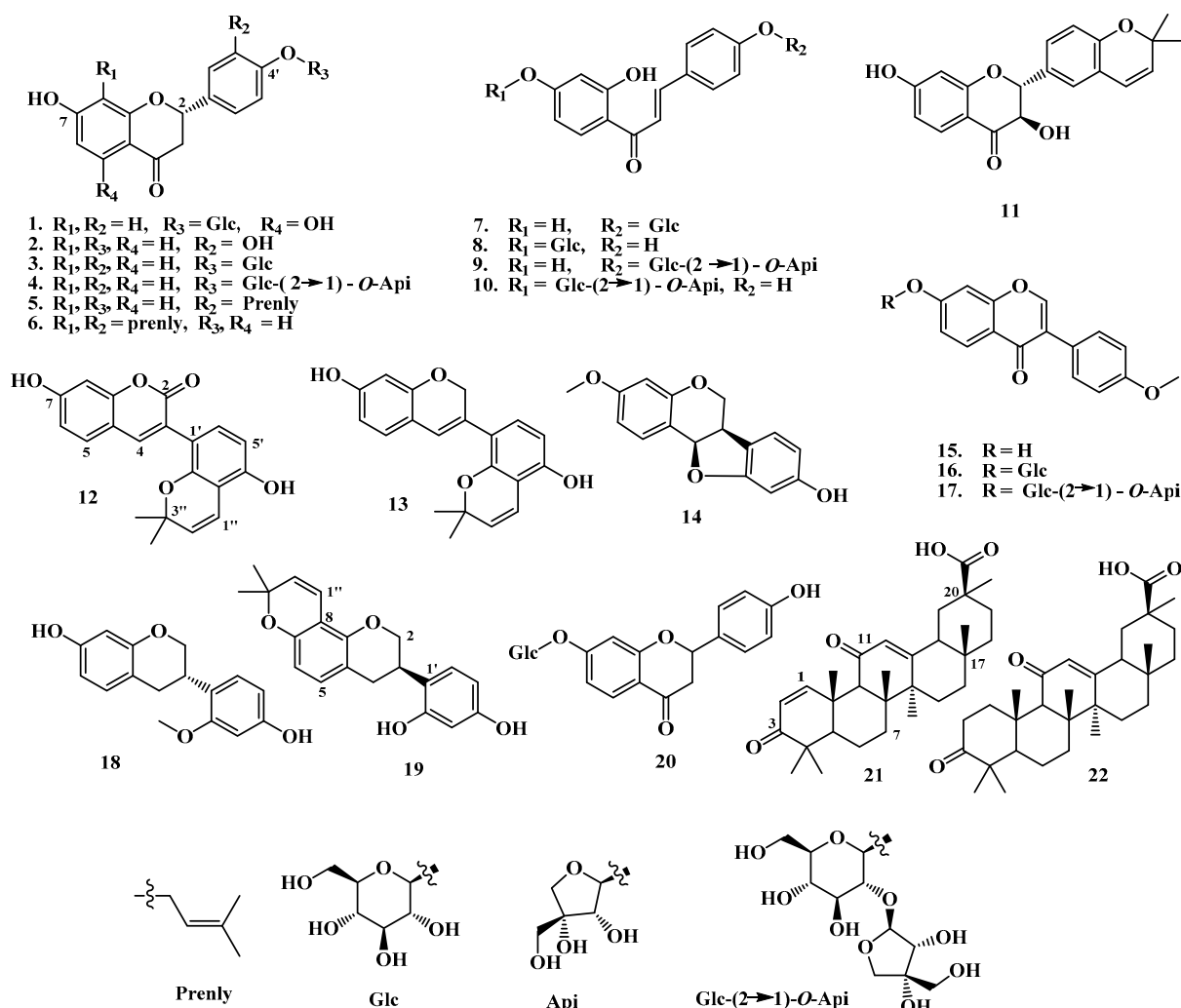


Figure 1. Chemical structures of the compounds isolated from *G. glabra*.

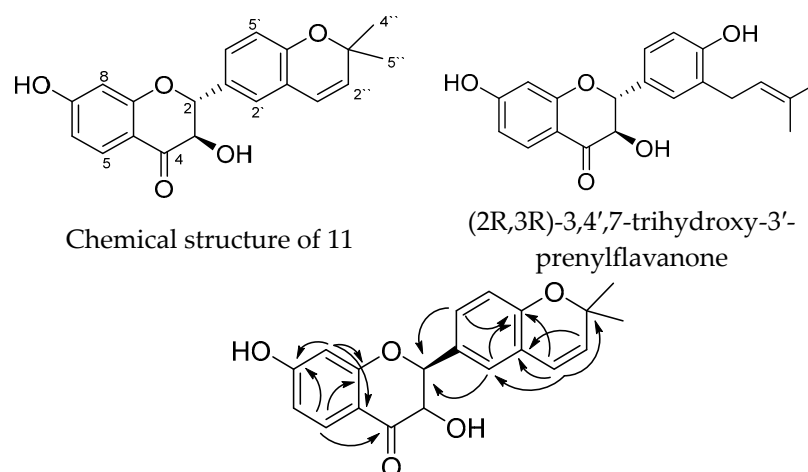
Compound **11** was isolated as a whitish-yellow powder, and its molecular formula was determined to be $C_{20}H_{18}O_5$ by its HRESIMS (High Resolution Electrospray Ionization Mass Spectrometry). The UV spectrum of **11** had absorption maxima at 313 and 276 nm, whereas the IR spectrum showed absorption bands at 3374, 1673, 1608, 1502, and 1463 cm^{-1} for OH, CO, and aromatic groups.

The 1H NMR spectrum of **11** showed signals assignable to six aromatic protons belonging to two 1,3,4-trisubstituted aromatic rings [δ_H 7.72 (*d*, $J = 8.5$ Hz), 6.51 (*dd*, $J = 8.5, 2.0$ Hz), and 6.30 (*d*, $J = 2.0$ Hz); δ_H 7.25 (*d*, $J = 7.9$ Hz), and 6.76 (*br d*, $J = 7.9$ Hz), 7.23 (*br s*)]; two oxygenated methines [δ_H 4.92 and 4.50 (*d* each, $J = 11.8$ Hz)], and a 2,2-dimethyl pyrane ring [δ_H 6.43 and 5.58 (*d* each, $J = 9.7$ Hz), and two methyls at 1.37 (6H, *s*)]. The ^{13}C NMR and DEPT-135 showed 20 carbons which could be classified as CO (192.7); 14 olefinic signals, 12 of them aromatics (see Table 1); three oxy carbons (83.7, 76.3 and 72.9); and two methyls (27.7, 2C's). The above data showed similarity with (2*R*,3*R*)-3,4',7-trihydroxy-3'-prenylflavanone isolated from the same source by Kuroda et. al. (2010) [51]; the only differences are lying on the chemical shifts of the prenyl signals. This similarity indicates that **11** could be a 3-hydroxyflavanol derivative attached to a pyrane ring. Heteronuclear Multiple Bond Correlation (HMBC) correlations between H-5 and C-4, C-7, C-9; H-8/C-7, C-9, C-10; H-6'/C-2, C-4'; H-2'/C-2, C-4'; H-1''/C-4', C-2', C-3'' indicate connectivity of the molecule and the linkage of the pyrane ring at C3', C4' of ring B (Figure 2).

Table 1. NMR data of compound **11** (DMSO-*d*₆) and 3,4',7-trihydroxy-3'-prenylflavanone (acetone-*d*₆).

Position	Compound 11		3,4',7-Trihydroxy-3'-prenylflavanone [51]	
	δ_C	δ_H , multi, J (Hz)	δ_C	δ_H , multi, J (Hz)
2	83.7	4.92 (<i>d</i> , 11.8)	84.6	5.03 (<i>d</i> , 11.9)
3	72.9	4.50 (<i>d</i> , 11.8)	73.4	4.59 (<i>d</i> , 11.9)
4	192.7	-	192.7	-
5	128.6	7.72 (<i>d</i> , 8.6)	129.3	7.74 (<i>d</i> , 8.6)
6	111.5	6.51 (<i>dd</i> , 8.6, 2.0)	111.2	6.64 (<i>dd</i> , 8.6, 2.2)
7	162.7	-	165.4	-
8	102.5	6.30 (<i>d</i> , 2.0)	103.1	6.41 (<i>d</i> , 2.2)
9	163.2	-	164.1	-
10	111.9	-	112.5	-
1'	129.6	-	128.9	-
2'	126.2	7.23 (<i>br s</i>)	130.0	7.35 (<i>d</i> , 2.0)
3'	120.7 <i>s</i>	-	128.1	-
4'	152.6	-	155.8	-
5'	115.5	6.76 (<i>d</i> , 7.9)	115.0	6.90 (<i>d</i> , 8.2)
6'	129.1 <i>d</i>	7.25 (<i>br d</i> , 7.9)	127.1	7.27 (<i>dd</i> , 8.2, 2.2)
1''	121.6	6.43 (<i>d</i> , 9.7)	28.7	3.38 (2H, <i>d</i> , 7.3)
2''	131.3	5.58 (<i>d</i> , 9.7)	123.1	5.39 (<i>m</i>)
3''	76.3	-	132.0	-
4''	27.7	1.37 (<i>s</i>)	25.4	1.72 (<i>br s</i>)
5''	27.7	1.37 (<i>s</i>)	17.4	1.74 (<i>br s</i>)

multi: multiplicity, J values in Hz, *s*: singlet, *br s*: broad singlet, *br d*: broad doublet, *d*: doublet, *dd*: doublet of doublet, *m*: multiple.

**Figure 2.** Chemical structure and important HMBC correlations of **11**, and the structure of 3,4',7-trihydroxy-3'-prenylflavanone.

The known compounds were identified based on the extensive NMR data, including 2D (HSQC, COSY, and HMBC). The ¹H NMR spectrum of **1** displayed a typical flavanone skeleton, as well as a 2-3 dihydro ring C pattern [δ_H 5.50 (*d*, *J* = 12.7, 2.2 Hz, H-2; δ_C 78.4), δ_H 2.69 (*dd*, *J* = 17.1, 2.2 Hz, H-3 β), and δ_H 3.24 (H-3 α , overlapped; δ_C 42.4)]. The peaks at δ_H 7.43 (*d*, *J* = 8.6 Hz, H-2', 6') and δ_H 7.07 (*d*, *J* = 8.6 Hz, H-3', 5') indicated a 1,4-disubstituted ring B. The two protons at δ_H 5.85 and 5.83 (*br s*, δ_C 95.8 and 96.7) suggested free 6,8-positions of ring A. Additionally, the proton at δ_H 4.89 (*d*, *J* = 7.2 Hz, δ_C 100.7) was identified as an anomeric proton of a glucose unit. The ¹³C NMR and DEPT-135 spectra revealed 19 signals corresponding to 21 carbons, including a signal at δ_C 196.1 (CO, C-4), supporting the presence of a flavanone skeleton. The attachment of the glucose unit to C-4' is confirmed by the HMBC correlation of the anomeric proton with C-4'. The NMR data (Table S1) are identical to those of naringenin-4'-O-glucoside, previously isolated from the same source and *Salvia patens* [29,30].

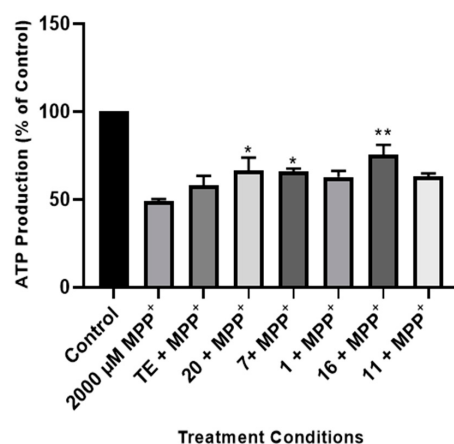


Figure 5. Effect of licorice TE and compounds on MPP⁺-induced depletion of ATP. Cells were pre-treated with 50 µg/mL of licorice TE and 2.5 µg/mL of compounds before exposure to 2000 µM of MPP⁺ for 24 h, and ATP levels were assessed. Each bar represents the mean percentage of ATP production relative to control, and the significance of the difference is indicated as * $p < 0.05$ and ** $p < 0.01$ when extract/compounds are compared to MPP⁺ and when MPP⁺-treated cells are compared to control.

2.2.4. Effect of TE and Compounds on Caspase 3/7 Activities in the Cells

To further ascertain the mechanism involved in the neuroprotective aspects of licorice TE and compounds in SH-SY5Y cells, the levels of apoptosis were assessed using caspase 3/7 as a marker. Caspases belong to the family of cysteine proteases, which drive apoptosis in cells and carry out their function by cleavage of substrates; thus, they are used as markers of apoptosis [24,53]. To investigate apoptosis, cells were pretreated with 50 µg/mL of the TE and 2.5 µg/mL of the compounds before exposure to MPP⁺, and caspase 3/7 activities were measured. Figure 6 shows that TE and compounds mitigate MPP⁺-increased levels of caspase 3/7 activity in the SH-SY5Y cells. Specifically, MPP⁺ increased levels of caspase 3/7 to about 3-fold of control and pre-treatment with TE reduced this activity to about 1.8-fold of control. Furthermore, all compounds also protected SH-SY5Y cells from MPP⁺-induced increase in caspase 3/7 activities. Altogether, these results indicate that inhibition of apoptosis is involved in the neuroprotective aspects of licorice TE and compounds.

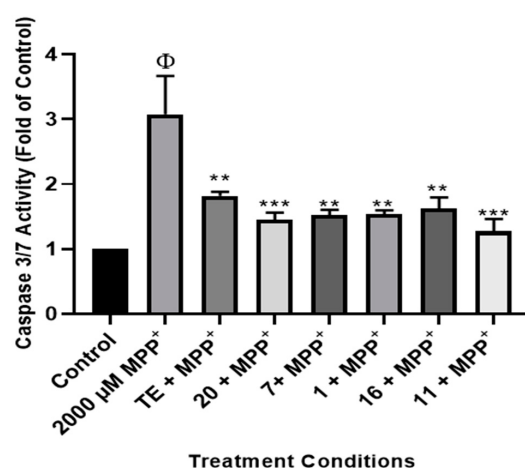


Figure 6. Licorice TE and compounds attenuate MPP⁺-induced increase in caspase 3/7 activities. Cells were pre-treated with 50 µg/mL of licorice TE and 2.5 µg/mL of compounds before exposure to 2000 µM of MPP⁺ for 24 h, and caspase 3/7 activities were determined. Each bar represents the level of caspase 3/7 expressed as a multiple of the control, and significance of difference is indicated by ** $p < 0.01$ and *** $p < 0.001$ when TE/compounds were compared to MPP⁺, and Φ when MPP⁺-treated cells were compared to control.

3. Discussion

The burden of PD continues to pose a challenge to the ageing population globally [54]. While the exact cause of the disease has yet to be fully elucidated, it has been proposed that PD is caused by a plethora of environmental events and genetic factors [55]. In clinical settings, PD is incurable, as it involves different clinical manifestations, including bradykinesia, resting tremor, and rigidity, along with postural instability; hence, medications are given to relieve symptoms [56]. One notable example of such medication is levodopa, but its prolonged use is associated with neuronal cell toxicity and other serious side effects, such as dyskinesias and psychosis [54]. Delaying the onset of PD is thought to effectively slow down the progression of the disease, so exploiting new approaches to achieve this remains critical.

In the present study, the neuroprotective effects of licorice TE and compounds were evaluated in an MPP⁺ model of PD. Twenty-two compounds were isolated from the plant, with compound **11** being a new compound. The compounds were screened for their cytotoxicity, and some of them were observed to be cytotoxic to the SH-SY5Y cells. These compounds include glabrol (**6**), abyssinone (**5**) [35], liquiritin apioside (**4**), glabrocoumarin (**12**), isomedicarpin (**14**), and 3,11-dioxooleana-1,12-die-29-oic acid (**21**). In support of these findings, liquiritin apioside has been reported to induce toxicity on human cancer cell lines (HSC-2, HSC-3, HSC-4, and HL-60) which are derived from oral squamous cancers of the tongue [57], while glabrol showed cytotoxicity against C6 rat glioma cells [58]. In addition, 18 β -glycyrrhetic acid and formononetin have been reported to have cytotoxic potential against various cancer cell lines [59–64]. Despite the cytotoxicity observed for the compounds mentioned above, other compounds from the *Glycyrrhiza* genus have been demonstrated to have neuroprotective effects [20–23]. To this end, our findings show that the compounds naringenin 4'-O-glucoside (**1**), neoliquiritin (**20**), glabrol (**6**), isoliquiritin (**7**), and 3'[O],4'-(2,2-dimethylpyrano)-3,7-dihydroxyflavanone (**11**), alongside the TE, when investigated for their neuroprotective potentials, conferred neuroprotection on the cells. Supporting our findings, studies have demonstrated that neoliquiritin (**20**) protected rat cardiac myocytes from doxorubicin-induced reduction in cell proliferation [65], while naringenin, which is the parent compound from which neoliquiritin (**20**) and naringenin 4'-O-glucoside (**1**) were derived, is widely known to have cytoprotective potentials, including protecting neurons from toxin-mediated cell death in Alzheimer's disease and PD [66–68]. Isoliquiritin, on the other hand, has also shown to have neuroprotective potentials, as it inhibits monoamine oxidase and ameliorates depression [69,70], while 7-hydroxy-4'-methoxyflavone (formononetin) (**15**) was able to alleviate neuroinflammation in lipopolysaccharide-stimulated microglial cells by inhibiting TLR4/MyD88/MAPK signaling and activating the Nrf2/NQO-1 pathway [71].

As a mechanism of action, MPP⁺ is known to alter cellular energy levels in neuronal cells following the induction of oxidative stress [72]. Oxidative stress leads to impairment of the mitochondrial electron transport chain and, consequently, a decline in ATP production, as well as increased mitochondrial dysfunction [73,74]. Alteration of cellular ATP negatively impacts the ability of cells to carry out their normal metabolic functions, and in relation to humans, this could lead to cognitive decline, as neurotransmission will be affected [75]. Importantly, studies have shown that the ability to ameliorate oxidative stress and improve cellular energy and metabolism counteracts the effects of neurotoxins and, at the same time, improves neurotransmission and neuron function [75,76]. In line with this postulate, we show that the tested compounds and TE improved cellular ATP levels following exposure to MPP⁺. In support of these findings, it has been suggested that isoliquiritin provides protective action against corticosterone-induced cell damage by reducing oxidative stress and regulating mitochondrial dysfunction by preventing dissipation of mitochondrial membrane potential [77].

Consequently, neuronal cells proceed to programmed cell death following a decline in ATP, and this contributes to the reduction of dopaminergic neuronal cells in the substantia nigra pars compacta of the midbrain [72,78]. Thus, slowing or preventing neurons with

respect to cell death via several mechanisms holds a future in the management of neurodegenerative diseases like PD. In the present study, our findings show that *G. glabra* TE and compounds **20**, **7**, **1**, **16**, and **11** inhibited elevated caspase 3/7 activities induced by MPP+. While this was the case for our study, previous studies have demonstrated that prunin (**20**) exerts its cytoprotective potential by inhibiting apoptosis triggered by cellular toxins in cardiac cells [65]. In addition, naringenin showed hepatoprotective and neuroprotective properties against lead-induced oxidative stress, inflammation, and apoptosis in rats [68]. Moreover, isoliquiritin was also demonstrated to attenuate apoptosis by the inhibition of the overload of intracellular calcium Ca^{2+} ions; down-regulation of Bax, caspase 3, and cytochrome protein expression; and up-regulation of Bcl protein expression [77]. Thus, the findings are sufficient to state that prevention of cellular apoptosis is a critical component of the neuroprotective mechanism of *G. glabra* and compounds.

4. Materials and Methods

4.1. Chemicals, Materials, and Reagents

Root powder of *G. glabra* was purchased from the local market in Khartoum, Sudan in May 2020; the specimen (H614) was identified by Prof Hatil Elkamali (Department of Botany, Omdurman Islamic University, Omdurman, Sudan). Organic solvents, including methanol, acetonitrile (HPLC grade, Merck, Cape Town, South Africa), hexane, dichloromethane, ethanol, and ethyl acetate (analytical grade (AR), obtained from a local merchant (Kimix, Cape Town, South Africa)) were acquired. Silica gel 60 (0.063–0.200 mm), Sephadex (LH-20), and Aluminum TLC plate, silica gel PF254 were supplied by Merck (Cape Town, South Africa). The 1D NMR (^1H , ^{13}C and DEPT-135) and 2D spectra were measured using a Bruker spectrometer (Rheinstetten, Germany) operating at 400 MHz (for ^1H) and 100 MHz (for ^{13}C).

4.2. Method

Extraction and Purification of Compounds

The root powder (0.5 Kg) was extracted with methanol at 60 °C (3 L $\times$ 2 h $\times$ 2 time). After concentration, it yielded ~104.3 g. A quantity of 50.0 g of the total extract (TE) was applied to the silica gel column and eluted using a gradient of hexane and ethyl acetate with increasing polarity. Collected fractions were pooled together according to their profiles on the TLC to afford 24 major fractions coded as I to XXIV. The fractions were re-chromatographed to yield twenty-three major compounds as follows: Fraction XXIII (3.50 g) underwent chromatography on silica gel using a hexane: EtOAc gradient (80:20 to 0:100). Subsequently, sub-fraction 10 was subjected to purification on Sephadex with an isocratic 80% aqueous ethanol, followed by semi-preparative HPLC (Shimadzu, Kyoto, Japan) utilizing a MeOH and de-ionized water (DIW) gradient (40:60 to 60:80 in 30 min, then to 80:90 in 10 min, and finally to 100% MeOH in 10 min). This process yielded compounds **1** (4.8 mg), **3** (100.8 mg), **7** (45.6 mg), **8** (80.3 mg), and **16** (5.2 mg).

The main fraction, XXIV (4.30 g), was chromatographed on silica gel, and subsequent subfractions 2 and 3 were individually subjected to chromatography on Sephadex and semi-prep HPLC, as described earlier. This resulted in the isolation of compounds **4** (50.3 mg), **9** (39.8 mg), **10** (20 mg), **17** (15.6 mg), and **20** (5.3 mg).

Fraction XV (2.40 g) underwent chromatography on silica gel. Subfraction 16 was subsequently chromatographed on Sephadex and subjected to semi-prep HPLC as outlined before, resulting in the isolation of compound **2** (10.5 mg).

Fraction X (1.40 g) was chromatographed on silica gel using a hexane:EtOAc gradient (80:20 to 0:100), and sub-fraction 7 underwent purification on Sephadex and semi-prep HPLC with a MeOH and DIW mixture (1:1, isocratic) to yield compound **14** (5.8 mg).

Fraction IX (7.30 g) was chromatographed on silica gel using a hexane:EtOAc gradient (80:20 to 0:100), and sub-fraction 6 underwent purification on Sephadex and semi-prep HPLC with a MeOH and DIW mixture (1:1, isocratic) to yield compound **19** (300.8 mg).

Fraction VIII (5.5 g) underwent chromatography on silica gel. Subfractions 4, 5, 8, and 11 were subsequently chromatographed separately on Sephadex and semi-prep HPLC, following the previously mentioned protocol. This led to the isolation of compound **13** (8.4 mg) from sub-fraction 4, the compounds **6** (5.4 mg), **11** (10.8 mg), **12** (5.3 mg), and **18** (9.8 mg) from sub-fraction 5; compounds **5** (10.2 mg) and **15** (5.8 mg) from sub-fraction 8; and compounds **21** (4.4 mg) and **22** (25.2 mg) from sub-fraction 11.

4.3. Compound **11**

Whitish yellow powder $[\alpha]_{25}^D -18.5$ (c 0.01, MeOH); UV (MeOH) $\lambda_{\max}$ 315, 277; FTIR (film) 3378, 1677, 1605, 1500, and 1463 cm^{-1} ; ^1H NMR ($\text{DMSO}-d_6$, 400 MHz), and ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz), see Table 1; $\text{C}_{20}\text{H}_{18}\text{O}_5$ by its HRFABMS (positive mode). HRFABMS m/z 339.1232 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{19}\text{O}_5$, 339.1227).

4.4. Cell Culture and Treatments

The human neuroblastoma SH-SY5Y cells were generously donated by the Blackburn Laboratory, University of Cape Town. Cells were grown in Dulbecco's Modified Eagle's medium (DMEM, Gibco, Life Technologies Corporation, Paisley, UK), supplemented with 10% fetal bovine serum (FBS, Gibco, Life Technologies Corporation, Paisley, UK), 100 U/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin (Lonza Group Ltd., Verviers, Belgium). Cultures were incubated at 37 °C in humidified air with 5% CO_2 , and cell growth medium was routinely changed every three days. Cells were sub-cultured when they attained 70 to 80 percent confluency, using a solution of 0.25% trypsin EDTA (Lonza Group Ltd., Verviers, Belgium).

4.5. Treatments

Stock solutions of 40 mg/mL and 10 mg/mL for total extract and compounds, respectively, were prepared in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA), from which final concentrations were made in cell growth media. To determine the optimum concentrations of licorice TE and compounds to be used for neuroprotection studies, SH-SY5Y cells were plated at a density of 10,000 cells/well and treated with concentrations of 12.5, 25, and 50 $\mu\text{g}/\text{mL}$ of the TE, and 2.5, 5, and 10 $\mu\text{g}/\text{mL}$ of the compounds. The vehicle-treated cells (cells treated with the same concentration of DMSO, similar to that of the highest concentration of extract) were used as control, and the treatments lasted 24 h. For MPP^+ , 2000 μM was chosen as the concentration used to establish neurotoxicity, which is in accordance with our published works [27,53]. For neuroprotection experiments, cells were plated as above and pre-treated with optimized concentrations of licorice TE and compounds for 2 h prior to the addition of 2000 μM MPP^+ . Treatments were incubated for 24 h, and the vehicle-treated cells served as control.

4.6. Cell Viability Assays

The MTT (Sigma-Aldrich, St. Louis, MO, USA) cell viability assay was used to determine the viability of cells following treatment with licorice TE- and compounds-only and the pre-treatment of cells with licorice TE and/or compounds and MPP^+ . Cells were seeded in 96-well plates and treated as stated above, after which the MTT assay was performed. After treatment, 10 or 20 μL (depending on well volume) of the 5 mg/mL MTT solution in phosphate-buffered saline (PBS) (Lonza Group Ltd., Verviers, Belgium) was added to each well and left to incubate in the dark at 37 °C for 4 h. After incubation, the medium containing the MTT dye was discarded, and the MTT formazan was solubilized with 100 μL of DMSO for an absorbance reading using a microplate reader (BMG Labtech Omega® POLARStar, Offenburg, Germany) at a wavelength of 570 nm. Cell viability was calculated and expressed as a percentage of control.

4.7. Adenosine Triphosphate Assay

The Mitochondrial ToxGlo ATP assay kit (Promega, Madison, WI, USA) was used to investigate cell ATP levels. Briefly, cells were plated at a density of 10,000 cells per well in

a white 96-well plate, and after attachment, cells were treated as per the neuroprotection assay above. After treatment, cells were processed according to the manufacturer's protocol by adding a volume of ATP detection reagent appropriate to the contents of each well and leaving the resulting mixture to incubate at 37 °C for 30 min. After incubation, luminescence intensity was read using the Promega GloMax[®] explorer multimode microplate reader, and luminescence values were expressed as percentages of control.

4.8. Caspase 3/7 Apoptosis Assay

To investigate apoptosis in the cells, the Caspase 3/7 assay kit (Promega, Madison, WI, USA) was used to estimate levels of caspase 3/7 activity in the cells, in a manner according with the manufacturer's instructions. Briefly, cells were plated in a white 96-well plate at a density of 10,000 cells per well and allowed to attach overnight, after which cells were pre-treated with licorice TE and compounds before the addition of 2000 µM MPP⁺. Treatments lasted for 24 h, and at the end of the experiments, equal volumes of Caspase 3/7 assay mix were added to each well. The luminescence intensity was read with the Promega GloMax → explorer multimode microplate reader. Luminescence values of the treated cells were expressed as a multiple of the control.

4.9. Statistical Analysis

Data generated from this study were analyzed using GraphPad Prism 6 and expressed as means and standard error values of means of three independent experiments performed in quadruplicate wells. One-way analysis of variance (ANOVA) was used to compare treated cells to either control or MPP⁺ alone. The significance of difference $p < 0.05$ was determined using Tukey's multiple comparisons test and was indicated with * when comparing treatments to control or Φ when comparing licorice and TE and compounds to MPP⁺ treated cells.

5. Conclusions

This study investigated the neuroprotective potentials of *G. glabra* TE and isolated compounds in an in vitro model of PD. The results show that some of the compounds were cytotoxic (including glabrol (6), abyssinone (5), liquiritin apioside (4), glabrocoumarin (12), isomedicarpin (14), and 3,11-dioxoleana-1,12-die-29-oic acid (21)), which might have implications for the treatment of cancer. However, some of the compounds (naringenin 4'-O-glucoside (1), isoliquiritin (7), 3'[O],4'-(2,2-dimethylpyrano)-3,7-dihydroxyflavanone (11), ononin (16), and neoliquiritin (20)) were further studied for their neuroprotective potentials. The protective effects were related not only to the inhibition of ATP degeneration, but also to the attenuation of MPP⁺-induced elevated caspase 3/7 activities. These results indicate that the compounds isolated from *G. glabra* may provide novel therapeutic strategies for the treatment of PD. One limitation of this study was that the biological characterization was only performed on cell lines; therefore, in the future, it would be interesting to see if these compounds would have similar effects on in vivo models of PD. In addition, we recommend that future studies further elucidate the mechanisms of action, with emphasis on the molecular and genetic events underpinning the actions of these compounds.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ph17070852/s1>, Table S1. ¹H and ¹³C spectral data of compounds 1–22, Figure S1. ¹H and ¹³C spectra of compounds 1–22 and Scheme S1. The details of the isolation processes for compounds 1–22 from licorice.

Author Contributions: A.O.E.E., conceptualization; A.A.H., project researcher and supervisor; R.C.L., co-supervisor; S.I.O., investigation, formal analysis, and data curation; T.N.A., funding, investigation, reviewing of draft. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Research Foundation, South Africa (grant number 106055). CPUT and WITS are acknowledged for financial and infrastructural support. TNA acknowledges the Friedel Sellschop Fellowship Award and NRF CSUR funding.

Institutional Review Board Statement: Ethics waiver for this study was obtained from the Wits Human Research Ethics Committee with approval number W-CBP-221024-01.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data associated with the isolated compounds, ^1H NMR, ^{13}C NMR spectra, and the biological data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Lamperty, R.N.L.; Chaulagain, B.; Trivedi, R.; Gothwal, A.; Layek, B.; Singh, J. A Review of the Common Neurodegenerative Disorders: Current Therapeutic Approaches and the Potential Role of Nanotherapeutics. *Int. J. Mol. Sci.* **2022**, *23*, 1851. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Kalia, L.V.; Lang, A.E. Parkinson's disease. *Lancet Neurol.* **2015**, *29*, 896–912. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Postuma, R.B.; Berg, D.; Stern, M.; Poewe, W.; Olanow, C.W.; Oertel, W.; Obeso, J.; Marek, K.; Litvan, I.; Lang, A.E.; et al. MDS clinical diagnostic criteria for Parkinson's disease. *Mov. Disord.* **2015**, *30*, 1591–1601. [\[CrossRef\]](#)
4. Li, J.-L.; Lin, T.-Y.; Chen, P.-L.; Guo, T.-N.; Huang, S.-Y.; Chen, C.-H.; Lin, C.-H.; Chan, C.-C. Mitochondrial Function and Parkinson's Disease: From the Perspective of the Electron Transport Chain. *Front. Mol. Neurosci.* **2021**, *14*, 797833. [\[CrossRef\]](#)
5. Keane, P.; Kurzawa, M.; Blain, P.; Morris, C. Mitochondrial dysfunction in Parkinson's disease. *Park. Dis.* **2011**, *2011*, 716871. [\[CrossRef\]](#)
6. Nonnekes, J.; Post, B.; Tetrud, J.W.; Langston, J.W.; Bloem, B.R. MPTP-induced parkinsonism: An historical case series. *Lancet Neurol.* **2018**, *17*, 300–301.
7. Reeve, A.; Simcox, E.; Turnbull, D. Ageing and Parkinson's disease: Why is advancing age the biggest risk factor. *Ageing Res. Rev.* **2014**, *14*, 19–30. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Thanvi, B.; Lo, N.; Robinson, T. Levodopa-induced dyskinesia in Parkinson's disease: Clinical features, pathogenesis, prevention and treatment. *Postgrad. Med. J.* **2007**, *83*, 384–388. [\[CrossRef\]](#)
9. Brahmachari, G. *Discovery and Development of Neuroprotective Agents from Natural Products*; Elsevier: Amsterdam, The Netherlands, 2017.
10. Abdolmaleki, A.; Akram, M.; Saeed, M.M.; Asadi, A.; Kajkolah, M. Herbal medicine as neuroprotective potential agent in human and animal models: A historical overview. *J. Pharm. Care* **2020**, *8*, 75–82. [\[CrossRef\]](#)
11. Bosch-Morell, F.; Villagrasa, V.; Ortega, T.; Acero, N.; Muñoz-Mingarro, D.; González-Rosende, M.E.; Castillo, E.; Sanahuja, M.A.; Soriano, P.; Martínez-Solís, I.P. Medicinal plants and natural products as neuroprotective agents in age-related macular degeneration. *Neural Regen. Res.* **2020**, *15*, 2207–2216.
12. Öztürk, M.; Altay, V.; Hakeem, K.; Akçiçek, E. *Liquorice: From Botany to Phytochemistry*; Springer: Berlin/Heidelberg, Germany, 2018.
13. Hayashi, H.; Hosono, N.; Kondo, M.; Hiraoka, N.; Ikeshiro, Y.; Shibano, M.; Kusano, G.; Yamamoto, H.; Tanaka, T.; Inoue, K. Phylogenetic relationship of six Glycyrrhiza species based on rbcL sequences and chemical constituents. *Biol. Pharm. Bull.* **2000**, *23*, 602–606. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Hayashi, H.; Miwa, E.; Inoue, K. Phylogenetic relationship of Glycyrrhiza lepidota, American licorice, in genus Glycyrrhiza based on rbcL sequences and chemical constituents. *Biol. Pharm. Bull.* **2005**, *28*, 161–164. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Avula, B.; Bae, J.-Y.; Chittiboyina, A.G.; Wang, Y.-H.; Wang, M.; Zhao, J.; Ali, Z.; Brinckmann, J.A.; Li, J.; Wu, C.; et al. Chemometric analysis and chemical characterization for the botanical identification of Glycyrrhiza species (*G. glabra*, *G. uralensis*, *G. inflata*, *G. echinata* and *G. lepidota*) using liquid chromatography-quadrupole time of flight mass spectrometry (LC-QToF). *J. Food Compos. Anal.* **2022**, *112*, 104679. [\[CrossRef\]](#)
16. Akhtar, N.; Ihsan-ul-Haq; Mirza, B. Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. *Arab. J. Chem.* **2018**, *11*, 1223–1235. [\[CrossRef\]](#)
17. Sharma, V.; Katiyar, A.; Agrawal, R.C. Glycyrrhiza Glabra: Chemistry and Pharmacological Activity. In *Sweeteners*; Mérillon, J., Ramawat, K.G., Eds.; Springer: Cham, The Netherlands, 2018; pp. 87–100.
18. Jiang, M.; Zhao, S.; Yang, S.; Lin, X.; He, X.; Wei, X.; Song, Q.; Li, R.; Fu, C.; Zhang, J.; et al. An “essential herbal medicine” Licorice: A review of phytochemicals and its effects in combination preparations. *J. Ethnopharmacol.* **2020**, *249*, 112439. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Hosseini, M.S.; Ebrahimi, M.; Samsampour, D.; Abadía, J.; Khanahmadi, M.; Amirian, R.; Ghafoori, I.N.; Ghaderi-Zefrehei, M.; Gogorcena, Y. Association analysis and molecular tagging of phytochemicals in the endangered medicinal plant licorice (*Glycyrrhiza glabra* L.). *Phytochemistry* **2021**, *183*, 112629. [\[CrossRef\]](#)
20. Chang, K.-H.; Chen, I.-C.; Lin, H.-Y.; Chen, H.-C.; Lin, C.-H.; Lin, T.-H.; Weng, Y.-T.; Chao, C.-Y.; Wu, Y.-R.; Lin, J.-Y.; et al. The aqueous extract of Glycyrrhiza inflata can upregulate unfolded protein response-mediated chaperones to reduce tau misfolding in cell models of Alzheimer's disease. *Drug Des. Dev. Ther.* **2016**, *10*, 885–896.
21. Chen, C.-M.; Weng, Y.-T.; Chen, W.-L.; Lin, T.-H.; Chao, C.-Y.; Lin, C.-H.; Chen, I.-C.; Lee, L.-C.; Lin, H.-Y.; Wu, Y.-R.; et al. Aqueous extract of Glycyrrhiza inflata inhibits aggregation by upregulating PPARC1A and NFE2L2–ARE pathways in cell models of spinocerebellar ataxia 3. *Free Radic. Biol. Med.* **2014**, *71*, 339–350. [\[CrossRef\]](#) [\[PubMed\]](#)

22. Kong, Z.-H.; Chen, X.; Hua, H.-P.; Liang, L.; Liu, L.-J. The oral pretreatment of glycyrrhizin prevents surgery-induced cognitive impairment in aged mice by reducing neuroinflammation and Alzheimer's-related pathology via HMGB1 inhibition. *J. Mol. Neurosci.* **2017**, *63*, 385–395. [\[CrossRef\]](#)
23. Ravanfar, P.; Namazi, G.; Atigh, M.; Zafarmand, S.; Hamed, A.; Salehi, A.; Izadi, S.; Borhani-Haghighi, A. Efficacy of whole extract of licorice in neurological improvement of patients after acute ischemic stroke. *J. Herb. Med.* **2016**, *6*, 12–17. [\[CrossRef\]](#)
24. Hasan, M.K.; Ara, I.; Mondal, M.S.A.; Kabir, Y. Phytochemistry, pharmacological activity, and potential health benefits of *Glycyrrhiza glabra*. *Heliyon* **2021**, *7*, 1–10. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Yi, Y.; Zhang, M.; Xue, H.; Yu, R.; Bao, Y.-O.; Kuang, Y.; Chai, Y.; Ma, W.; Wang, J.; Shi, X.; et al. Schaftoside inhibits 3CLpro and PLpro of SARS-CoV-2 virus and regulates immune response and inflammation of host cells for the treatment of COVID-19. *Acta Pharm. Sin. B* **2022**, *12*, 4154–4164. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Xiang, C.; Qiao, X.; Ye, M.; Guo, D. Classification and distribution analysis of components in *Glycyrrhiza* using licorice compounds database. *Acta Pharm. Sin.* **2012**, *47*, 1023–1030.
27. Omoruyi, S.I.; Ibrakaw, A.S.; Ekpo, O.E.; Boatwright, J.S.; Cupido, C.N.; Hussein, A.A. Neuroprotective activities of crossyne flava bulbs and amaryllidaceae alkaloids: Implications for parkinson's disease. *Molecules* **2021**, *26*, 3990. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Egunlusi, A.O.; Malan, S.F.; Omoruyi, S.I.; Ekpo, O.E.; Palchykov, V.A.; Joubert, J. Open and rearranged norbornane derived polycyclic cage molecules as potential neuroprotective agents through attenuation of MPP+-and calcium overload-induced excitotoxicity in neuroblastoma SH-SY5Y cells. *Eur. J. Med. Chem.* **2020**, *204*, 112617. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Oyama, K.-i.; Kondo, T. Total synthesis of apigenin 7, 4'-di-O- β -glucopyranoside, a component of blue flower pigment of *Salvia patens*, and seven chiral analogues. *Tetrahedron* **2004**, *60*, 2025–2034. [\[CrossRef\]](#)
30. Chen, K.; Hu, Z.-m.; Song, W.; Wang, Z.-l.; He, J.-b.; Shi, X.-m.; Cui, Q.-h.; Qiao, X.; Ye, M. Diversity of O-glycosyltransferases contributes to the biosynthesis of flavonoid and triterpenoid glycosides in *Glycyrrhiza uralensis*. *Am. Chem. Soc. Synth. Biol.* **2019**, *8*, 1858–1866. [\[CrossRef\]](#)
31. Wu, J.-Y.; Ding, H.-Y.; Wang, T.-Y.; Cai, C.-Z.; Chang, T.-S. Application of Biotransformation-Guided Purification in Chinese Medicine: An Example to Produce Butin from Licorice. *Catalysts* **2022**, *12*, 1–10. [\[CrossRef\]](#)
32. Tian, G.; Zhang, U.; Zhang, T.; Yang, F.; Ito, Y. Separation of flavonoids from the seeds of *Vernonia anthelmintica* Willd by high-speed counter-current chromatography. *J. Chromatogr. A* **2004**, *1049*, 219–222. [\[CrossRef\]](#)
33. Ji, S.; Li, Z.; Song, W.; Wang, Y.; Liang, W.; Li, K.; Tang, S.; Wang, Q.; Qiao, X.; Zhou, D.; et al. Bioactive Constituents of *Glycyrrhiza uralensis* (Licorice): Discovery of the Effective Components of a Traditional Herbal Medicine. *J. Nat. Prod.* **2016**, *79*, 281–292. [\[CrossRef\]](#)
34. Pastorino, G.; Cornara, L.; Soares, S.; Rodrigues, F.; Oliveira, M.B.P. Liquorice (*Glycyrrhiza glabra*): A phytochemical and pharmacological review. *Phytother. Res.* **2018**, *32*, 2323–2339. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Park, J.-H.; Wu, Q.; Yoo, K.-H.; Yong, H.-I.; Cho, S.-M.; Chung, I.-S.; Baek, N.-I. Cytotoxic effect of flavonoids from the roots of *Glycyrrhiza uralensis* on human cancer cell lines. *J. Appl. Biol. Chem.* **2011**, *54*, 67–70. [\[CrossRef\]](#)
36. Asada, Y.; Li, W.; Yoshikawa, T. Biosynthesis of the dimethylallyl moiety of glabrol in *Glycyrrhiza glabra* hairy root cultures via a non-mevalonate pathway. *Phytochemistry* **2000**, *55*, 323–326. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Nomura, T.; Fukai, T.; Akiyama, T. Chemistry of phenolic compounds of licorice (*Glycyrrhiza* species) and their estrogenic and cytotoxic activities. *Pure Appl. Chem.* **2002**, *74*, 1199–1206. [\[CrossRef\]](#)
38. Lee, J.E.; Lee, J.Y.; Kim, J.; Lee, K.; Choi, S.U.; Ryu, S.Y. Two minor chalcone acetylglycosides from the roots extract of *Glycyrrhiza uralensis*. *Arch. Pharmacol. Res.* **2015**, *38*, 1299–1303. [\[CrossRef\]](#)
39. Wang, D.; Liang, J.; Zhang, J.; Wang, Y.; Chai, X. Natural chalcones in Chinese materia medica: Licorice. *Evid.-Based Complement. Altern. Med.* **2020**, *2020*, 3821248. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Kaur, P.; Kaur, S.; Kumar, N.; Singh, B.; Kumar, S. Evaluation of antigenotoxic activity of isoliquiritin apioside from *Glycyrrhiza glabra* L. *Toxicol. Vitro* **2009**, *23*, 680–686. [\[CrossRef\]](#) [\[PubMed\]](#)
41. Fu, B.; Li, H.; Wang, X.; Lee, F.S.C.; Cui, S. Isolation and Identification of Flavonoids in Licorice and a Study of Their Inhibitory Effects on Tyrosinase. *J. Agric. Food Chem.* **2005**, *53*, 7408–7414. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Li, G.; Simmler, C.; Chen, L.; Nikolic, D.; Chen, S.-N.; Pauli, G.F.; van Breemen, R.B. Cytochrome P450 inhibition by three licorice species and fourteen licorice constituents. *Eur. J. Pharm. Sci.* **2017**, *109*, 182–190. [\[CrossRef\]](#)
43. Montoro, P.; Maldini, M.; Russo, M.; Postorino, S.; Piacente, S.; Pizza, C. Metabolic profiling of roots of liquorice (*Glycyrrhiza glabra*) from different geographical areas by ESI/MS/MS and determination of major metabolites by LC-ESI/MS and LC-ESI/MS/MS. *J. Pharm. Biomed. Anal.* **2011**, *54*, 535–544. [\[CrossRef\]](#)
44. Kinoshita, T.; Tamur, Y.; Mizutani, K. The Isolation and Structure Elucidation of Minor Isoflavonoids from Licorice of *Glycyrrhiza glabra* Origin. *Chem. Pharm. Bull.* **2005**, *53*, 847–849. [\[CrossRef\]](#)
45. Fukai, T.; Sheng, C.-B.; Horikoshi, T.; Nomura, T. Isoprenylated flavonoids from underground parts of *Glycyrrhiza glabra*. *Phytochemistry* **1996**, *43*, 1119–1124. [\[CrossRef\]](#)
46. Adesanya, S.; O'Neill, M.J.; Roberts, M.F. Structure-related fungitoxicity of isoflavonoids. *Physiol. Mol. Plant Pathol.* **1986**, *29*, 95–103. [\[CrossRef\]](#)
47. Piccinelli, A.L.; Fernandez, M.C.; Cuesta-Rubio, O.; Hernández, I.M.; Simone, F.D.; Rastrelli, L. Isoflavonoids Isolated from Cuban Propolis. *J. Agric. Food Chem.* **2005**, *53*, 9010–9016. [\[CrossRef\]](#)

48. Liaoa, W.C.; Linb, Y.-H.; Changc, T.-M.; Huang, W.-Y. Identification of two licorice species, *Glycyrrhiza uralensis* and *Glycyrrhiza glabra*, based on separation and identification of their bioactive components. *Food Chem.* **2012**, *132*, 2188–2193. [\[CrossRef\]](#)
49. Chintharlapalli, S.; Papineni, S.; Jutooru, I.; McAlees, A.; Safe, S. Structure-dependent activity of glycyrrhetic acid derivatives as peroxisome proliferator-activated receptor γ agonists in colon cancer cells. *Mol. Cancer Ther.* **2007**, *6*, 1588–1598. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Baltina, L.A.; Budaev, A.S.; Mikhailova, L.R.; Baltina, J.L.A.; Spirikhin, L.V.; Makara, N.S.; Zarudii, F.S. New stereoisomeric glycyrrhetic acid derivatives. *Chem. Nat. Compd.* **2014**, *50*, 1042–1046. [\[CrossRef\]](#)
51. Kuroda, M.; Mimakia, Y.; Honda, S.; Tanaka, H.; Yokota, S.; Mae, T. Phenolics from *Glycyrrhiza glabra* roots and their PPAR-c ligand-binding activity. *Bioorganic Med. Chem.* **2010**, *18*, 962–970. [\[CrossRef\]](#)
52. Hoglinger, G.U.; Carrard, G.; Michel, P.P.; Medja, F.; Lombès, A.; Ruberg, M.; Friguet, B.; Hirsch, E.C. Dysfunction of mitochondrial complex I and the proteasome: interactions between two biochemical deficits in a cellular model of Parkinson's disease. *J. Neurochem.* **2003**, *86*, 1297–1307. [\[CrossRef\]](#)
53. Omoruyi, S.I.; Akinfenwa, A.O.; Ekpo, O.E.; Hussein, A.A. Aspalathin and linearthin from *Aspalathus linearis* (Rooibos) protect SH-SY5Y cells from MPP⁺-induced neuronal toxicity. *South Afr. J. Bot.* **2023**, *157*, 53–63. [\[CrossRef\]](#)
54. Kouli, A.; Torsney, K.M.; Kuan, W.-L. Parkinson's disease: Etiology, neuropathology, and pathogenesis. *Exon Publ.* **2018**, 3–26. [\[CrossRef\]](#)
55. Pang, S.Y.-Y.; Ho, P.W.-L.; Liu, H.-F.; Leung, C.-T.; Li, L.; Chang, E.E.S.; Ramsden, D.B.; Ho, S.-L. The interplay of aging, genetics and environmental factors in the pathogenesis of Parkinson's disease. *Transl. Neurodegener.* **2019**, *8*, 23. [\[CrossRef\]](#)
56. Massano, J.; Bhatia, K. Clinical approach to Parkinson's disease: Features, diagnosis, and principles of management. *Cold Spring Harb. Perspect. Med.* **2012**, *2*, a008870. [\[CrossRef\]](#) [\[PubMed\]](#)
57. HOHno; Arahō, D.; Uesawa, Y.; Kagaya, H.; Ishihara, M.; Sakagami, H.; Yamamoto, M. Evaluation of Cytotoxicity and Tumor-specificity of Licorice Flavonoids Based on Chemical Structure. *Anticancer Res.* **2013**, *33*, 3061–3068.
58. Goel, B.; Sharma, A.; Tripathi, N.; Bhardwaj, N.; Sahu, B.; Kaur, G.; Singh, B.; Jain, S.K. In-vitro antitumor activity of compounds from *Glycyrrhiza glabra* against C6 glioma cancer cells: Identification of natural lead for further evaluation. *Nat. Prod. Res.* **2021**, *35*, 5489–5492. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Lee, C.S.; Kim, Y.J.; Lee, M.S.; Han, E.S.; Lee, S.J. 18 β -Glycyrrhetic acid induces apoptotic cell death in SiHa cells and exhibits a synergistic effect against antibiotic anti-cancer drug toxicity. *Life Sci.* **2008**, *83*, 481–489. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Kowalska, A.; Kalinowska-Lis, U. 18 β -Glycyrrhetic acid: Its core biological properties and dermatological applications. *Int. J. Cosmet. Sci.* **2019**, *41*, 325–331. [\[CrossRef\]](#) [\[PubMed\]](#)
61. Tay, K.-C.; Tan, L.T.-H.; Chan, C.K.; Hong, S.L.; Chan, K.-G.; Yap, W.H.; Pusparajah, P.; Lee, L.-H.; Goh, B.-H. Formononetin: A Review of Its Anticancer Potentials and Mechanisms. *Front. Pharmacol.* **2019**, *10*, 820. [\[CrossRef\]](#) [\[PubMed\]](#)
62. Oh, J.-S.; Kim, T.-H.; Park, J.-H.; Lim, H.; Cho, I.-A.; You, J.-S.; Lee, G.-J.; Seo, Y.-S.; Kim, D.K.; Kim, C.S.; et al. Formononetin induces apoptotic cell death through the suppression of mitogen activated protein kinase and nuclear factor κ B phosphorylation in FaDu human head and neck squamous cell carcinoma cells. *Oncol. Rep.* **2020**, *43*, 700–710. [\[CrossRef\]](#)
63. Jiang, D.; Rasul, A.; Batool, R.; Sarfraz, I.; Hussain, G.; Tahir, M.M.; Qin, Z.S.T.; Ali, M.; Li, J.; Li, X. Potential Anticancer Properties and Mechanisms of Action of Formononetin. *BioMed Res. Int.* **2019**, *2019*, 854315. [\[CrossRef\]](#)
64. Hsu, Y.-C.; Hsieh, W.-C.; Chen, S.-H.; Li, Y.-Z.; Liao, H.-F.; Lin, M.-Y.; Sheu, S.-M. 18 β -glycyrrhetic Acid Modulated Autophagy is Cytotoxic to Breast Cancer Cells. *Int. J. Med. Sci.* **2023**, *20*, 444–454. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Han, X.; Ren, D.; Fan, P.; Shen, T.; Lou, H. Protective effects of naringenin-7-O-glucoside on doxorubicin-induced apoptosis in H9C2 cells. *Eur. J. Pharmacol.* **2008**, *581*, 47–53. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Sugumar, M.; Sevanan, M.; Sekar, S. Neuroprotective effect of naringenin against MPTP-induced oxidative stress. *Int. J. Neurosci.* **2019**, *129*, 534–539. [\[CrossRef\]](#) [\[PubMed\]](#)
67. Hassan, H.M.; Elnagar, M.R.; Abdelrazik, E.; Mahdi, M.R.; Hamza, E.; Elattar, E.M.; ElNashar, E.M.; Alghamdi, M.A.; Al-Qahtani, Z.; Al-Khater, K.M.; et al. Neuroprotective effect of naringin against cerebellar changes in Alzheimer's disease through modulation of autophagy, oxidative stress and tau expression: An experimental study. *Front. Neuroanat.* **2022**, *16*, 1012422. [\[CrossRef\]](#) [\[PubMed\]](#)
68. Mansour, L.; Elshopakey, G.; Abdelhamid, F.; Albukhari, T.; Almeshmadi, S.; Refaat, B.; El-Boshy, M.; Risha, E. Hepatoprotective and Neuroprotective Effects of Naringenin against Lead-Induced Oxidative Stress, Inflammation, and Apoptosis in Rats. *Biomedicines* **2023**, *11*, 1080. [\[CrossRef\]](#) [\[PubMed\]](#)
69. Li, Y.; Song, W.; Tong, Y.; Zhang, X.; Zhao, J.; Gao, X.; Yong, J.; Wang, H. Isoliquiritin ameliorates depression by suppressing NLRP3-mediated pyroptosis via miRNA-27a/SYK/NF- κ B axis. *J. Neuroinflammation* **2021**, *18*, 1. [\[CrossRef\]](#) [\[PubMed\]](#)
70. Prajapati, R.; Seong, S.H.; Park, S.E.; Paudel, P.; Jung, H.A.; Choi, J.S. Isoliquiritigenin, a potent human monoamine oxidase inhibitor, modulates dopamine D1, D3, and vasopressin V1A receptors. *Sci. Rep.* **2021**, *11*, 23528. [\[CrossRef\]](#) [\[PubMed\]](#)
71. Qu, Z.; Chen, Y.; Luo, Z.-H.; Shen, X.-L.; Hu, Y.-J. 7-methoxyflavanone alleviates neuroinflammation in lipopolysaccharide-stimulated microglial cells by inhibiting TLR4/MyD88/MAPK signalling and activating the Nrf2/NQO-1 pathway. *J. Pharm. Pharmacol.* **2020**, *72*, 385–395. [\[CrossRef\]](#)
72. Pörtl, D.; Schildknecht, S.; Karreman, C.; Leist, M. Uncoupling of ATP-depletion and cell death in human dopaminergic neurons. *NeuroToxicology* **2012**, *33*, 769–779. [\[CrossRef\]](#)

73. Babu, V.; Khurana, N. A review on mitochondrial dysfunction and oxidative stress due to complex-i in Parkinson disease. *Res. J. Pharmacol. Pharmacodyn.* **2021**, *13*, 167–170. [[CrossRef](#)]
74. Dorszewska, J.D.P.; Kowalska, M.; Predecki, M.; Piekut, T.; Kozłowska, J.; Kozubski, W. Oxidative stress factors in Parkinson's disease. *Neural Regen. Res.* **2021**, *16*, 1383–1391. [[CrossRef](#)] [[PubMed](#)]
75. Błaszczyk, J.W. Energy metabolism decline in the aging brain—Pathogenesis of neurodegenerative disorders. *Metabolites* **2020**, *10*, 450. [[CrossRef](#)] [[PubMed](#)]
76. Jiménez-Delgado, A.; Ortiz, G.G.; Delgado-Lara, D.L.; González-Usigli, H.A.; González-Ortiz, L.J.; Cid-Hernández, M.; Cruz-Serrano, J.A.; Pacheco-Moisés, F.P. Effect of Melatonin Administration on Mitochondrial Activity and Oxidative Stress Markers in Patients with Parkinson's Disease. *Oxidative Med. Cell. Longev.* **2021**, *2021*, 1–7. [[CrossRef](#)] [[PubMed](#)]
77. Zhou, Y.-z.; Li, X.; Gong, W.-x.; Tian, J.-s.; Gao, X.-x.; Gao, L.; Zhang, X.; Du, G.-h.; Qin, X.-m. Protective effect of isoliquiritin against corticosterone-induced neurotoxicity in PC12 cells. *Food Funct.* **2017**, *8*, 1235–1244. [[CrossRef](#)]
78. Abramov, A.; Angelova, P.R. Mitochondrial dysfunction and energy deprivation in the mechanism of neurodegeneration. *Turk. J. Biochem.* **2019**, *44*, 723–729. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.