Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i19.3593

EFFICACY OF A POLYHERBAL FORMULATION IN ALLEVIATING SEIZURES IN A RAT MODEL OF EPILEPSY

Taufiq Ahmad[1], Sultan Ayaz[1*], Abid Rashid[2], Hafiz Muhammad Asif[3], Seyd Muhammad Ali Shah[1]

[1*]Department of Eastern Medicine, Faculty of Medical Sciences, Government College University Faisalabad, Punjab, Pakistan
[2]Faculty of Medical Sciences, Government College University Faisalabad, Punjab, Pakistan
[3] University College of Conventional Medicine, The Islamia University of Bahawalpur, Pakistan

***Corresponding Author:** Sultan Ayaz
Email: research.publication1994@gmail.com

Abstract
Epilepsy poses a significant global health burden, necessitating the exploration of alternative therapeutic approaches. This research investigates the antiepileptic and neuropharmacological potential of a polyherbal extract comprising *Catharanthus roseus* (CR), *Ocimum sanctum* (OS), *Valeriana officinalis* (VO), and *Withania somnifera* (WS) in a rat model. The study employs behavioral assays, forebrain biogenic amine level assessments, and neuropharmacological tests to comprehensively evaluate the effects of the polyherbal extract. Leg extension time, stupor time, flexion time, and righting reflex recovery time are among the seizure-related metrics that significantly decreased in the polyherbal extract and diazepam-treated groups as compared to the control group, indicating the antiepileptic activity. Serotonin, dopamine, and noradrenaline levels significantly rise following the injection of polyherbal extract, according to forebrain biogenic amine analysis, indicating a possible regulation of neurotransmitter systems. Increased squares crossed in the Open Field Test, increased rearing behaviour, decreased cage crossing activity, longer travel times in the Traction Test, and longer periods of immobility in the Forced Swimming Test are all indicators of anxiolytic and sedative effects revealed by neuropharmacological evaluations. These results are consistent with the antioxidant, neuroprotective, and anxiolytic qualities seen in each of the polyherbal formulation's constituent ingredients. This research sets the stage for the exploration of phytotherapeutic alternatives in epilepsy treatment, emphasizing the potential of natural remedies in neurological disorder management.

Keywords: Epilepsy, Polyherbal extract, *Catharanthus roseus*, *Ocimum sanctum*, *Valeriana officinalis*, *Withania somnifera*

1. Introduction

Epilepsy, a prevalent neurological disorder, imposes a substantial global burden on disability and mortality (Moon et al., 2023). It is characterized by frequent and spontaneous seizures that cause abnormal electrical activity in the brain. This can cause motor abnormalities, altered awareness, and behavioral disturbances (Adiga & Nandit, 2023) (Guerrini et al., 2023). The disturbance of the homeostatic balance between inhibitory and excitatory currents, favoring the latter, is a basic feature of the pathophysiology of epilepsy (Guerrini et al., 2023) (Dossi & Huberfeld, 2023).

Despite having several antiepileptic medications available in modern medicine that can control seizures and postpone epileptogenesis, such as sodium valproate, phenobarbital, phenytoin, and benzodiazepines, their effectiveness is restricted to treating only 70% of cases (Łukasiuk & Lasoń, 2023) (Stafford, 2008) (Aderibigbe, 2016). Moreover, apprehensions over their toxicity, which includes adverse reactions varying from increased body weight to signs of neuropsychiatry, highlight the necessity for safer substitutes (Beppe et al., 2015). Instead of stopping or slowing the progression of epilepsy, the main focus of these drugs is on its symptoms (Pinzon et al., 2019). The lack of a reliable and effective antiepileptic medication, despite intensive efforts by scientists and pharmaceutical corporations, highlights the need for new management options (Eldeen et al., 2005).
In response to the limitations of conventional drugs, a growing focus on plant-based medicine has gained prominence. With over 80% of the global population relying on herbal remedies (Amuri et al., 2017), researchers are increasingly exploring phytotherapeutic alternatives as potentially safer and more effective options for epilepsy management.
Utilizing the medicinal potential of four powerful botanicals, *Ocimum sanctum, Withania somnifera, Valeriana officinalis*, and *Catharanthus roseus*, the polyherbal mixture examined in this study. For their distinct medicinal qualities, these plants have all been valued in a variety of traditional medical systems. *Ocimum sanctum*, also referred to as holy basil or tulsi, is prized for its anti-inflammatory and neuroprotective qualities (Sarris et al., 2011). Ashwagandha, or *Withania somnifera*, is well known for its adaptogenic properties, which include reducing stress and perhaps having anti-epileptic benefits (Behl et al., 2020) (Bashir et al., 2023). *Catharanthus roseus*, or Madagascar periwinkle, is known for its anti-inflammatory and antioxidant qualities (Tolambiya et al., 2023) (Ahmed et al., 2022), while *Valeriana officinalis*, or Valerian, offers sedative and anticonvulsant qualities (Das et al., 2021). Combining different plant elements into a polyherbal formulation, the current study aims to leverage each one's unique characteristics in order to fully treat the intricate pathophysiology of epilepsy. This synergistic approach holds promise in providing a natural and potentially efficacious alternative to conventional antiepileptic medications, underscoring the significance of exploring herbal therapies in the pursuit of improved epilepsy management.

2. Methodology

2.1. Plants used in the research and Preparation of polyherbal extract

Four medicinal plants were chosen based on their historical use and medicinal properties:
Catharanthus roseus (CR) - Sadabahar or Rantanjot
Ocimum sanctum (OS) - Tulsi
Valeriana officinalis (VO) - Bal-char
Withania somnifera (WS) - Ashwagandha
Whole plants were utilized for extraction, specifically focusing on roots and leaves. Plants were ethically harvested from various regions in Pakistan, ensuring diversity and potency. Collected plant parts were thoroughly washed with distilled water and dried in an oven at 40 degrees Celsius overnight. Dried leaves and roots were ground into a fine powder using a domestic grinder, maintaining consistency across all samples. The powdered leaves and roots of each herb underwent hydroethanolic extraction for 72 hours, utilizing a shaker with an ethanol:water ratio of 80:20. Maintained a solute-to-solvent ratio of 1:10 for all extracts to optimize extraction effectiveness. To ensure maximum extraction, the residue was re-extracted twice after filtering. The solvent was evaporated using a rotary evaporator under decreasing pressure, resulting in a semi-solid substance (Boota et al., 2022; Mustafa et al., 2021; Nisar et al., 2023).

2.2. Animal Selection and Grouping

Thirty number of healthy wistar rats weighing between 200 to 250 grams (6 to 8 weeks age) were selected for the study to establish a strong baseline. rats were housed in controlled conditions with standard temperature and humidity. Each rat received water and standard pellet meals. The research, conducted at the University of Balochistan, Quetta, Pakistan, adhered to ethical standards and regulations governing animal research. The rats were divided into three groups (n=10). Groups 1 was

the control group receiving 1 mL of 5% CMC as i.p injection daily. Group 2 was the experimental group receiving 450mg/kg of the polyherbal extract as i.p injection daily and the group 3 was the standard control group receiving diazepam as standard anticonvulsant drug at the dose of 3mg/kg daily. All the rats were observed and treated for 15 days, noting any changes in awareness, mood, movement, posture, motor coordination, muscle tone, and reflexes. On day 16 half of the rats from each group (n=5) were induced seizures by maximal electric shock (MES) using an electroconvulsometer to give single, 12-mA, 50 Hz stimulus for 0.2 seconds. A droplet of an electrolyte solution (0.9 percent NaCl) having lignocaine was administered before applying the ocular electrodes to the rats. That's why there are fewer deaths overall, but more communication. The sums of the times spent in each phase of recurrent epilepsy were calculated. The length of the clonic phase, the duration of the convulsions as a whole, and the beginning of tonic hindlimb extension (THE) were all calculated. The remaining half rats (n=5) from each group were treated with Pentylenetetrazole (PTZ), at the dose of 70mg/kg as a single i.p injection to induce seizures. The reaction to PTZ was assessed an hour after oral sodium valproate delivery using the PTZ-induced seizure paradigm. To assess the anti-epileptic impact, Clonic seizure presence/absence, seizure onset latency, and seizure duration were employed. After all the behavior test the animals were decapitated by cervical dislocation to collect brain tissue for the evaluation of serotonin, Dopamine and noradrenaline. The brain tissue was stored at -20°C until further analysis.

2.3. Open Field Activity Test:

In an open field of 40 cm in length, 40 cm in breadth, and 45 cm in height, with 16 smaller squares measuring exactly 10 cm by 10 cm, mice were examined for their exploratory behavior, motor coordination, and locomotor activity (Nkwingwa et al., 2023). Animals were tested in an open field setting one hour following the conclusion of the new object recognition task session. For five minutes, animals could investigate the box. The number of lines crossed, rearing, grooming, and time spent in the center were all scored using manually controlled counters and stopwatches.

2.4. Cage Crossing Movement Test:

Maze traversal abilities were assessed in an elevated plus-maze, measuring the impact on walking abilities (Nkwingwa et al., 2023).

2.5. Rearing Test:

Rearing behavior was observed, and the number of jumps recorded to assess behavioral responses (Nkwingwa et al., 2023).

2.6. Swimming Test:

Daily forced swimming was implemented to gauge the effects on physical endurance and response to exercise. Muscle and central nervous system (CNS) effects were studied through a swimming test subjected to external forces (Acar et al., 2022).

2.7. Traction Test:

The time taken for rats to slide down an iron pole was recorded to evaluate the impact on motor coordination (Han et al., 2021).

2.8. Biogenic Amine Levels

For the evaluation of serotonin, dopamine and noradrenaline, ELISA kits from elabsciences (Cat.No.: E-EL-R1140; Cat.No.: E-EL-0343; and Cat.No.: E-EL-0047 respectively)

2.9. Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze the data, and post hoc tests were used for multiple comparisons. When $p<0.05$, effects were supposed significant.

3. Results

3.1. Anti-Epileptic Activity

The analysis of variance (ANOVA) and the t test were used for comparisons and the results thus obtained are expressed in table 1. The time for leg extension was decreased significantly (P≤0.001) in polyherbal extract treatment group (128.5±0.22*** sec) and diazepam treated group (258.79±2.28*** sec) as compared with the control group (389.24±2.86 sec). Similarly, the time for stopor was also decreased significantly (P≤0.01) in the polyherbal extract treated group (1.20±0.64*** sec) as compared with the control group (5.76±1.05 sec). The flexion time was also decreased significantly (P≤0.001) in polyherbal extract treatment group (65.04±0.07*** sec) and diazepam treated group (84.65±0.86*** sec) as compared with the control group (166.47±1.62 sec). A substantial reduction in righting reflex recovery time was observed, lending credence to the hypothesis that Polyherbal extract was effective in avoiding MES-induced seizures.

Table. 1: Polyherbal Extract's Influence on MES-Susceptible Rats with Seizures.

Group		Extension	Stupor	Flexion	Clonus	Recovery
		Time in seconds				
I	Control	389.24±2.86	5.76±1.05	166.47±1.62	35.85±0.57***	176.6
II	Polyherbal extract	128.5±0.22***	1.20±0.64***	65.04±0.07***	54.43±1.24***	175.2
III	Diazepam	258.79±2.28***	21±1.67**	84.65±0.86***	71.83±1.12***	145.63

*P< 0.05, **P< 0. 01, ***P < 0. 001.

3.2. Forebrain biogenic amine levels

It was shown that extract administration substantially enhanced (P≤0.001) forebrain serotonin, dopamine, and noradrenaline levels in rats (Table 4.2).

Table. 2: Polyherbal's impact on forebrain biogenic amine levels in a rat model of epilepsy.

Groups		Serotonin, mg-g^{-1} of wet brain tissue	Dopamine, mg-g^{-1} of wet brain tissue	Noradrenaline, mg-g^{-1} of wet brain tissue
		ng/mL	pg/mL	pg/mL
I	Control	162.43±1.52	48.15± 2.67	30.64±0.65***
II	Polyherbal-extraxt	61.02±0.57***	131.6± 0.31***	54.37±1.42***
III	Diazepam	74.61±0.89***	265.79±2.13***	72.70±1.22***

*P< 0.05, **P< 0. 01, ***P < 0. 001.

Polyherbal extract's substantial protection against MES-induced seizures led to the hypothesis that it might significantly (P < 0. 001) decrease brain levels of serotonin (61.02±0.57), and increased levels of dopamine (131.6± 0.31 pg/mL), and noradrenaline (54.37±1.42pg/mL) as compared with the control group (162.43±1.52 ng/mL, 48.15± 2.67 pg/mL, and 30.64±0.65 pg/mL respectively) (Table. 2). By inhibiting monoamine oxidase (MAO), an enzyme responsible for the breakdown of biogenic amines, increased monoamine synthesis in the brain reduces the threshold for epileptic seizures.

3.2. Open Filed Test results

The control group had an average of 123.2±4.38 squares crossed, 187.7±3.31* for a polyherbal-extract at the dose of 450 mg/kg, and 79.3±3.32 for a group receiving a conventional medicine (Diazepam). The test results were significant when compared to the control group and traditional treatment (P < 0.05).

Table. 3: The Impact of Neuropharmacological Interventions on Free-Roaming Behavioral Testing.

Groups		Open Field Test (Mean ± SEM)
I	Control	123.2±4.38
II	Polyherbal extract	187.7±3.31*
III	Diazepam	79.3±3.32**

*P< 0.05, **P< 0. 01,

3.4. Rearing Test results

The test showed that the control group raised animals in an average amount of 133.2 ± 3.38, but the polyherbal extract raised animals to 177.7 ± 2.31. The results of the trial reveal that the polyherbal extract's sedative effects at both dosages were ($P < 0.05$) different from diazepam conventional medicine.

Table. 4: Neuropharmacological Activities' Effect on the Rearing Test

Groups		Rearing test (Mean ± SEM)
I	Control	133.2 ± 3.38
II	Polyherbal extract	177.7 ± 2.31*
III	Diazepam	77.3 ± 2.32**

*P< 0.05, **P< 0. 01,

3.5. Cage Crossing Test

The outcomes of the trial showed that the control set of animals crossed the cages an average of 26 ±2.23 times, the polyherbal (Test Group) crossed the cages an average of 15.6 ± 2.79 times, and the diazepam-treated group crossed the cages an average of 15.8 ± 2.35 times. Results reveal that, in comparison to the control, the conventional treatment and the polyherbal extract treatment significantly ($P < 0.05$) reduced cage crossing activity.

Table. 5: Neuropharmacological Activities' Effect on the Cage Crossing Test

Groups		Cage crossing Test (Mean ± SEM)
I	Control	26 ±2.23
II	Polyherbal extract	15.6 ± 2.79*
III	Diazepam	15.8 ± 2.35**

*P< 0.05, **P< 0. 01,

3.6. Traction Test

Control group crossing time was 7 ± 8.04 seconds, and 13.4 ± 2.31 seconds for polyherbal extract group while 16.1± 2.05 seconds in the diazepam-treated group. The traction test's travel time was prolonged after taking Polyherbal extract, demonstrating the sedative effects of the substance. The outcomes were significant ($P < 0.05$) to those of the reference drug diazepam (Table. 6).

Table. 6: Traction Test Results

Groups		Traction test (Mean ± SEM) (seconds)
I	Control	7 ± 8.04
II	Polyherbal extract	13.4 ± 2.31*
III	Diazepam	16.1± 2.05**

*P< 0.05, **P< 0. 01,

3.7. Forced Swimming Test

The results of the study demonstrate that the control group shifted their position after 4.23±1.008 minutes and remained still for an average of 3.31±1.007 minutes. After receiving 450 mg/kg of

polyherbal extract, the average time to move was 4.04±1.02 minutes, whereas the average time to stay still was 3.20±1.01 minutes. In the group given diazepam (a popular medicine), the time spent moving about was 3.27±1.27 minutes and the time spent sitting still was 4.28±1.02 minutes. Based on the results of the investigation, the polyherbal extract had sedative properties. The outcomes were significant ($P < 0.05$) to those of the reference drug diazepam (Table. 7).

Table. 7: Results of Forced Swimming Test

Groups		Mobility Mean ±SEM (min)	Immobility Mean ±SEM (min)
I	Control	4.23±1.008	3.31±1.007
II	Polyherbal extract	4.04±1.02*	3.20±1.01*
III	Diazepam	3.27±1.27**	4.28±1.02**

*P< 0.05, **P< 0. 01,

4. Discussion

The seizure was induced by a suprathreshold electrical shock (current intensity=70 mA, duration=0.2 s) delivered through ear clip electrodes 60 minutes after the administration of an automated external defibrillator (AED) or a combination of many herbal drugs. The incidence, onset latency, and duration of tonic hind limb extension (THLE) in rats was analyzed. THLE is defined as the animal's hind limbs being extended 70 mA to the plane of the body axis. Many different kinds of plants were used in this study. Data from a comparison of the Polyherbal extract to the gold standard are shown in Table 4.1, where a dose-dependent significant P is (0.01 and 0.001) decrease in many stages of epileptic episodes was found. The results of the present study provide valuable insights into the potential antiepileptic and neuropharmacological effects of the polyherbal formulation.

A strong antiepileptic impact is indicated by the considerable decrease in recovery times for leg extension, stopor, flexion, and righting reflex in the diazepam and polyherbal extract treatment groups as compared to the control group. These results are consistent with research showing the anticonvulsant qualities of the polyherbal formulation's constituent ingredients. For example, in animal models, the active ingredient in Withania somnifera (WS), withaferin A, has demonstrated antiepileptic benefits through regulating GABAergic transmission and lowering oxidative stress (Speers et al., 2021). Furthermore, a number of studies have documented *Ocimum sanctum*'s (OS) anxiolytic and antiepileptic properties (Panigrahi et al., 2022). The idea that the polyherbal extract effectively attenuated seizures generated by MES is supported by the observed reduction in the recovery time of the righting reflex.

The substantial increase in forebrain serotonin, dopamine, and noradrenaline levels in rats following the injection of polyherbal extract are notable. This rise could be a factor in the study's antiepileptic effects. Research has indicated that changes in dopamine and serotonin levels are important factors in epilepsy. As an illustration, serotonin functions as a neuromodulator, and a lack of it has been connected to a higher risk of seizures (Meftahi et al., 2023). Furthermore, dopamine has been linked to the regulation of seizures, therefore raising its levels could be a factor in the antiepileptic benefits seen in this investigation (Krabbe et al., 2015). The results corroborate the theory that the polyherbal extract has antiepileptic effects by regulating biogenic amine levels.

The Open Field Test results indicating increased squares crossed in the polyherbal extract and diazepam treated groups compared to the control group suggest anxiolytic and exploratory effects. Studies have demonstrated the anxiolytic properties of *Valeriana officinalis* (VO) (Marcucci et al., 2023), supporting the observed effects. The Rearing Test further supports the sedative effects of the polyherbal extract, with increased rearing behavior compared to the control group. The anxiolytic and sedative effects observed are consistent with studies on *Withania somnifera* (Ha et al., 2022) (Bhattacharya et al., 2000) and *Ocimum sanctum* (Yuniarti et al., 2021). The Cage Crossing Test's reduction in activity in both the polyherbal and diazepam treated groups aligns with the sedative effects reported in previous studies (Al-Attraqchi et al., 2020).

The findings of the Forced Swimming Test, where the groups treated with diazepam and herbal extract spent more time sitting still, were supported by the Traction Test results, which showed that the

polyherbal extract group travelled for a longer period of time, indicating sedative effects. These results are consistent with research describing the sedative qualities of *Valeriana officinalis* and *Withania somnifera* (Gupta et al., 2003) (Sarris et al., 2011). All of the data point to the polyherbal formulation's potential as a neuropharmacological agent by indicating that it has sedative and anxiolytic properties. The findings of this extensive investigation highlight the complex impact of the polyherbal extract on epilepsy and related behaviors. A comprehensive strategy for managing epilepsy is made possible by the manipulation of biogenic amine levels in addition to sedative and anxiolytic effects. Nevertheless, additional research, such as clinical trials, is necessary to confirm the polyherbal extract safety and effectiveness in human patients. Furthermore, clarifying the precise mechanisms behind the effects that have been noticed would advance our knowledge of the formulation's potential as a therapy.

Conclusion

In conclusion, our study underscores the promising antiepileptic and neuropharmacological properties of the polyherbal formulation derived from *Catharanthus roseus, Ocimum sanctum, Valeriana officinalis,* and *Withania somnifera*. In behavioural tests, the formulation showed notable seizure control, altered levels of forebrain biogenic amines, and had sedative and anxiolytic effects. These results confirm the formulation's potential as a complete therapy strategy for treating epilepsy and related behavioral changes. To establish its safety and effectiveness in human subjects, however, more research is necessary, especially in the form of clinical trials. This will open the door to creative and comprehensive approaches to the care of neurological disorders.

References

1. Acar, S., Kapucu, A., & Akgün-Dar, K. (2022). The effects of regular swimming exercise during sodium valproate treatment on seizure behaviors and EEG recordings in pentylenetetrazole-kindled rats. *Epilepsy Research*, *179*, 106830.
2. Aderibigbe, A. (2016). Neurobehavioral and Anticonvulsant effects of ethanol extract of Albizia adianthifolia leaves in experimental animals. *Archives of Basic and Applied Medicine*, *4*(2), 77–85-77–85.
3. Adiga, U., & Nandit, P. (2023). An Overview of Pathogenesis of Epilepsy. *International Neurourology Journal*, *27*(4), 88-94.
4. Ahmed, M., Sahibzada, M. U. K., Rasheed, H. M., Khan, T., Wahid, F., Farooq, U., Khusro, A., Uddin, J., Afzal, S., & Khan, A. (2022). Inhibition of Inflammation Associated Corneal Neovascularization by Dalbergia sissoo and Catharanthus roseus Leaf Extracts in an Animal Model. *South African Journal of Botany*, *151*, 379-386.
5. Al-Attraqchi, O. H., Deb, P. K., & Al-Attraqchi, N. H. A. (2020). Review of the Phytochemistry and Pharmacological Properties of Valeriana officinalis. *Current Traditional Medicine*, *6*(4), 260-277.
6. Amuri, B., Maseho, M., Simbi, L., Okusa, P., Duez, P., & Byanga, K. (2017). Hypoglycemic and antihyperglycemic activities of nine medicinal herbs used as antidiabetic in the region of Lubumbashi (DR Congo). *Phytotherapy research*, *31*(7), 1029-1033.
7. Bashir, A., Nabi, M., Tabassum, N., Afzal, S., & Ayoub, M. (2023). An updated review on phytochemistry and molecular targets of Withania somnifera (L.) Dunal (Ashwagandha). *Frontiers in Pharmacology*, *14*, 1049334.
8. Behl, T., Sharma, A., Sharma, L., Sehgal, A., Zengin, G., Brata, R., Fratila, O., & Bungau, S. (2020). Exploring the multifaceted therapeutic potential of withaferin A and its derivatives. *Biomedicines*, *8*(12), 571.
9. Beppe, G. J., Dongmo, A. B., Foyet, H. S., Dimo, T., Mihasan, M., & Hritcu, L. (2015). The aqueous extract of Albizia adianthifolia leaves attenuates 6-hydroxydopamine-induced anxiety, depression and oxidative stress in rat amygdala. *BMC Complementary and Alternative Medicine*, *15*(1), 1-13.
10. Bhattacharya, A., Ramanathan, M., Ghosal, S., & Bhattacharya, S. (2000). Effect of Withania somnifera glycowithanolides on iron-induced hepatotoxicity in rats. *Phytotherapy Research: An*

International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, *14*(7), 568-570.

11. Boota, M., Shah, S. M. A., Rashid, A., Akram, M., Ayaz, S., Mustafa, I., Nisar, J., & Nisar, Z. (2022). The Hepatoprotective and Anti-Nephrotoxic Potential of Methanolic Extract of a Polyherbal Preparation in CCl4-Induced Liver Injury Model of Wistar Rats. *Dose-Response*, *20*(3), 15593258221124728.
12. Das, G., Shin, H.-S., Tundis, R., Gonçalves, S., Tantengco, O. A. G., Campos, M. G., Acquaviva, R., Malfa, G. A., Romano, A., & Robles, J. A. H. (2021). Plant species of sub-family Valerianaceae—A review on its effect on the central nervous system. *Plants*, *10*(5), 846.
13. Dossi, E., & Huberfeld, G. (2023). GABAergic circuits drive focal seizures. *Neurobiology of Disease*, 106102.
14. Eldeen, I., Elgorashi, E., & Van Staden, J. (2005). Antibacterial, anti-inflammatory, anti-cholinesterase and mutagenic effects of extracts obtained from some trees used in South African traditional medicine. *Journal of Ethnopharmacology*, *102*(3), 457-464.
15. Guerrini, R., Conti, V., Mantegazza, M., Balestrini, S., Galanopoulou, A. S., & Benfenati, F. (2023). Developmental and epileptic encephalopathies: from genetic heterogeneity to phenotypic continuum. *Physiological Reviews*, *103*(1), 433-513.
16. Gupta, S. K., Dua, A., & Vohra, B. P. (2003). Withania somnifera (Ashwagandha) attenuates antioxidant defense in aged spinal cord and inhibits copper induced lipid peroxidation and protein oxidative modifications. *Drug metabolism and drug interactions*, *19*(3), 211-222.
17. Ha, J. W., Yu, J. S., Lee, B. S., Kang, D.-M., Ahn, M.-J., Kim, J. K., & Kim, K. H. (2022). Structural Characterization of Withanolide Glycosides from the Roots of Withania somnifera and Their Potential Biological Activities. *Plants*, *11*(6), 767.
18. Han, K., Jin, X., Guo, X., Cao, G., Tian, S., Song, Y., Zuo, Y., Yu, P., Gao, G., & Chang, Y.-Z. (2021). Nrf2 knockout altered brain iron deposition and mitigated age-related motor dysfunction in aging mice. *Free Radical Biology and Medicine*, *162*, 592-602.
19. Krabbe, S., Duda, J., Schiemann, J., Poetschke, C., Schneider, G., Kandel, E. R., Liss, B., Roeper, J., & Simpson, E. H. (2015). Increased dopamine D2 receptor activity in the striatum alters the firing pattern of dopamine neurons in the ventral tegmental area. *Proceedings of the National Academy of Sciences*, *112*(12), E1498-E1506.
20. Łukasiuk, K., & Lasoń, W. (2023). Emerging Molecular Targets for Anti-Epileptogenic and Epilepsy Modifying Drugs. *International Journal of Molecular Sciences*, *24*(3), 2928.
21. Marcucci, C., Rademacher, M., Kamecki, F., Pastore, V., Bach, H. G., Ricco, R. A., Wagner, M. L., Knez, D., Gobec, S., & Colettis, N. (2023). Biological Evaluation of Valeriana Extracts from Argentina with Potent Cholinesterase Inhibition for the Treatment of Neurodegenerative Disorders and Their Comorbidities—The Case of Valeriana carnosa Sm.(Caprifoliaceae) Studied in Mice. *Pharmaceuticals*, *16*(1), 129.
22. Meftahi, G. H., Hatef, B., & Jahromi, G. P. (2023). Creatine Activity as a Neuromodulator in the Central Nervous System. *Archives of Razi Institute*, *78*(4).
23. Moon, S. G., Kim, J.-K., Lee, S.-Y., & Kim, H. K. (2023). Trends of Epilepsy-Related Mortality in South Korea. *Journal of Clinical Neurology (Seoul, Korea)*, *19*(2), 174.
24. Mustafa, I., Faisal, M. N., Hussain, G., Muzaffar, H., Imran, M., Ijaz, M. U., Sohail, M. U., Iftikhar, A., Shaukat, A., & Anwar, H. (2021). Efficacy of Euphorbia helioscopia in context to a possible connection between antioxidant and antidiabetic activities: a comparative study of different extracts. *BMC Complementary Medicine and Therapies*, *21*, 1-12.
25. Nisar, J., Ali Shah, S. M., Ayaz, S., Akram, M., Rashid, A., Mustafa, I., & Nisar, Z. (2023). In vitro comparative evaluation of Tamarix gallica extracts for antioxidant and antidiabetic activity. *Experimental Biology and Medicine*, *248*(3), 253-262.
26. Nkwingwa, B. K., Wado, E. K., Foyet, H. S., Bouvourne, P., Jugha, V. T., Mambou, A. H. M. Y., Bila, R. B., & Taiwe, G. S. (2023). Ameliorative effects of Albizia adianthifolia aqueous extract against pentylenetetrazole-induced epilepsy and associated memory loss in mice: Role of

GABAergic, antioxidant defense and anti-inflammatory systems. *Biomedicine & Pharmacotherapy*, *165*, 115093.

27. Panigrahi, A. K., Satapathy, S., Mishra, S. K., & Jena, S. K. (2022). Effect of Ocimum sanctum leaf extract in animal models of anxiety. *National Journal of Physiology, Pharmacy and Pharmacology*, *12*(7), 1084-1084.
28. Pinzon, R. T., Buwana, F., Sanyasi, R. D. L. R., Dharmawan, A., & Wijono, J. (2019). Antiepileptic drugs and quality of life in patients with epilepsy: A tertiary care hospital-based study. *Asian Journal of Pharmacy and Pharmacology*, *5*(2), 249-254.
29. Sarris, J., Panossian, A., Schweitzer, I., Stough, C., & Scholey, A. (2011). Herbal medicine for depression, anxiety and insomnia: a review of psychopharmacology and clinical evidence. *European neuropsychopharmacology*, *21*(12), 841-860.
30. Speers, A. B., Cabey, K. A., Soumyanath, A., & Wright, K. M. (2021). Effects of Withania somnifera (Ashwagandha) on stress and the stress-related neuropsychiatric disorders anxiety, depression, and insomnia. *Current neuropharmacology*, *19*(9), 1468.
31. Stafford, R. S. (2008). Regulating off-label drug use—rethinking the role of the FDA. *New England Journal of Medicine*, *358*(14), 1427-1429.
32. Tolambiya, P., Jagetiya, B., & Mathur, S. (2023). EXTRACTION AND IDENTIFICATION OF VARIOUS PHYTOPHARMACEUTICAL CONSTITUENT OF CATHARANTHUS ROSEUS L.(ALBA) LEAVES. *Plant Archives*, *23*(2), 158-165.
33. Yuniarti, W. M., Krismaharani, N., Ciptaningsih, P., Celia, K., Veteriananta, K. D., Ma'ruf, A., & Lukiswanto, B. S. (2021). The protective effect of Ocimum sanctum leaf extract against lead acetate-induced nephrotoxicity and hepatotoxicity in mice (Mus musculus). *Veterinary World*, *14*(1), 250.