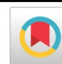

Cannabis Sativa: A Remedy for Convulsion by Inhibition of GABAA Receptor and Significantly Delayed Onset of Seizure Latency and Death - An Experimental Validation and Molecular Docking

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Abstract

This study aimed to investigate the anticonvulsant potentials of Cannabis sativa and its possible mechanism using the in vivo and in-silico models. A total of 20 male albino mice was used for this study and were divided randomly into five groups. Group 1 as negative control receiving 0.2ml 0f 10% ethanol only, and groups 2 and 3, received 200mg/kg/ body weight low dose of C. Sativa extract and 400mg/kg bw low dose of C. sativa extract, while groups 4 received 50 mg/kg body weight of phenobarbitone and group 5 received 50 mg/kg body weight of phenobarbitone+ 200 mg/kg body weight of the extract. Administration of C. Sativa extract was done via orogastric feeding; GABA-ergicseizure, was induced by administration of 80 mg/kg/bodyweight of Pentylenetetrazole (PTZ) via the intra-peritoneal route to all the groups. This was done about thirty minutes after the administration of treatment agents. Each animal was observed individually for one hour to determine the onset of muscular jerks and tonic hind limb extensions and death. Animal study results revealed that C. sativa may likely possess' anticonvulsant potentials as it is significantly seizure latency and death in PTZ treated mice. For the in silico studies, about 49 compounds of C. sativa were obtained from the PubChem library and were docked on various targets site of proteins; Glutaminase, 4-Aminobtyrate Aminotransferase, Perioxisome proliferator Activated receptor (PPAR), Succinic semialdehyde dehydrogenase, Gama amino butyric acid – A receptor (GABA-A), Histone deacetylases (HDAC), Phosphoribosyl pyrophosphate amido transferase and human GAFT-1. Furthermore, the in-silico study revealed that compounds like Cannabisin A and D showed anticonvulsant potential activation of GABA.

Key Words: Convulsions, Anticonvulsant, Cannabis Sativa, in-silico studies, Glutaminase

Significance | Development of anticonvulsant herbal drug.

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Introduction

Convulsion is defined as a paroxysmal involuntary disturbance of brain function that manifests as an impairment or loss of consciousness, abnormal motor activity, behavioural abnormalities, sensory disturbances, or autonomic dysfunction (Anagilaje and Anagilaje, 2012). A convulsion is a general term that people use to describe uncontrollable muscle contractions; some may use it interchangeably with "seizure" or "epilepsy". Seizure refers to an electrical disturbance in the brain,

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while, epilepsy is a group of related disorders in the brain's electrical system that are characterized by a tendency to cause a recurrent seizure. Seizures cause changes in movement, behaviour, sensation, or awareness, including loss of consciousness or convulsions; that is to say, seizures may cause a person to have convulsions, but this is not always the case (as in absence seizures). Convulsions can also happen to a specific part of a person's body or affect their whole body (Huzar, 2019).

Convulsions occur as a result of overexcitation of brain cells. Although following the physiology of the human body, the primary mechanism of neuronal excitability is the action potential, a hyperexcitable state can result from increased excitatory synaptic neurotransmission, decreased inhibitory neurotransmission, an alteration in voltage-gated ion channels, or an alteration of intra- or extra-cellular ion concentrations in favour of membrane depolarization (Guyton, 2012). A hyperexcitable state can also result when several synchronous subthreshold excitatory stimuli occur, thus allowing their temporal summation in the postsynaptic neurons. An action potential occurs due to depolarization of the neuronal membrane, with membrane depolarization propagating down the axon to induct the axon terminal. The action potential occurs in an all or non-fashion due to local changes in membrane potential brought about by net positive inward ion fluxes. Membrane potential is altered by the variation accompanied with activation of ligand-gated channels whose conductance is affected by binding to neurotransmitters; or with activation of voltage gated channels whose conductance is affected by changes in trans-membrane potential; or with changes in intracellular ion compartmentalization (Guyton, 2012).

Neurotransmitters are substances that are released by the presynaptic nerve terminal at a synapse and subsequently bind to specific postsynaptic receptors for that ligand. Ligand binding results in channel activation and passage of ions into or out of the cells. The major neurotransmitters in the brain are glutamate, gamma-amino-butyric-acid (GABA), acetylcholine, norepinephrine, dopamine, serotonin, and histamine. Other molecules, such as neuropeptides and hormones, play modulatory roles that modify neurotransmission over more extended periods. The major excitatory neurotransmitter is the amino acid glutamate. There are several subtypes of glutamate receptors. Glutamate receptors can be found postsynaptically on principal excitatory cells and inhibitory interneurons and have been demonstrated on certain glial cells (Guyton, 2012). Substances which are GABA receptor agonists, such as barbiturates and benzodiazepines, are well known to suppress seizure activity (Broomfield, 2006).

In this study, the treatment of convulsion was demonstrated using the Cannabis extract (Eubanks 2006). In 2017, an Australian Nationwide survey on medical cannabis use for epilepsy was carried out, including 976 responders (patients with epilepsy and/or parents/guardians of patients with epilepsy). It showed that around 15% of patients used cannabis irrespective of their physician's knowledge to control their multi-drug resistant seizures and get rid of the adverse effects of traditional antiepileptic drugs. In addition, most of them reported an improvement in their seizures (Suray*et al.*, 2017). The molecular target activity of the Cannabis was determined in this study using molecular docking analysis. The objective of the study was to demonstrate the convulsion mechanism and efficacy of *Cannabis sativa*.

Materials And Methods

Materials used for this study are drugs; Pentylenetetrazole (PTZ)Sigma-Aldrich Co. LLC, USA. Phenobarbitone, ethanol (70%), Syringes, orogastric cannula, water bath and calibrated stop watch.

Ethanol preparation of *Cannabis sativa*

Permission to use *Cannabis sativa* was approved and provided by National Drug Law Enforcement Agency (NDLEA) Calabar, Cross River State Command Nigeria, and was sent to Botany Department University of Calabar for identification and issued a Herbarium number 7. It was ground to powder and was dissolved in 250 ml of ethanol for 72 hours, then filtered with a filter paper and evaporated to dryness in a water bath (B-Bran Scientific and Instrument Company, England) at 60^0C. The brownish residue were weighed and kept in an air-tight bottle in the refrigerator until use. This method was recently used by Mobisson, *et al.*, (2018).

Laboratory animals

Twenty male albino mice weighing 18–30g were used for this study. The animals were housed in the Department of Pharmacology Animal house, Madonna University, Nigeria. Standard animal cages with wood dust as bedding were used in keeping the animals. They were allowed *ad libitum* access to rat chow and clean water, and exposed to 12/12-hr light/dark cycle. The animals were acclimatized for 7 days. The animals were kept in line with laid down principles for animal care as prescribed in Helsinki's 1964 declaration. The animal ethics committee of University of Calabar, Ehthics committee– 040PHY3719..

Experimental design and *Cannabis sativa* Extract administration

The animals were randomly assigned into five (5) groups of four animals each. First group serves as control received 0.2ml 0f 10% n-hexaneand represented as (**n-hex**) second and third groups received 200 mg/Kg and 400 mg/kg body weight of *C. sativa* extract dissolved in n-hexane solvent respectively and represented as (**200 mg CS and 400 mg CS**). The fourth group received

50mg/kg body weight phenobarbitone (**50 mg PB**) while the fifth group received 200 mg/Kg body weight of *C. sativa* extract + phenobarbitone 50 mg/Kg body weight (**200 mg CS + 50 mg PB**). The administration of *C. sativa* extract was done via orogastric feeding; whereas, 80 mg/kg bw of Pentylenetetrazole (PTZ to all subjects) and phenobarbitone were administered via intra-peritoneal route. This was done about thirty minutes after administration of test agent (C. sativa extract) and standard agent (phenobarbitone). Each animal was observed individually for one hour to determine the onset of muscular jerks and tonic hind limb extensions (THLE) or tonic-clonic phase of seizures or death. The seizure latency, the onset of tonic clonic seizure and death time from induced seizure were observed using well calibrated stop watch.

Statistical Analysis

Statistical analysis was conducted with Graph pad prism (version 5.01).

Ligand Library Generation

Identified secondary metabolites of *C. sativa* employed for this study were determined from published literature and were used to create the ligand library. Forty-nine (49) secondary metabolites were retrieved from NCBI PubChem library, in Standard Database Format (2D).

a-pinene, linalool, limonene, trans-ocimene, a-humulene(Novak *et al.*, 2001). Trigonelline, muscarine, isoleucine, N-trans-feruloyltyramine, N-trans-caffeoltyramine, N-trans-coumaroyltyramine, cannabisin A and D, Piperadine, methylamine, ethylamine, pyrrolidine.Mannitol, D-rhamnose, pentanal, zeatin, Xylanaldehydes (acetaldehyde, isobutyraldehyde) gluconic acid, phloroglucinolPhytosterols (campesterol, ergosterol beta-sitosterol, stigmasterol)

Vitamin K, xanthophylls, carotene, cannabitriol, cannabicycol, cannabichromene, cannabielson, cannabigerol, cannabidiol, cannabinodiol, cannabistilbene-1 and II, delta9-Tetrahydrocannabinol(Brenneisen, 2007).b -phellandrene, L-quebrachitol(Mediavilla and Stenmenn, 1997).Nonacosane, heptacosane, pentacosane(Elsohly and El-feraly, 2005). Hexacosane(Hendricks et al., 1977)

The ligand library generated was imported to docking software (Maestro) and prepared using the method described by Brooks *et al.,*(2008).

Protein Preparation

Structure of the proteins (i.e. gamma-aminobutyrate aminotransferase (1.90 Å), Human PPAR (2.01 Å), Human histone deacetylase 2 (HDAC2) (2.05 Å), NADP+-dependent succinic semialdehyde dehydrogenase from Streptococcus pyogenes(2.40 Å), Glutamate A2 AMPA receptor (6.8 Å), human $GABA_AR$(3.58 Å), human Glutamine-fructose-6-phosphate transaminase 1.GFAT-1 (2.50 Å), human Glutaminase C(GAC; the first enzyme in glutaminolysis2.50 Å), Escherichia Coli Glutamine Phosphoribosylpyrophosphate (PRPP) Amidotransferase(2.40 Å)) bound with ligands were retrieved from the Protein Data Bank according to Berman *et al.*, (2000). With the PDB ID: 1SFF, 2ZNN, 3MAX, 4YWU, 5KBV, 6HUP, 6R4F, 6UL9, 1ECC. They were prepared using the Protein Preparation Wizard as described by (Sastry*et al.*, 2013). Module in maestro 11.5 was used to prepare each protein complex. Missing hydrogen atoms, missing loop, and missing side-chains of protein structure were fixed while the added hydrogen atoms were optimized at pH 7.0. Optimized structures were then minimized using the OPLS3 force field by converging heavy atoms to root mean square deviation (RMSD) of 0.3Å (Sastry*et al.*, 2013).

Pharmacokinetic parameters (ADME/TOX Prediction)

The pharmacokinetic properties of the hit compounds were estimated using the Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) of the hit ligands were predicted using the Qikprop module in maestro 11.5.

Molecular Docking

Molecular docking methods are commonly used for predicting binding modes to proteins and energies of ligands (Chou, 2004). Using the Autodockvina program compiled under Ubuntu 14.04 LTS, different Cannabis phytochemicals were docked into the target proteins to get the respective binding affinity. The binding affinity predicts the strength of the molecular interaction of the ligand-protein complex. The binding results were validated using the chembl Database. The fasta sequence of the protein was gotten from Pubmed and blast on www.ebi.ac.uk/chembl/, and the search result was downloaded in the text format, using the IC50 chembl activity type. The smile format of the compounds were converted to sdf using Data warrior software and saved as 2D. These 2D structures were converted to pdb and pdbqt using Babel and lig prep command lines, respectively to generate the 3D structure of the compounds. The 3Dgenerated compounds were docked into the protein targets using the vina command line, and the corresponding docking score was plotted against their PubChem values to get the correlation value(Chou, 2004). The results were analyzed using binding energy. For each ligand, a docking experiment consisting of 100 simulations was performed and the analysis was based on binding free energies and root mean square deviation (RMSD) values, and the ligand molecules were then ranked in the order of increasing docking energies. The binding energy of each cluster is the mean binding energy of all the conformations present within the cluster, the cluster with the lowest binding energy and higher number of conformations within it was selected as the docked pose of that particular ligand.

The clusters were ranked by the lowest-energy representative of each binding mode. The rest of the parameters were set as default values. At the end of a docking experiment with multiple runs, a cluster analysis was performed. Substrate docking with natural Plant phytochemicals was performed on a protein model with same parameters and PMV 1.4.5 viewer was then used to observe the interactions of the docked compound to the protein model (Chou, 2004).

Results

Figure 1 showed the seizure latency in rats fed with 400mg/kg of C. sativa (C), 50mg/kg of phenobarbitone (D) and 50mg/kg phenobarbitone + 200mg/kg bw of C. sativa (E) respectively were significantly ($p<0.05$) higher compared to control. Although, the group administered with 50mg/kg dose of phenobarbitone had higher latency time compared to groups that were given400mg/kg of C. sativa and 50mg/kg of phenobarbitone + 200mg/kg bw of C. sativa. Furthermore, the group fed with 200mg/kg of *C. sativa* had no significant difference from control.

Figure 2 showed that the onset of tonic clonic seizure in rats in experimental groups was significantly ($p<0.05$) higher than in the control group. Although, the group administered with 50mg/kg dose of phenobarbitone+ 200mg/kg bw of *C. Sativa* (E) had higher tonic clonic seizure time compared to groups that were given 200mg/kg and 400mg/kg of *C. sativa* and 50mg/kg of phenobarbitone. Furthermore, the group fed with 200mg/kg and 400mg/kg of *C. Sativa* were significantly ($p<0.001$) reduced compared to the standard (group D).

Figure 3 showed that the death time from rats' induced seizures in experimental groups was significantly ($p<0.05$) higher than control. Although, the groups administered with50mg/kg of phenobarbitone (D) and 50mg/kg dose of phenobarbitone+ 200mg/kg bw of *C. Sativa* (E) had higher significant ($p<0.001$) death time from seizures compared to groups that were given 200mg/kg and 400mg/kg of *C. sativa*. Furthermore, the group fed with 200mg/kg and 400mg/kg of *C. sativa* were significantly ($p<0.001$) reduced compared to the standard (group D).

The free energy of binding of phytochemicals of *C. sativa* docked into the substrate binding, of gamma-aminobutyrate aminotransferase complex, compared with the agonist, aminooxyacetate and represented as heat map. (The scale is a spectrum from purple (-2 kcal/mol) to red (-8 kcal/mol). The free energy of binding of phytochemicals of C. sativa docked into the substrate binding sites of different ligand (i.e. Human PPAR alpha, HDAC2, succinic semialdehyde dehydrogenase, GluA2, GABA(A)R, GFAT-1, GAC, Escherichia coli glutamine amidotransferase and assessed the potential molecular binding efficacy (Figure 4, supplementary files).

Discussion

There are many anticonvulsants for drug therapy of epilepsy, but all anticonvulsants can harm cognitive performance. If any cognitive deficits before treatment existed, these deficits may be exacerbated. The most common negative effect of anticonvulsants is a decrease in information processing speed, reaction speed and concentration. Most treatment-related cognitive disorders are reversible and fade after dose reduction or completely disappear after a change in substance. Only visual field defects with Vigabatrine are irreversible, which is why this substance has been used only in individual cases and under strict ophthalmologic control (Katjia, 2020).

The effect of *C. sativa* compounds such as Δ(9) - tetrahydrocannabinol (THC) and cannabidiol (CBD) have been reported in the amelioration of seizures (Huzar, 2019). This study explored the anticonvulsant potential of *C. sativa,* by *in-vivo* studies and potential effects of otherphytoconstituents that may be important in this mechanism in silico studies. In the in vivo study, using chemoconvulsant animal model, convulsion was induced using Pentelynetetrazol (PTZ) with Phenobarbitone as positive control (D) and the anticonvulsant potential of *C. sativa* was tested. In figure 1 above, seizure latency was significantly increased compared to control group (A). However, The Mice in groups C, D and E showed increased significant difference to the Control in terms of delay of onset of seizure. In figure 2, mice in groups A, B and C showed decreased significant differenceof onset of tonic clonic seizure when compared to the standard control (Phenobarbitone) $p < 0.001$ while group C showed a significant difference of $p < 0.05$ to the negative control and group E showed significant difference of $p < 0.001$ to the negative control. In figure 3, mice in groups A, B and C exhibited significant difference of $p < 0.001$ in the death time from induced seizures when compared with a standard Control while mice in groups C, D and E exhibited significant difference of $p < 0.001$ when compared with a negative control (ethanol).

The animal study results indicate that 400mg/kg *C. sativa* extract; 50mg/kg Phenobarbitone and 50 mg/kg Phenobarbitone + 200 mg/kg *C. sativa* extract had only but a slight relative effect on seizure latency to ethanol. It also reveals that 400 mg/kg *C. sativa* extract was significantly higher on the effect of ethanol on onset of tonic clonic seizure. Furthermore, mice that received 50 mg/kg Phenobarbitone and 50 mg/kg Phenobarbitone + 200 mg *C. sativa* exhibited a higher significant difference on the onset of tonic clonic seizure. Moreover, the reveal also increased death time from induced seizure in mice that received 400 mg/kg *C. sativa*, 50 mg/kg Phenobarbitone and 50 mg/kg Phenobarbitone + 200 mg/kg *C. sativa* extract. The results is in conformity with the study

Fig 1: Effect of C sativa on seizure latency.
Figure 1shows the seizure latency in rats fed with 400mg/kg of C. sativa (C), 50mg/kg of phenobarbitone (D) and 50mg/kgphenobarbitone + 200mg/kg bw of C. sativa (E) respectively were significantly ($p<0.05$) higher compared to control. Although, the group administered with 50mg/kg dose of phenobarbitonehad higher latency time compared to groups that were given400mg/kg of C. sativa and 50mg/kg of phenobarbitone + 200mg/kg bw of C. sativa. Furthermore, the group fed with 200mg/kg of C. Sativa had no significant difference compared to control. Animals per group (n) = 4. The values are mean ± SEM.; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; when compared with control group. Same significance values; ap<0.05, bp< 0.01, cp< 0.001; when compared with a standard control. (One-way ANOVA followed by Bonferroni's Multiple Comparison Test)

Fig. 2 Effect of C. sativa on onset of tonic clonic seizure latency.
The onset of tonic clonic seizure of rats in experimental groups were significantly ($p<0.05$) higher compared to control. Although, the group administered with 50mg/kg dose of phenobarbitone+ 200mg/kg body weight of C. sativa (E) had highertonic clonic seizure time compared to groups treated with 200mg/kg and 400mg/kg of C. sativa and 50mg/kg of phenobarbitone. Furthermore, the group fed with 200mg/kg and 400mg/kg of C. Sativa were significantly ($p<0.001$) reduced compared to the standard (group D). Animals per group (n) = 4. The values are mean ± SEM.; *p < 0.05, **p < 0.01, ***p < 0.001; when compared with control group. Same significance values; ap<0.05, bp< 0.01, cp< 0.001; when compared with a standard control. (One-way ANOVA followed by Bonferroni's Multiple Comparison Test)

Fig. 3 Death time from induced seizures.
The death time from induced seizures of rats in experimental groups were significantly ($p<0.05$) higher compared with the control. Although, the groups administered with50mg/kg of phenobarbitone (group D) and 50mg/kg dose of phenobarbitone+ 200mg/kg body weight of C. sativa (E) had higher significant ($p<0.001$) death time from seizures compared to groups that were given 200mg/kg and 400mg/kg of C. sativa. Furthermore, the group fed with 200mg/kg body weight and 400mg/kg body weight of C. sativa extract were significantly ($p<0.001$) reduced compared to the standard phenolbabitone (group D).

Fig. 4: Heat map representation of docking result for compound interaction with structure of GABAAR
The free energy of binding of phytochemicals of C. sativa docked into the substrate binding sites of GABA(A)R bound to ligand binding domain complex and compared with an agonist, diazepam and represented as heat map. (The scale is a spectrum from purple (-2 kcal/mol) to red (-9 kcal/mol).

Table 1. showing the ADMETOX predictions of the compounds present in *C. sativa*

Entry Name	mol MW	donorHB	accptHB	QPlogPo/w	HOA	ROF
Coligand__1sff	322.211	4	10.45	0.167	1	0
Coligand__2znn	503.68	2	5.25	7.457	1	2
Coliagnd__3max	288.348	2.5	3.5	3.597	3	0
Coligand_4ywu	102.09	1	4	-0.437	2	0
Coligand_5kbv	409.258	3	13.2	0.318	2	0
Coligand_6hup	284.744	0	4	2.997	3	0
Coligand_6r4f	260.137	5	12.8	-2.363	1	1
Coligand_6ul9	507.584	2	9	4.573	1	1
Coligand_IECC	171.155	3	6.75	-1.476	2	0
Acetaldehyde	44.053	0	2	0.059	3	0
Alpha-humulene	204.355	0	0	5.191	1	1
Alpha-pinene	136.236	0	0	3.634	3	0
B-sitosterol	414.713	1	1.7	7.621	1	1
Beta-caryophyllene	204.355	0	0	5.125	1	1
Beta-phellandrene	136.236	0	0	3.972	3	0
Campesterol	400.687	1	1.7	7.301	1	1
Cannabichromene	314.467	1	1.5	6.03	1	1
Cannabicyclol	314.467	1	1.5	5.566	1	1
Cannabidiol	314.467	2	1.5	5.336	3	1
Cannabielsoin	330.466	2	2.25	5.117	3	1
Cannabigerol	316.483	2	1.5	5.894	1	1
Cannabinodiol	310.435	2	1.5	5.288	3	1
Cannabisin A	594.62	8	9.5	2.997	1	2
Cannabisin D	624.689	6	9.5	4.183	1	2
Cannabistilbene I	312.408	2	2.25	4.696	3	0
Cannabistilbene II	304.342	2	3.75	3.381	3	0
Cannabitriol	346.466	3	3.95	3.996	3	0
Carotene	536.882	0	0	16.745	1	2
D-Rhamnose	164.158	3	7.8	-1.483	2	0
delta9-Tetrahydrocannabinol	314.467	1	1.5	5.724	1	1
Ergosterol	396.655	1	1.7	7.18	1	1
Ethylamine	45.084	2	1	-0.131	3	0
Gluconic acid	196.157	5	9.5	-1.755	2	1
Hexacosane	366.713	0	0	14.92	1	1
Isobutyraldehyde	72.107	0	2	0.307	3	0
Isoleucine	131.174	3	3	-1.524	2	0
L-Quebrachitol	194.184	5	10.2	-1.858	2	0
Limonene	136.236	0	0	3.986	3	0
Linalool	154.252	1	0.75	3.14	3	0
Mannitol	182.173	6	10.2	-3.086	1	1
Methylamine	31.057	2	1	-0.571	2	0
N-p-coumaroyltyramine	283.326	3	4	2.682	3	0
N-trans-caffeoyltyramine	299.326	4	4.75	1.977	3	0
N-trans-Feruloyltyramine	313.352	3	4.75	2.843	3	0

Nonacosane	**408.793**	**0**	**0**	**16.59**	**1**	**1**
Pentacosane	**352.686**	**0**	**0**	**14.403**	**1**	**1**
Pentanal	**86.133**	**0**	**2**	**0.812**	**3**	**0**
Phloroglucinol	**126.112**	**3**	**2.25**	**-0.02**	**2**	**0**
Piperidine	**85.149**	**1**	**1.5**	**0.837**	**3**	**0**
Pyrrolidine	**71.122**	**1**	**1.5**	**0.458**	**3**	**0**
Stigmasterol	**412.698**	**1**	**1.7**	**7.564**	**1**	**1**
Trans-ocimene	**136.236**	**0**	**0**	**4.374**	**3**	**0**
Vitamin K	**450.703**	**0**	**4**	**8.319**	**1**	**1**
Xanthophyll	**568.881**	**2**	**3.4**	**10.681**	**1**	**2**
Xylan	**150.131**	**4**	**8.5**	**-1.758**	**2**	**0**
Zeatin	**219.246**	**3**	**5.7**	**0.549**	**3**	**0**

done by (Sidra et al., 2018; Russo, 2017), where they reported a positive effect on *Cannabis* used in the treatment of seizures.

These positive results then gave grounds for further in silico study which involved virtual screening, the purpose of which was to ascertain the different targets of anticonvulsant drugs, by implicating the various pathways and channels involved in the process of convulsion. The enzyme and target sites discovered were Succinic semialdehyde dehydrogenase (SSADH), Phosphoribosyl pyrophosphate amidotransferase, Glutaminase enzyme, Gama Amino Butyric Acid Receptor A (GABA A), 4-Aminobutyrate Aminotransferase, Histone deacetylases (HDAC), Peroxisome proliferator-activated receptor (PPAR), and Human GAFT-1. The virtual screening yielded 49 constituents of *C. sativa*. Some of the constituents are Cannabidiol, Cannabitrol, Cannabicycol, Cannabichromene, Isoleucine, etc. The enzyme SSADH is involved in the breakdown of GABA to succinic acid via the intermediate succinic semialdehyde by GABA-transaminase, and in its absence, GABA is alternatively converted to GHB. There is a resultant build-up of both GABA and GHB in physiological fluids and brain parenchyma and one of the clinical manifestations of this is convulsion (Philip et al., 2011). Valproate, one of the major antiepileptic drugs used today, has antiepileptic effect on the GABAergic system and the effect on enzymes like succinate semialdehyde dehydrogenase (SSADH), GABA transaminase (GABA-T), and alpha-ketoglutarate dehydrogenase, related to the tricarboxylic acid (TCA) cycle and thereby cerebral metabolism. In vitro studies have shown that VPA is a potent inhibitor of SSA-DH and any other drug/compound with an anticonvulsant effect will also inhibit it. As shown in figure 8 above, Trigonelline, Cannabistilbene I and Acetaldehyde possess potential anticonvulsant action because of their high interaction (docking score) with the SSADH enzyme, the one with the highest docking score comes first.

Glutamate and gamma-aminobutyric acid (GABA) are the major inhibitory neurotransmitters in the brain. Inhibitory GABA and excitatory glutamate work together to control many processes, including the brain's overall excitation level. Astrocytes generate glutamate via *de novo* synthesis or by "recycling" glutamine from GABA and glutamate after reuptake; glutamine is converted to glutamate by phosphate-activated glutaminase, an enzyme which is expressed preferentially in neurons. Antiepileptic drugs inhibit glutaminase to stop this conversion, thus inhibiting excitation involved in convulsion, like Sodium valproate (Kyamme*et al.*, 2001). The Molecular docking result of the compounds Cannabisin A, L-Quebrachitol and Phloroglucinol had a high docking score or interaction with the glutaminase enzyme; this suggests their anticonvulsant potential through inhibition of the enzyme as shown in figure 8 and figure 11.

The $GABA_A$ receptor ($GABA_AR$) is a major target of antiepileptic drugs. A variety of agents that act at $GABA_ARs$ are used to terminate or prevent seizures. Many acts at distinct receptor sites determined by the subunit composition of the holoreceptor. For the benzodiazepines, barbiturates, and Loreclezole, actions at the $GABA_AR$ are the primary or only known mechanism of antiseizure action. For topiramate, felbamate, and retigabine, $GABA_AR$ modulation is one of several possible antiseizure mechanisms. Cannabistilbene I, II and Cannabielsoin have a high interaction (docking score) when docked with the $GABA_A$ receptor as shown in figure 9.

Epigenetic mechanisms, such as alterations in histone acetylation based on histone deacetylases (HDACs) activity, have been linked to normal brain function and several brain disorders including epilepsy and the epileptogenic process. HDAC inhibitors (HDACi), namely sodium butyrate (NaB), valproic acid (VPA) deter the development of absence seizures and related psychiatric/neurologic comorbidities. According to fig. 4.6, N-trans-feruloyltyramine, N-trans-caffeoyltyramine and

Phloroglucinol possess potential anticonvulsant action because of their high interaction (docking score) with the HDAC enzyme with N-trans-caffeoyltyramine coming first.

4-Aminobutyrate aminotransferase (GABA-transaminase) is a pyridoxal 5'-phosphate (PLP)-dependent enzyme that degrades GABA, the principal inhibitory neurotransmitter in mammalian cells. When the concentration of GABA falls below a threshold level, convulsions can occur. Conversely, inhibition of GABA-T raises GABA levels in the brain, which can terminate seizures and have potential therapeutic applications in treating other neurological disorders, including drug addiction. Vigabatrin is a known inactivator of GABA-AT and approved drug (Sabril) to treat infantile spasms and refractory adult epilepsy. As shown figure 4, the compounds Cannabisin A, D, and Cannabistilbene I showed high interaction with the enzyme GABA-T, with Cannabisin A having the highest docking score. These compounds can therefore be said to possess potential anticonvulsant effect based in their interaction with GABA-T. This result agrees with the findings of Railton, 2018 and Schauer et al., 2016.

Peroxisome proliferator-activated receptor-α (PPAR-α) has been regarded as a drug target for control of epilepsy through lift elevation of seizure threshold; thus; PPAR-α agonist is valuable in controlling seizure frequency. PPAR-α is a nuclear receptor encoded by PPAR-A gene, Endogenous activators of PPAR-α are arachidonic acid, poly-unsaturated fatty acids. Therefore, PPAR-α agonists have a role in treating coronary heart disease, non-alcoholic fatty liver disease, and play a role in preventing epiliptogenesis(Puligheddu*et al*., 2013). In figure 7, the compounds Cannabisin A, D, and Cannabistilbene I showed a high interaction with the PPAR target site, thus having a potential agonist effect on this receptor to inhibit convulsion.

These Promising constituents of *C. sativa* were subsequently accessed based on the Lipinski’s rule of five, also known as Pfizer’s rule of five (RO5) which is a rule of thumb to evaluate drug-likeness or determine if a compound with a certain pharmacological or biological activity has chemical and physical properties that would make it a likely orally active drug in humans. It considers 5 key physiochemical parameters- molecular weight, lipophilicity, polar surface area, hydrogen bonding, and charge). Its tenets are, molecular weight <500, not more than 5 hydrogen bond donors, not more than 5 hydrogen bond acceptors and a partition coefficient (log P) value<5(Lipinski, 2001).

In table 1 above, the ADMETOX predictions of the various compounds in *C. sativa*, all of the compounds in the list of compounds with potentials presented above were within the acceptable RO5 violation limit. In the sense that, neither of them exceeded 2 violations of the RO5;. However, the ones that violated up to 2 rules (egCannabisin A and Cannabisin D) recorded low Human Oral Absorption (HOA) level (1), while those without any violation (L-Quebrachitol and N-trans-caffeoyltyramine), recorded High HOA value. This study therefore agrees with the work of (Emilio 2017) and (Huntsman et al., 2019) to solidify the potential anticonvulsant claim of *Cannabis sativa*.

Conclusion

Based on the positive result of protection from PTZ induced convulsion, *C. sativa* can be considered to possess anticonvulsant properties and potential at least as a synergistic element or adjunct compound. Because the mice in Group E had no significant difference from group D (standard control) in regards to seizure latency, onset of tonic clonic seizure and death time from induced seizures whereas mice in groups B and C did. The positive interaction between the identified *C. sativa* constituents and the identified target sites implicated in convulsion indicates the promising anticonvulsant potential of this plant called *Cannabis sativa*.

Author Contributions

Ben Enoluomen Ehigiator designed and worked on data analysis for the study, Samuel Kelechi Mobisson performed the statistical analysis, Iheanyichukwu Wopara wrote the protocol and the manuscript's first draft. Eruotor Ogheneochuko Harrison and Iheanyichukwu Wopara managed laboratory experiments and data analysis of the study. Chinonye Cynthia Chibuife and Awarajih Uwaezuoke Chukwuekele managed the literature searches for methodology. All authors read and approved the final manuscript.

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Competing financial interests

The author(s) declare no competing financial interests.

Supplementary Information

Please download supplementary information online.

References

Anigilaje E, Anigilaje OO. (2012). Childhood convulsion: inquiry about the concerns and home management among mothers in tegbesun, a periurban community in Ilorin, Nigeria. Paediatrics (4):209609.https://doi.org/10.5402/2012/209609

Berman HM, Westbrook, J, Feng Z, Gillialand G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. (2000). The Protein data bank.Nucleic Acids Research 28(1):235-242.https://doi.org/10.1093/nar/28.1.235

BrenneisenR. (2007). "Chemistry and analysis of phytocannabinoids and other cannabis constituents," in Marijuana and the Cannabinoids Forensic Science and Medicine, ed. M. ElSohly (New York, NY: Humana Press), 17-49. doi: 10.1007/978-1-59259-947-9.https://doi.org/10.1007/978-1-59259-947-9

Bromfield EB. (2006). Basic mechanisms underlying seizures and epilepsy -An introduction to epilepsy.American Epilepsy Society: B (slide 2)

Brooks, WH, Daniel KG, Sung SS, Guida, WC. (2008). Computational validation of the importance of absolute stereochemistry in virtual screening.Journal of Chemical Information and Modeling; 48: 639-45.https://doi.org/10.1021/ci700358r

Chou KC. (2004). Structural bioinformatics and its impact to biomedical science.Current Medicinal Chemistry.11(16):2105-2134.https://doi.org/10.2174/0929867043364667

Edward HR. (2000). The ILAE/IBE/WHO global campaign against epilepsy: bringing epilepsy "out of the shadows". Epilepsy & Behavior, 1(4), S3-S8.https://doi.org/10.1006/ebeh.2000.0104

Ekeh BC. (2015). The knowledge, attitude and perception towards Epilepsy amongst medical students in Uyo, southern Nigeria. Advances in Medicine :876135.https://doi.org/10.1155/2015/876135

Elsohly M, El-Feraly MA. (2005). Chemical constituents of marijuana- the complex mixture of natural cannabinoids. The Haworth Press, Inc., Binghamton, New York, pages 27-36.https://doi.org/10.1016/j.lfs.2005.09.011

Emilio P. (2017). Cannabinoids in the treatment of epilepsy: hard evidence at last. Journal of Epilepsy Research 7(2); 61-76.https://doi.org/10.14581/jer.17012

EubanksLM, Rogers C J, Beuscher AE, Koob GF, Olson AJ, Dickerson TJ, Janda KD. (2006). "A Molecular Link Between the Active Component of Marijuana and Alzheimer's Disease Pathology". Molecular Pharmaceutics3 (6): 773-7.https://doi.org/10.1021/mp060066m

Guyton AC, Hall JE. (2012). Textbook of medical physiology (10thed.). Philadelphia: W. B. Saunders, 386 - 387.

Hendricks H, Malingre TM, Batterman S, Bos R. (1977). Alkanes of the essential oil of cannabis sativa. Available at https://www.semanticscholar.org/paper/alkanes-of-the-essential-oil-of-cannabis-sativa accessed [14th November, 2020].https://doi.org/10.1016/S0031-9422(00)89239-0

Huntsman R, Tang-wai R, ZentenoJ. (2019).Cannabis for pediatric and adult epilepsy.Available on https://www.intechopen.com doi:10.5772/intechopen.85719.https://doi.org/10.5772/intechopen.85719

HuzarT. (2019). Everything you need to know about convulsions. Available at https://www.medicalnewstoday.com/articles/324330.

Katja EB. (2020). Cognitive and Psychological Side Effects of Antiepileptic Drugs-Update on classification, etiologies, instrumental diagnosis and treatment.Intechopen, doi:10.5772/intechopen.94308.https://doi.org/10.5772/intechopen.94308

Kvamme E, Torgner I, Roberg B. (2001). Kinetics and localization of brain phosphate activated glutaminase. Journal of Neuroscience Research; 66:951-958.https://doi.org/10.1002/jnr.10041

Lipinski CA. (2001). Avoiding investment in doomed drugs.Current drug discovery, 1; 17-19.

Mediavilla V, Steinmann S. (1997). Essential oil of cannabis sativa L. strains.Journal international hemp association 4:80-82

Mobisson SK, Obembe AO, Ukoh IE, Duru, GO. (2018). The Role of Hypothalamic Ptuitary Gonadal Axis in Aqueous Extract of Cannabis Sativa Induced Male Reproductive Dysfunction of Albino Wistar Rats. European Journal of Pharmaceutical and Medical Research,5(1), 71-78.

Novak J, Zitter I-Eglseer K, Deens S.G, Franz C.M. (2001). Essential oils of different cultivars of cannabis sativa L. and other antimicrobial activity; flavour and fragrance journal 16; 259-262.https://doi.org/10.1002/ffj.993

Perucca E. (2017). Cannabinoids in the Treatment of Epilepsy: Hard Evidence at Last?. Journal of epilepsy research, 7(2), 61-76.https://doi.org/10.14581/jer.17012.https://doi.org/10.14581/jer.17012

Phillip L, Shukla, L, William, H, Jakobs C, Gibson K. (2011). Epilepsy in Succinic Semialdehyde Dehydrogenase Deficiency, a Disorder of GABA Metabolism.US National Library of Medicine 33(9); 796-805.https://doi.org/10.1016/j.braindev.2011.04.013

Puligheddu, M, Pillolla G, Melis M. (2013). PPAR-alpha agonists as novel antiepileptic drugs: Preclinical findings. PLoS ONE;8(5):64541.https://doi.org/10.1371/journal.pone.0064541

Railton D. (2018). Marijuana: good or bad. Medical News Today.BMC Psychiatry, 11 (1), 25.Available at https://www.medicalnewstoday.com .

Russo EB. (2017). Cannabis and Epilepsy: An ancient treatment returns to the fore. Epilepsy behaviour, 70: 292-297.https://doi.org/10.1016/j.yebeh.2016.09.040

Sastry GM, Adzhigrey M, Day T, Annabhimoju R, Sherman W. (2013). Protein and Ligand preparation: Parameters, protocols, and influence on virtual screening enrichments.Journal of Computer-Aided Molecular Design , 27(3),221-234.https://doi.org/10.1007/s10822-013-9644-8

Schauer G, King B, Bunnel R, Promoff G, McAfee T. (2016). Toking, vaping, and eating for health or fun: marijuana use patterns in adults, U.S., American Journal of Preventive Medicine. 50(1):1-8.https://doi.org/10.1016/j.amepre.2015.05.027

Sidra Z, Deepark K, Muhammad TK, Pirthvi RG. Fnu, K. (2018). Epilepsy and cannabis: A Literature Review. Neurology, 10(9): e3278.

Suraev AS, Todd L, Bowen MT, Alsop DJ, McGregor IS, Ireland C, Lintzeris N. (2017). Australian nationwide survey on medicinal cannabis use for epilepsy: history of antiepileptic drug treatment predicts medicinal cannabis use. Epilepsy behaviour 70:334-334.https://doi.org/10.1016/j.yebeh.2017.02.005

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