Research Article

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Phytochemical, in Vivo, and in Silico Anticonvulsant Activity Screening of *Albizia Amara* Leave's Ethanolic Extract

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ABSTRACT

Relying on the previous literature, *Albizia amara* possesses phytochemical constituents having significant pharmacological activities. Our study preliminary screened the phytochemical constituents and investigated the potential anticonvulsant activity using the pilocarpine-induced seizures on albino rats. The qualitative phytochemical screening of the extract indicated the presence of flavonoids, alkaloids, saponins, tannins, and sterols. The anticonvulsant activity assessment revealed that the extract (400 mg/kg i.p.) increased the time to onset of seizure(latency) and significantly decreased the duration of the seizure. Moreover, the extract reduced the mortality rate during the first observed 24 hours. Furthermore, to predict the probable mechanism of action, in silico study was conducted. The results indicated that the phytochemical compounds having the highest contribution in the activity are budmunchiamine A, 1, 2-benzenedicarboxylic acid, mono (2-Ethylhexyl) ester, hexadecanoic acid methyl ester, and pithecolobine. The predicted targets that involved in the antiepileptic activity are neural acetylcholine receptor subunits alpha-4 and alpha-7, 5-hydroxytryptamine receptor 3A, cannabinoid receptor 1, kinase C gamma & epsilon subunits, and glutamate carboxypeptidase-2 enzyme. Unfortunately, budmunchiamine A was predicted to be cardiotoxic and hepatotoxic. Pithecolobine was predicted to be cardiotoxic. In conclusion, the findings demonstrate the potentiality of *A.amara's* ethanolic leaves extract in the attenuation of Pilocarpine induced convulsion.

Keywords: *Albizia amara;* Phytochemical screening; Anticonvulsant activity; Pilocarpine-induced seizure and in silico study.

1. INTRODUCTION

Epilepsy is a chronic brain disorder characterized by periodic or unpredictable seizures that have been classified into partial and generalized as well as simple and complex seizures. Globally, about 50 million people are suffering from epileptic seizures that affect their quality of life via several psychosocial complications. Hence, there is a reasonable requirement for the treatment of epilepsy [1-2].

The pharmacological treatment of epilepsy is based on the administration of antiepileptic drugs that act by enhancing the activity of gamma aminobutyric acid (GABA)and the decreasing the activity of voltage-gated ion channels [3]. About 30% of epileptic patients do not respond to common treatments [4-5]. Moreover, they are suffering from their associated side effects and interactions [1]. Consequently, the investigation for effective and safe newer antiepileptic drugs of a natural source is an interesting issue, since many phytochemical groups like saponin and flavonoids are known to have an antiepileptic activity [5].

Albizia amara is a valuable medicinal plant belongs to the Fabaceae family. It is widely distributed in African countries, including Sudan [6]. It contains several phytochemical groups including macrocyclic spermine alkaloids, flavonoids, terpenoids, phenols, triterpenoid saponins, tannins, steroids, quinines, and cardiac glycosides [7-8-9]. The pharmacological screening revealed that it possesses significant activities involving the antimicrobial, anti-inflammatory, antihyperlipidemic activity, antioxidant activity [7-10]. Traditionally, in Tanzania, the aqueous extract of *A.amara* leaves was used for the treatment of diarrhoea, epilepsy, severe backache, loin pain and other abdominal problems [7].

The screening of anticonvulsant activity is conducted via various seizure models, including the pilocarpine-induced seizures that is a commonly used model. it is based on the induction of seizures via "the direct cholinergic system activation and the leakage of the blood-brain barrier (BBB)" [11-12-13]. This study aims to screen the anticonvulsant activity of *A. amara* leaves' ethanolic extract utilizing the Pilocarpine-induced seizures at albino. In addition, it aims to preliminary screen the phytochemical constituent of the plant. After the screening of the pharmacological activity, understanding mechanism of action is critical aspect, which can be achieved by several methods including computational tools that are valuable in the identification of drug targets [14]. Subsequently, an *in-silico* study is performed to identify the probable targets responsible for the mechanism of action.

2. MATERIALS AND METHODS

The study was ethically approved by the Department of Pharmacology, Omdurman Islamic University, Khartoum, Sudan, under the code (No: OIU/I.A.E.C./Exp.Ph.2019/1) and conducted in complying with the prescribed guidelines.

2.1 Preparation of the Plant Material

A.amara leaves were collected from the National Botanical Garden, Khartoum, Sudan in March 2017 and authenticated at the Medicinal and Aromatic Plant and Traditional Medicine Research Institute, Khartoum, Sudan. A voucher specimen was deposited at the institute herbarium.

400g of the leaves were shade dried, grounded into coarse powder and extracted using the maceration method with 4000 L of 70% ethanol (CARLO ERBA REAGENTS, France). The ethanol was evaporated, and the extract was concentrated at reduced pressure using the rotatory evaporator (IKA-WERKE, Germany). The dried extract was saved at the well-closed container in the refrigerator. The dried extract then was dissolved in propylene glycol as solvent and administered to rats. for that a vechicle control group was administered propylene glycol to control the experiment.

2.2 The preliminary phytochemical screening

The phytochemical screening of A.amara leaves extract for different secondary metabolites including alkaloids, flavonoids, tannins, triterpenoids, saponins, steroids, and anthraquinone were carried out according to standard procedures described by Tiwari *et al.* [15]. The results were listed in table 1.

2.3 Evaluation of anticonvulsant activity

2.3.1 Experimental design and treatment

Twenty five healthy Wistar albino rats of mixed gender (77-147g) were purchased from the experimental animal house, Faculty of Pharmacy, University of Khartoum, Khartoum, Sudan. The dose of 200 mg/kg was selected based on safety and well-established in vivo studies of the dose in the other pharmacological activities for *Albizia amara* leaves extract [16-17].

Moreover, it is reported that the dose of 400 mg/kg of *Albizia glaberrima*; a plant species belongs to the same genus of *Albizia amara*, showed considerable anticonvulsant activity .so, we added the dose 400 mg/kg as second does to be tested believing that they might be sharing the similar content of secondary and primary metabolites [18].

To screen the anticonvulsant activity, the pilocarpine induced seizure was used in this study. The rats were divided randomly into 5 main groups(n=5) with 3:2 male to female rats' ratio. Group I was administered pilocarpine (Amman phar. Industries, Jordan) 400 mg/kg/i.p. as a negative control. Group II was administered propylene glycol and pilocarpine in normal saline (1:1/ i.p, at same volume given in extract doses) as vehicle control. Group III was administered Diazepam (Shanghai, Sudan) (4 mg/kg) that was dissolved in a mixture of propylene glycol and normal saline (4: 1/i.p.) followed by pilocarpine as a positive control. Group IV and V were given pilocarpine after the administration of *A.amara* leaves' ethanolic extract at the doses 200 and 400 mg/kg (i.p.) respectively as a single dose. Intraprotenial doses of pilocarpine (400mg/kg) was given at thirty minutes after *A.amara* and diazepam doses.

2.3.2 Observation of the change in the convulsive behaviours

The onset and duration of total seizures attacks were observed during first 5 hours in open field arena then, the rats were transferred to the normal standard cage and allowed to freely access to water and food to reduce the animal suffering time. also, locomotor activity and the mortality rate were recorded in first hour and after 24 hours via the observation and the automated activity cage (AccuScan Instruments, USA). The results were listed in Tables II and III.

2.3.3 Statistical Analysis

The obtained data were expressed as mean ± S.E.M and were analysed using the one-way ANOVA test followed by the level of significance that taken as $p \leq 0.05$. All the treated groups were compared to the negative control group. The statistical analysis was conducted at GraphPad prism software version 5. The results were illustrated in figure I.

2.4 The *in-silico* study

In silico study was conducted according to a detailed protocol described by M. Al-Nour et.al [19].

2.4.1 Ligand-based virtual screening and Blood-Brain Barrier permeability prediction

Initially, the chemical structures of the reported phytochemical constituents of *A.amara* leaves (33 compounds) were drawn using Marvin Sketch software version 18.5 (licensed under Academic License) and saved as a mol. file format [20-21-22]. Subsequently, their smile format was submitted to the TargetNet web server (open webserver) to identify the probable targets that they are bind with to exert the anticonvulsant activity [23]. The targets that involved in the pathophysiology of epilepsy (9 targets) and having the highest probability scores were selected for further confirmation. Moreover, the screening of the Blood-Brain Barrier (BBB) permeability was conducted using the SwissADME web server (open webserver) [24]. The results are listed in table 4.

2.4.2 Molecular Docking

The structures of the reported phytochemical constituents were prepared for molecular docking and minimized accurately using Cresset Flare software (licensed under Academic License) [25]. The 3D structures of the predicted targets were downloaded from the RCSB protein data bank (open database) as PDB format, however, for the 3D structures that were not determined practically, Phyre2 webserver (open webserver) was used to model the structure [26-27]. The PDB IDs are listed in table 5 at lines above each docking's scores. The obtained 3D structures were prepared and energetically minimized for molecular docking at Cresset Flare software. The

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molecular docking calculations were conducted in Cresset Flare software at the normal type and default settings [25]. The natural and co-crystallized ligands were used as positive controls. The 2D interaction between the phytochemical constituents and the predicted targets is conducted via PoseView software at the ProteinsPlus web portal (open webserver) [28-29]. The results are listed in table 5 and showed in figures II and III.

2.4.3 The Pharmacokinetics, Toxicity, and Drug-likeness prediction

The prediction of the intestinal absorption, the apparent volume of distribution, clearance, and CYP-450 enzyme inhibition were carried out via pkCSM (open webserver) and SwissADME web servers [24-30]. The major organ toxicity of the phytochemical constituents (Cardiotoxicity, Hepatotoxicity, and Renal toxicity) was predicted using pkCSM and eMolTox webservers (open webserver) [31]. Moreover, the probability of the phytochemical constituents to be like a drug was predicted via SwissADME webserver. The results were listed in table VI.

3. Results and Discussion

The literature reported that *A.amara* is affluent with multiple phytochemical groups. In an agreement with the literature, the preliminary phytochemical screening indicates that it contains alkaloids, flavonoids, saponins, and diterpenes. In contrast, the Anthraquinone Glycosides were not detected (Table 1).

Several studies on medicinal plant families, including the Fabaceae were conducted to discover safe and effective antiepileptic drugs [32]. Within the Fabaceae family, studies on the genus Albizia revealed its promised anticonvulsant activity [33].

In this study, to evaluate the anticonvulsant activity of *A.amara's* ethanolic extract, the onset and duration of convulsions, the horizontal locomotor activity as well as the mortality rate were recorded from albino rats administered Pilocarpine as seizures inducing agent.

The results revealed that the onset and duration of convulsion at Group IV and V that administered *A.amara's* ethanolic extract, when compared with Group I that administered pilocarpine only, vary with the doses. At Group I, the first seizure appeared after 16±1.90 minutes from the administration. In contrast, at Group IV and V, it was appeared after 22±1.50 and 24±1.30 minutes respectively, meaning that there was a noticeable delaying in the first seizures at Group IV and V.

In addition, the duration of total convulsions attacks was observed for 5 hours as maximum period and the test were performed in open field arena, then the rats transferred to their normal cages, some animals groups continue to have minor attacks after the observation period (5 hours) which expressed as (> 5 hrs) instead of 5±0.00 hrs (Mean ±SD) to be more descriptive.

Group I continued for more than 5 hours. Group IV was similar to Group I, however, the duration at Group V was less than one-hour, meaning that the dose 400 mg/kg of *A.amara's* ethanolic extract exhibits an apparent reduction in the duration of convulsions. No convulsions were recorded at Group III that was administered Diazepam (4 mg/kg).

Moreover, at Group I and Group IV, no survival was recorded, at Group I V, 60% survival was recorded. In contrast, Group V exhibits 100% survi-

Table 1. The preliminary phytochemical screening of *A. amara* leaves' ethanolic extract

Phytochemical group	Test/ Reagent	Result
Alkaloids	Hager's reagent	Positive
	Mayer's reagent	Positive
	Wagner's reagent	Positive
Flavonoid	Alkaline reagent test	Positive
Saponins	Foaming test	Positive
Diterpenes	Cu acetate test	Positive
Anthraquinone Glycosides	Modified Bontrager's test	Negative

val. This finding indicates that the dose of 400 mg/kg of *A.amara's* ethanolic extract reduced the mortality rate from pilocarpine-induced seizures to 40% (Table 2).

To determine the significance of the anticonvulsant activity exhibited by *A.amara's* ethanolic extract, a biostatistics analysis was performed. The results showed that the dose 400 mg/kg of *A.amara's* ethanolic extract, when compared to the positive and negative controls, exhibits a significant reduction in the seizures. The efficacy of 400 mg/kg dose is supported more by the extract's compounds screening data from in silico study which elucidate the potential interaction between several *A.amara's* phytochemical constituents and well-known epileptic targets. one of them is neural acetylcholine receptor subunits alpha-4, and also the ability of these compounds to cross BBB and reach out these targets.

In contrast, the dose of 200 mg/kg has no considerable reduction to onset of seizure(latency) nor duration of the total seizures attacks, suggesting that it might be not valuable as a therapeutic dose (figure 1). The reason for this may be due to the lower diffusion rate characteristic of extract from peritoneal cavity into the surrounding tissues and subsequently to the blood circulation, which in this low dose leads to lower plasma concentration than the minimum effective concentration required to exert the anticonvulsant activity. In addition the extract may has a high binding affinity to blood proteins, so in dose of 200 mg/kg there is no free amount remaining to bind with target in the side of action to exert the anticonvulsant activity which may be dose dependent. In order to reduce the number of animal used in the experiment in complying with the 3 R principles as possible, only two different doses were selected as starting point which will be followed by more comprehensive future research plan including different extraction methods and using of other epilepsy models to test other doses.

Regarding the epileptic behaviours, the dose 400 mg/kg of *A.amara's* ethanolic extract was associated with delaying in the appearing of tremor, salivation, diarrhoea, respiratory changes, S-shape, head nodding, and the oro-facial movement (table III).

To identify the probable targets that the phytochemical constituents bind to exert the antiepileptic activity, a virtual screening via the TargetNet web server that based on QSAR models with molecular docking via Cresset Flare software were conducted.

The results indicated that budmunchiamine A interacts with the neural acetylcholine receptor subunit alpha-7 increasing the GABAergic inhibition at the somatic or somatodendritic regions of the basolateral amygdala interneurons making it probably effective in seizures from amygdalar hyperactivity [34]. Besides, it interacts with 5-hydroxytryptamine receptor 3A (5HT-3A) receptor that has a vital role in epilepsy's control [35].

With the neural acetylcholine receptor subunit alpha-7, budmunchiamine A was predicted to interact hydrophobically with the amino acids Leu36B, Trp53B, Tyr91A, Trp145A, Cys186A, and Tyr191A as well as Tyr184A via hydrogen bonds. This interaction was stronger than the interaction exhibited

Table 2. The onset, the duration of convulsion, and the mortality rate of *A. amara* ethanolic extract at different doses compared with Pilocarpine, Propylene glycol, and Diazepam.

Compound	The onset of convulsion[a] (min)	The duration of total convulsions (hrs.)[b]	The mortality rate (%)
Pilocarpine 400 mg/kg	16±1.90	> 5h	100
Propylene glycol 1:1	24±1.20	>5h	50
Diazepam 4 mg/kg	Nil[c]	Nil	0
A. amra ethanolic Extract 200 mg/kg	22±1.50	>5h	100
A. amraethanolic Extract 400 mg/kg	24±1.30[d]	<1h	40

a. The value expressed as Mean ±SD (n=5)
b.The duration of total convulsions attacks were observed for 5 hrs maximumly.
c. In Diazepam the dose of 4 mg/kg no convulsions occurred (Nil : Nothing was observed).
d. $p < 0.0001$ vs Pilocarpine (one-way ANOVA followed by Dunnetts Multiple comparison)

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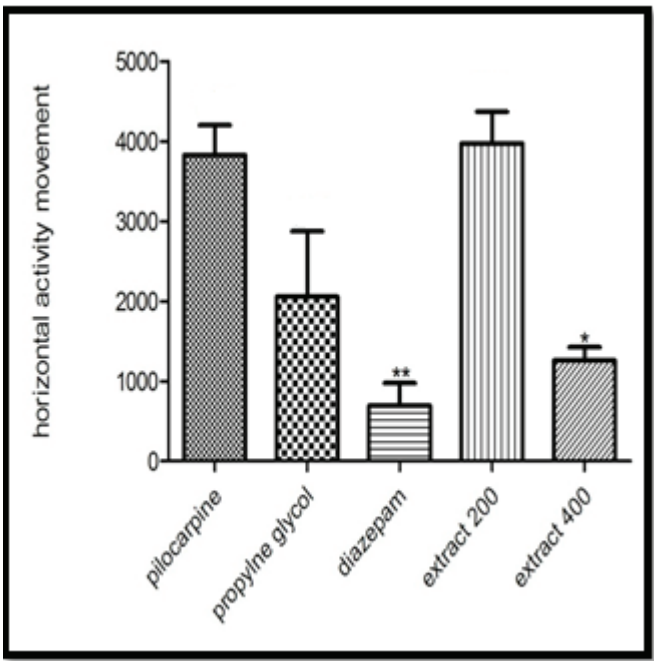

Figure 1. The anticonvulsant activity of *A.amara* ethanolic extracts, propylene glycol, and diazepam against pilocarpine-induced seizure in albino rats. NS; means statistically is not significant, NC; negative control. At **; P≤ 0.01 and at *; P≤ 0.05.

Table 3. The comparison of the observational signs between Pilocarpine, Diazepam, and *A.amara* ethanolic extract.

Groups / Time in minutes / Signs	Pilocarpine (400 mg/kg)			Extract (200 kg/kg)			Extract (400mg/kg)			Diazepam (0.4 mg/kg)		
	15	30	60	15	30	60	15	30	60	15	30	60
Convulsion	+			+			_			_		
Tremor	+			+				+		+		
Salivation	+			+				+		+		
Diarrhoea		+		_					+			+
Urination			+		+		_			_		
Respiratory Change	+			+					+	+		
Jerking	+			+			+			+		
Head nodding	+			+				+		_		
Oro-facial movement		+		+					+			+
Rearing	+			+					+	_		
S-shape	+				+			+		_		
Mortality	_					+			+	_		

by acetylcholine that interacts with the amino acid Tyr191A hydrophobically as via hydrogens bonds with Tyr91A and Tyr184A (figure IIa and b); hence the molecular docking score of budmunchiamine A (-9.391) was better than acetylcholine (-4.688) (Table IV). Both budmunchiamine A and acetylcholine interacted with neural acetylcholine receptor subunit alpha-7at the same binding site.

Moreover, pithecolobine interacts with neuronal acetylcholine receptor subunit alpha-4 that upon inhibition leads to the main increase in the sensitivity to GABA receptor blockers [6] and cannabinoid receptor 1 that participates in the reduction of the neuronal activity [36]. At the binding site of neuronal acetylcholine receptor subunit alpha-4, it interacts with the amino acids Trp25D, Asp96D, Trp93D,

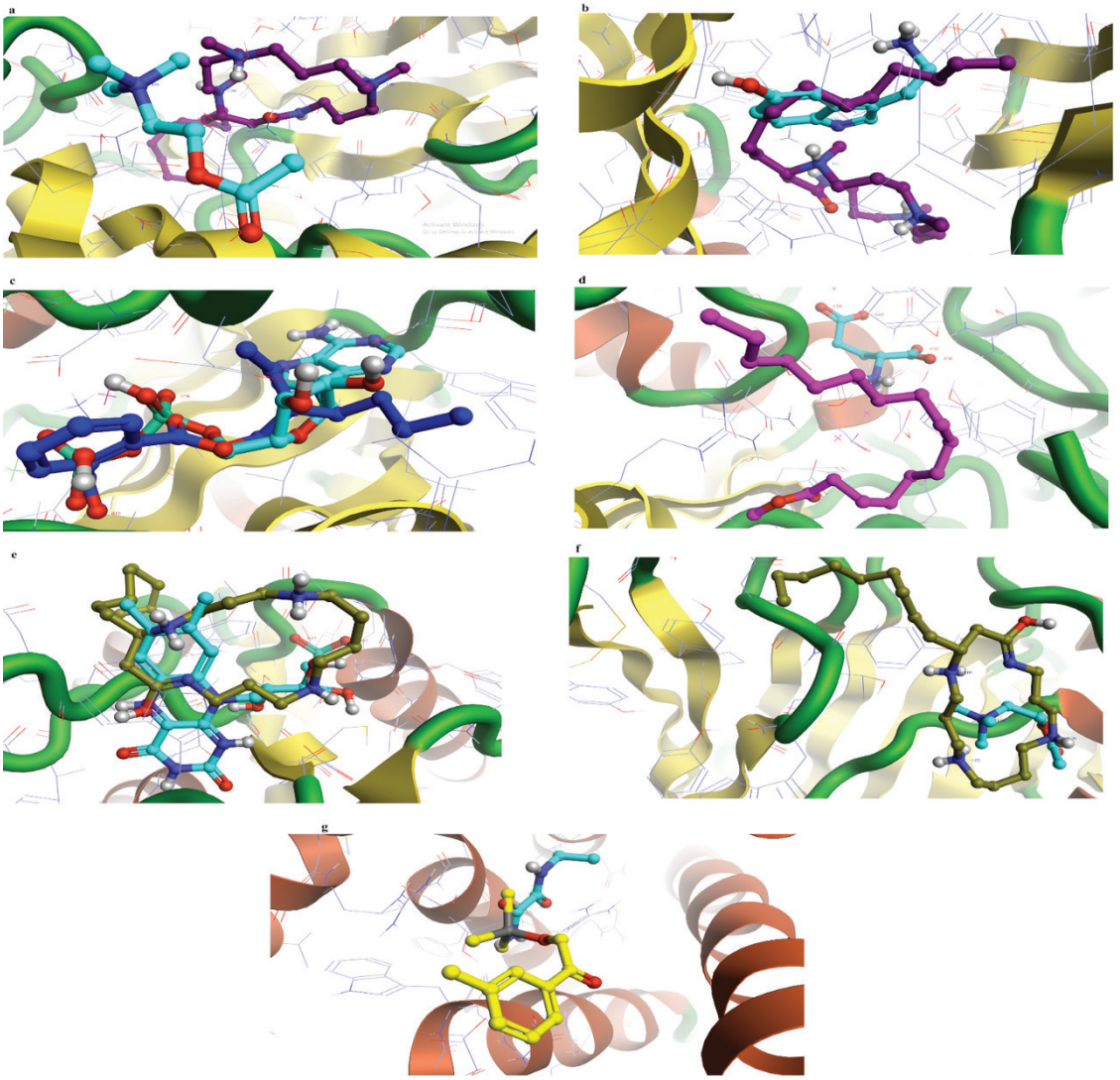

Figure 2. The 2D interaction between the predicted *A. amara's* phytochemical constituents and their predicted anticonvulsant targets.

a, b: Budmunchiamine A and Acetylcholine respectively with Neural acetylcholine receptor subunit alpha-7.

c: 1, 2-benzenedicarboxylic acid mono (2-Ethylhexyl) ester with Protein kinase C gamma & epsilon.

d: Hexadecanoic acid methyl ester with Glutamate carboxypeptidase 2 enzyme.

e: Pithecolobine with Neuronal acetylcholine receptor subunit alpha-4.

f, g: 5-methyl-2-trimethylsilyloxy-acetophenone and Ethoxamide with Voltage-dependant T-type calcium channel subunit alpha-H.

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Table 4. The results of ligand-based virtual screening (LBVS) and blood-brain barrier Permeability (BBB permeab.) prediction.

Target	Compound	LBVS score	BBB permeab.
1) Neural acetylcholine receptor subunit alpha-7.	1) Budmunchiamine A	0.999	Yes
	2) Cyclodecasiloxane, Eicosamethyl	0.964	No
2) 5-hydroxytryptamine receptor 3A	1) Budmunchiamine A	0.994	Yes
	2) Cyclodecasiloxane, Eicosamethyl	0.977	No
3) Protein kinase C gamma & epsilon subunits	1) 1, 2-benzenedicarboxylic acid, mono (2-Ethylhexyl) ester.		Yes
4) Glutamate carboxypeptidase 2 enzyme	1) 1, 2-benzenedicarboxylic acid, mono (2-Ethylhexyl) ester.	1.0	Yes
	2) 9-octadecenoic acid methyl ester (E).	0.999	No
	3) Heptadecanoic acid 14-methyl methyl ester.	1.0	No
	4) Heptadecanoic acid methyl ester.	1.0	No
	5) Hexadecanoic acid methyl ester.	1.0	Yes
	6) n-hexadecanoic acid.	1.0	Yes
	7) Octadecanoic acid methyl ester.	1.0	No
5) Cannabinoid receptor 1	1) 1, 2-benzenedicarboxylic acid, mono (2-Ethylhexyl) ester.	0.887	Yes
	2) 1,4-bis(trimethylsilyl)benzene.	0.908	Yes
	3) 1-bromoeicosane.	1.0	No
	4) 6-methyl-6-nonenamide.	0.966	Yes
	5) 9, 12-octadecadienoic acid (Z, Z)-methyl ester.	1.0	No
	6) 9-octadeceamide (Z).	1.0	Yes
	7) 9-octadecenoic acid methyl ester (E).	1.0	No
	8) Cyclodecasiloxane, Eicosamethyl.	1.0	No
	9) Eicosane.	0.895	No
	10) Heptadecanoic acid methyl ester .	1.0	No
	11) Hexadecanoic acid 14-methyl methyl ester.	1.0	No
	12) Pithecolobine.	0.809	Yes
6) Neuronal acetylcholine receptor subunit alpha-4	1) 1,4-bis(trimethylsilyl)benzene.	1.0	Yes
	2) 1-bromoeicosane.	0.992	No
	3) 6-nitro-2,3-dihydrophthalazine-1,4-dione.	0.857	No
	4) Cyclodecasiloxane, Eicosamethyl.	1.0	No
	5) Eicosane.	0.999	No
	6) Pithecolobine.	1.0	Yes
7) 5-hydroxytryptamine receptor 2C	1) 1,4-bis(trimethylsilyl)benzene	0.97	Yes
	2) 1-bromoeicosane	0.89	No
	3) Cyclodecasiloxane, Eicosamethyl	0.979	No
	4) Eicosane	0.955	No
8) Voltage-dependant T-type calcium channel subunit alpha-H	1) 1,4-bis(trimethylsilyl)benzene	0.873	Yes
	2) 5-methyl-2-trimethylsilyloxy-acetophenone	1.0	Yes
	3) Cyclodecasiloxane, Eicosamethyl	0.967	No
9) Inotropic glutamate receptor NMDA 1	1) 6-nitro-2,3-dihydrophthalazine-1,4-dione	1.0	No
	2) Cyclodecasiloxane, Eicosamethyl	1.0	No
	3) Tetrasiloxane, decamethyl	0.996	Yes

Thr157D, Trp158D, and Lys162D hydrophobically and Thr157D and Tyr158D via hydrogen bonds (figure 2e). These multiple interactions are responsible for the increase in the docking binding score (-7.438) when compared to the natural ligand Acetylcholine (-5.162) (Table IV, V), making it a probable candidate.

Besides that, 1, 2-benzenedicarboxylic acid mono (2-Ethylhexyl) ester interacts with the Protein kinase C gamma & epsilon subunits that its inhibition leads to depression of the epileptogenesis's course [37]. It interacts with the binding site's amino acids Asn383A, Phe552A, and Gly261A hydrophobically, Tyr265A, and LysA283A via hydrogen bonds

Table 5. The molecular docking results of the phytochemical constituents with their predicted targets.

Target	Compound	Docking score	
1) Neural acetylcholine receptor subunit alpha-7.		5AFG	5AFN
	Budmunchiamine A	-9.668	-9.391
	Acetylcholine	-5.466	-4.688
2) 5-hydroxytryptamine receptor 3A receptor		4PIR	6NP0
	Budmunchiamine A	-7.317	-10.093
	5-hydroxytryptamine	-9.129	-6.513
3) Protein kinase C gamma & epsilon		2UZP	5LIH
	1, 2-benzenedicarboxylic acid, mono (2-Ethylhexyl) ester.	-7.637	-7.275
	A PLP 1294	-10.07	----
	A ADP 601	----	-11.167
4) Glutamate carboxypeptidase 2		2C6G	4NGQ
	1, 2-benzenedicarboxylic acid, mono (2-Ethylhexyl) ester.	-5.826	-5.399
	Hexadecanoic acid methyl ester	-6.56	-8.021
	n-hexadecanoic acid	-4.708	-3.633
	Glutamate	-6.418	-3.046
5) Cannabinoid receptor 1		5TGZ	5XR8
	1, 2-benzenedicarboxylic acid, mono (2-Ethylhexyl) ester.	-7.613	-6.773
	1,4-bis(trimethylsilyl)benzene	-4.306	-4.509
	6-methyl-6-nonenamide	-5.795	-5.175
	9-octadeceamide(Z)	-7.351	-6.73
	Pithecolobine	-7.053	-9.988
	A ZDG 2001	-9.62	----
	A FMN 1201	----	-9.541
6) Neuronal acetylcholine receptor subunit alpha-4		6CNJ	5KXI
	1, 4-bis (trimethylsilyl) benzene	-4.008	-3.191
	Pithecolobine	-7.438	-7.884
	Acetylcholine	-5.162	-4.365
7) 5-hydroxytryptamine receptor 2C		6BQG	6BQH
	1, 4-bis (trimethylsilyl) benzene	-3.175	-3.487
	5-hydroxytryptamine	-7.50	-7.113
8) Voltage-dependant T-type calcium channel subunit alpha-H		5KLB	Phyre2
	1, 4-bis (trimethylsilyl) benzene	-3.135	-4.352
	5-methyl-2-trimethylsilyloxy-acetophenone	-4.709	-6.385
	Ethoxamide	-4.112	-5.582
9) Inotropic glutamate receptor NMDA 1		4TLM	5I59
	Tetrasiloxane, decamethyl	-2.78	-6.139
	Glutamate	-5.316	-4.954

as well as the co-factors Mn603A and Al605A via cation-pi interaction (figure 2c). Hexadecanoic acid methyl ester interacts with Glutamate carboxypeptidase 2 enzyme that responsible for the generation of the free Glutamate in the brain making it a probable neuroprotective agent [38]. The interaction is exhibited hydrophobically with the amino acids Leu83A, Ala84A, Phe426A, Ser380A, and Trp381A as well as via hydrogen bond with Ser118A (figure 2d). 5-methyl-2-trimethylsilyloxy-acetophenone interacts with the Voltage-dependant T-type calcium channel subunit Alpha-H that has an imperative role in the neuronal excitability [39]. At the active site, it interacts with the amino acids His1067A, Asp1074A hydrophobically, and via hydrogen bonds Arg1063A and Ser1077A. On the contrary, Ethoxamide that was used as a control compound interacts with the amino acids Arg08A and Asp1080A via hydrogen bonds (fi-

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gure 2g and h). Since Budmunchiamine A, Pithecolobine, 1, 2-benzenedicarboxylic acid, Hexadecanoic acid methyl ester, 5-methyl-2-trimethylsilyloxy-acetophenone have the best molecular docking score, they were best predicted phytochemical constituents contribute to the antiepileptic activity (Table IV).

In the interaction of the predicted phytochemical constituents and the used control compounds, a ligand superimposing occurred (e.g. figure 3b, c, and f).

In contrast, the molecular docking score of 1, 4-bis (trimethylsilyl) benzene (-3.175) with 5-hydroxytryptamine receptor 2C and Tetrasiloxane, decamethyl (-2.78) with Inotropic glutamate receptor NMDA 1 are lower than the natural ligands 5-hydroxytryptamine (-7.50) and Glutamate (-5.316) respectively (table IV), hence they are less probable candidates.

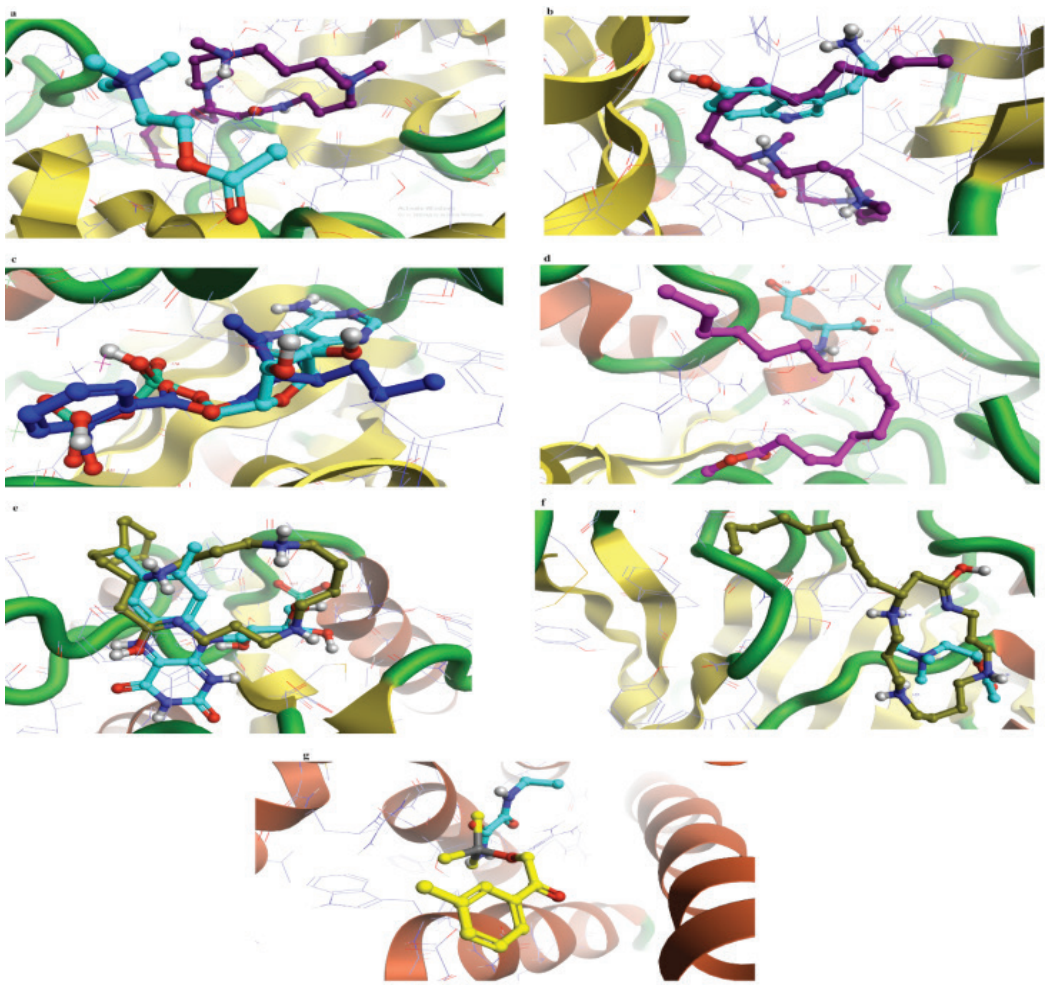

Figure 3: The 3D interaction between the predicted *A.amara's* phytochemical constituents and their predicted anticonvulsant targets.
Budmunchiamine A (violet) with neural acetylcholine receptor subunit alpha-7. Acetylcholine (turquoise) as control.
a. Budmunchiamine A (violet) with 5-hydroxytryptamine receptor 3A. 5-hydroxytryptamine (turquoise) as control.
b. 1, 2-benzenedicarboxylic acid, mono (2-Ethylhexyl) ester (blue) with Protein kinase C gamma & epsilon. The co-crystallized ligand A ADP 601 (turquoise) as control.
c. Hexadecanoic acid methyl ester (pink) with Glutamate carboxypeptidase 2. Glutamate (turquoise) as control.
d. Pithecolobine (dark yellow) with Cannabinoid receptor 1. The co-crystallized ligand A FMN 1201 (turquoise) as control.
e. Pithecolobine (dark yellow) with Neuronal acetylcholine receptor subunit alpha-4. Acetylcholine (turquoise) as control.
f. 5-methyl-2-trimethylsilyloxy-acetophenone (yellow) with Voltage-dependant T-type calcium channel subunit alpha-H. Ethoxamide (turquoise) as control. 5-methyl-2-trimethylsilyloxy-acetophenone (yellow) with Voltage-dependant T-type calcium channel subunit alpha-H. Ethoxamide (turquoise) as control.

Besides the interaction and pharmacodynamics, the respectable pharmacokinetics [40]. and safety profiles [41]. are important issues for the druggable molecules, hence a predictive study was conducted via pkCSM, SwissADME and eMolTox web servers [42]. The results showed that the predicted phytochemical constituents have a high intestinal absorption (above 90%) leading to high blood concentration, Budmunchiamine A and Pithecolobine have a high volume of distribution leading to a high CNS tissue supply, Hexadecanoic acid methyl ester, 9-octadeceamide(Z), and 6-methyl-6-nonenamide have the highest predicted clearance volume [42]. In contrast, Budmunchiamine A was predicted as Cardiotoxic and Hepatotoxic molecule, 9-octadeceamide (Z) and Pithecolobine was Cardiotoxic.

The predicted CYP-450 enzyme inhibition by phytochemical constituents leads to caution in co-administration with other drugs to avoid drug-drug interaction, accumulation, and toxicity [43].

Since the pilocarpine when is used as a seizure-inducing agent causes leakage of the blood-brain barrier (BBB), other non-BBB permeable phytochemical constituents may contribute to the activity in the studied model, however, they may not be effective at well intact BBB.

Furthermore, to identify the drug candidates, a drug-likeness prediction study was conducted. The results showed that 1, 2-benzenedicarboxylic acid, mono (2-Ethylhexyl) ester, 5-methyl-2-trimethyl silyloxy-acetophenone, and Tetrasiloxane, decamethyl could be a drug candidate (Table VI).

According to the results of in silico study, Budmunchiamine A, 1, 2-benzenedicarboxylic acid, mono (2-Ethylhexyl) ester, Hexadecanoic acid me-

Table 6. The results of intestinal absorption (Intestinal absorp), apparent volume of distribution (Vd), clearance (Cl), major organ toxicity, CYP-450 enzymes inhibition, and Drug-likeness prediction.

Compound	Intestinal absorp. (%)	Vd (L/Kg)	Cl (ml/ min /kg)	Major organ toxicity	CYP-450 enzymes inhibition	Drug-likeness
Budmunchiamine A	High (90.862)	18.07	2.864	Cardiotoxic (hERG II inhibitor), Hepatotoxic	No	No (MR>130, atoms>70, XLOGP3>5)
1,4-bis (trimethylsilyl benzene)	High (96.218)	2.654	1.905	No	CYP1A2	No
1, 2-benzenedicarboxylic acid, mono (2-Ethylhexyl) ester.	High (96.919)	0.059	7.586	No	CYP2C9	Yes
Hexadecanoic acid methyl ester	High (92.335)	2.157	72.61	No	CYP1A2	No
n-hexadecanoic acid	High (91.957)	0.275	54.94	No	CYP1A2	No
6-methyl-6-nonenamide	High (93.792)	1.306	43.15	No	No	No (MW<200)
9-octadeceamide (Z)	High (90.17)	2.118	90.99	Cardiotoxic (hERG II inhibitor)	CYP1A2	No
Pithecolobine	High (90.19)	15.03	3.589	Cardiotoxic (hERG II inhibitor)	No	No (MR>130, atoms>70)
5-methyl-2-trimethyl silyloxy-acetophenone	High (94.514)	1.425	1.782	No	CYP1A2	Yes
Tetrasiloxane, decamethyl	High (95.728)	1.828	14.09	No	CYP1A2, CYP2C19	Yes
2,4,6-cycloheptatrien-1-one-3,5-bistrimethylsilyl	High (99.757)	1.135	1.841	No	CYP1A2	No (Heteroatoms<2)

M R and XLOGP3 indicate the molar refractivity and lipophilicity, respectively.

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thyl ester, and Pithecolobine were the best *A.amara* phytochemical constituents that contribute to its antiepileptic activity. However, the predicted hERG II inhibition by Budmunchiamine A and Pithecolobine that causes possible fatal ventricular arrhythmias, the predicted hepatotoxicity of Budmunchiamine A, as well as the increased atoms' number, lipophilicity, and molar refractivity reduces their score to be as drug candidates [44]. The predicted hERG II inhibition may contribute to the recorded mortality rate that was 100% at the doses 200 mg/kg and 40% at the doses 400 mg/kg (Table II).

Though the used software packages for the in-silico study is established to have reputable efficiency and accuracy, a practical study at the wet lab is desired to validate the results [19-23-25-45-46].

4. Conclusion

A.amara's leaves ethanolic extract contains alkaloids, flavonoids, saponins, and diterpenes. The dose 400 mg/kg of it showed a significant reduction in the seizures when compared to the negative control pilocarpine and the positive control Diazepam.

The predicted targets that responsible for the anticonvulsant activity are neural acetylcholine receptor subunits alpha-4 and alpha-7, 5-hydroxytryptamine receptor 3A, Cannabinoid receptor 1, kinase C gamma & epsilon subunits, and Glutamate carboxypeptidase 2 enzymes.

The predicted phytochemical constituents have the highest contribution in antiepileptic activity are budmunchiamine A, 1, 2-benzenedicarboxylic acid, mono (2-Ethylhexyl) ester, hexadecanoic acid methyl ester, and pithecolobine. However, budmunchiamine A and pithecolobine were pharmacodynamically efficient, their predicted toxicity will reduce their score as candidate drugs. Future research studies for isolation of specific phytomolecules responsible for observed anticonvulsant activity and their precise mechanism(s) of action will be recommended.

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