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ORIGINAL ARTICLE

Jimson weed (*Datura stramonium* L.) alkaloid extracts modulate cholinesterase and monoamine oxidase activities in vitro: possible modulatory effect on neuronal function

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Abstract Despite the well-established medicinal values of Jimson weed (*Datura stramonium* L.), this medicinal plant has been associated with neurological effects such as hallucination and anxiety in folklore. This study examined the effect of alkaloid extracts from the leaf and fruit of Jimson weed on critical enzymes of the monoaminergic [monoamine oxidase (MAO)] and cholinergic [acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)] systems of neurotransmission. Alkaloid extracts were prepared by solvent extraction method and their interaction with the activities of MAO, AChE, and BChE were assessed (in vitro). Gas chromatography coupled with mass spectroscopic (GC-MS) characterization of the extracts was also carried out. The results revealed that the extracts inhibited the activity of the enzymes assayed for in a concentration-dependent manner. Considering the IC_{50} values, the fruit extract had more potent ($P<0.05$) inhibitory effect on the enzymes' activities, compared to the leaf extract. GC-MS characterization revealed the presence of atropine, scopolamine, amphetamine, 3-methoxyamphetamine, 3-ethoxyamhetamine cathine, spermine, phenylephrine, and 3-piperidinemethanol, among others in the extracts. The alteration of activities of these critical enzymes of the cholinergic and monoaminergic signaling may be responsible for the reported neurological effects of this medicinal plant in folklore; nevertheless, the fruit extract exhibited more neuromodulatory effect than the leaf.

Keywords Jimson weed · Alkaloid extracts · Neuromodulation · Monoamine oxidase · Cholinesterase

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Introduction

Brain neuronal activities, especially neurotransmission processes are organized into systems of individually unique neuronal cells, neurotransmitters, neuromodulators, receptor molecules, and secondary messengers. Notable among these systems include the monoaminergic and cholinergic systems. Central to the cholinergic system of neurotransmission is the neurotransmitter acetylcholine. Acetylcholine (ACh) operates as a rapidly acting neurotransmitter at the neuromuscular synapses, autonomic ganglia, and the brain (Changeux 2010). ACh is hydrolyzed by a family of enzymes called cholinesterase. The prolonged inhibition of acetylcholinesterase (AChE) could ultimately lead to excessive accumulation of ACh at the synaptic cleft which will lead to overstimulation of the cholinergic neuron (Rodrigues et al. 2011). The continual overstimulation of these neurons could lead to neurodegeneration and eventual death of the organism (Nunes et al. 2003). The monoamine system of neurotransmission consists of network of neurotransmission systems, each mediated by a specific monoamine neurotransmitter. Notable among them are the dopaminergic, noradrenergic, and serotonergic systems which are mediated by dopamine, noradrenaline, and serotonin neurotransmitters, respectively. The enzyme monoamine oxidase (MAO) exists in two isoforms (MAO A and MAO B) and are both involved in the oxidative deamination of biogenic amines (neuroamines, vasoactive, and exogenous amines); thus, regulating the concentration of amine neurotransmitters as well as

several amine drugs (McCabe-Sellers et al. 2006). Despite the well-reported therapeutic potentials of MAO inhibitors in the management of neurological disorders, there are several toxicological complications such as serotonin toxicity (Boyer and Shannon 2005) and hypertensive crisis (McCabe-Sellers et al. 2006) that can arise from excessive MAO inhibition which can occur as a result of excessive intake of MAO inhibitors. Furthermore, plant extracts such as from Syrian rue (*Peganum harmala*) (Herraiz et al. 2010) and Passion flower (*Passiflora incarnata*) (Dhawan et al. 2004) contain a family of effective MAO inhibitors called beta-carboline alkaloids which have records of causing adverse health effects (Santillo et al. 2014).

Jimson weed (*Datura stramonium* L.) is an annual herbaceous plant reported for its many pharmacological properties (Soni et al. 2012). All parts of the plant, notably the leaf, fruit, and seed are reported to be rich in alkaloids, especially the tropane alkaloids (Steenkamp et al. 2004). Despite the fact that some researchers have reported the medicinal properties of this plant (Soni et al. 2012; Altameme et al. 2015), there exist some serious neurological effects such as hallucination and anxiety, which have been reported in folklore. In addition, intentional use of the plant for its hallucinating effects has also been well documented, especially in adolescents (Adegoke and Alo 2013), while cases of *Datura*-induced toxicity in humans as a result of consumption of farm produce such as tea and soybean contaminated with *Datura* seeds have been reported (Soni et al. 2012). However, despite these serious adverse effects of this plant on psycho-activity, there is dearth of information on the effect of its phytoconstituents on critical enzymes involved in the maintenance of normal neuronal function. Consequently, this study aims to investigate the effect of crude alkaloid extracts from the leaf and fruit of Jimson weed on key enzymes of the monoaminergic (MAO) and cholinergic (AChE and BChE) systems of neurotransmission.

Materials and methods

Materials

Collection and preparation of samples

Jimson weed (*D. stramonium* L.) plant was collected at the stage of opening of first capsule, from local farm settlement in Akure, Ondo State (Southwest) Nigeria, during the late raining season (August) of year 2014. The plant was authenticated at the Forest Research Institute of Nigeria (FRIN), Ibadan, Oyo State (Southwest) Nigeria. A sample voucher was deposited at the institute's herbarium (voucher number FHI 110111). Leaves and fruits of the plant were carefully separated, washed with water to remove dirt, and dried under shade for several days until a constant weight was obtained. Thereafter, the dried samples were pulverized in an electronic stainless steel blender, and stored in air-tight dark containers in the refrigerator at 4 °C for alkaloid extraction.

Chemicals and reagents

Chemicals and reagents used such as semicarbazide, benzylamine, acetylthiocholine iodide, and butyrylthiocholine iodide were procured from Sigma-Aldrich, Inc., (St. Louis, MO); trichloroacetic acid (TCA) was sourced from Sigma-Aldrich, Chemie GmbH (Steinheim, Germany); 2,4-dinitrophenyl hydrazine (DNPH) from ACROS Organics (NJ, USA); and methanol and acetic acid were sourced from BDH Chemicals Ltd., (Poole, England). All other chemicals were of analytical grade while the water used for all analysis was glass distilled.

Methods

Preparation of alkaloid extracts

Alkaloid extract of samples were prepared according to the method of Harborne (1998), with slight modifications. Briefly, 50 g pulverized sample was defatted with *n*-hexane for 24 h. Thereafter, 10 g of defatted samples were weighed into a 250-ml beaker and 100 ml of 10 % acetic acid in ethanol was added and covered. These were vigorously shaken, venting the mounted pressure and allowed to stand for 24 h to allow for sufficient extraction. The mixtures were thereafter, filtered first using muslin cloth and then filter paper (Whatman No. 1) to obtain a clear filtrate which was concentrated under vacuum using rotary evaporator (Laborota 4000 Efficient, Heidolph, Germany) at 45 °C. Concentrated ammonium hydroxide was subsequently added drop wise to the concentrated filtrate until the precipitate was completed. The whole solution was allowed to settle and the precipitate was collected and rinsed with dilute ammonium hydroxide to obtain the alkaloid extracts. The extracts were collected and stored in the refrigerator at 4 °C for further analysis.

Monoamine oxidase assay (in vitro)

The effect of the alkaloid extracts from the leaf and fruit of Jimson weed MAO (EC 1.4.3.4) activity was measured according to a previously reported method (Green and Haughton 1961) with slight modification. In brief, the reaction mixture contained 0.025 M phosphate buffer (pH 7.0), 0.0125 M semicarbazide, 10 mM benzylamine, 0.67 mg of the enzyme and 0–100 μl of extracts. After 30 min incubation, acetic acid was added and incubated for 3 min in boiling water bath followed by centrifugation. The resultant supernatant (1 ml) was mixed with equal volume of 2,4-DNPH, and 1.25 ml of benzene was added after 10 min incubation at room temperature. After separating the benzene layer, it was mixed with

equal volume of 0.1 N NaOH. Alkaline layer was decanted and incubated at 80 °C for 10 min. The orange–yellow color developed was measured at 450 nm in a UV/visible spectrophotometer (Jenway 6305 model). The MAO activities were thereafter expressed as percentage inhibition of the reference; thus,

$$\%\ \text{inhibition} = \left[\text{ABS}_{\text{ref}} - \text{ABS}_{\text{sample}}\right) \Big/ \text{ABS}_{\text{ref}} \Big] \times 100$$

where ABS_{ref} = absorbance of reference and $\text{ABS}_{\text{sample}}$ = absorbance of sample.

Cholinesterase assay (in vitro)

The effect of the extracts on cholinesterase [acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)] activities was assessed by a modified colorimetric method (Perry et al. 2000). The cholinesterase activity was determined in a reaction mixture containing 200 μl of AChE (EC 3.1.1.7) or BChE (EC 3.1.8) solution in 0.1 M phosphate buffer (pH 8.0), solution of 5,5′-dithio-bis(2-nitrobenzoic) acid (DTNB 3.3 mM), sample extracts (0–100 μl), and phosphate buffer, pH 8.0. After incubation for 20 min at 25 °C, acetylthiocholine iodide (for AChE activity assay) or butyrylthiocholine iodide (for BChE activity assay) was added as the substrate, and enzyme activity was determined in a UV/visible spectrophotometer (Jenway 6305 model) at 412 nm. The AChE and BChE activities were thereafter expressed as percentage inhibition of the reference; thus,

$$\%\ \text{inhibition} = \left[\text{ABS}_{\text{ref}} - \text{ABS}_{\text{sample}}\right) \Big/ \text{ABS}_{\text{ref}} \Big] \times 100$$

Where ABS_{ref} = absorbance of reference and $\text{ABS}_{\text{sample}}$ = absorbance of sample.

GC-MS characterization of alkaloid extracts

This analysis was performed using HP 6890 series gas chromatograph coupled with 5975C inert mass spectrometer (with triple axis detector) with electron-impact source (Agilent Technologies). The stationary phase of separation of the compounds was HP-5 capillary column coated with 5 % phenyl methyl siloxane (30 m length × 0.32 mm diameter × 0.25 μm film thickness) (Agilent Technologies). The carrier gas was helium used at constant flow of 1.6 ml/min at an initial nominal pressure of 2.84 psi and average velocity of 46 cm/s. The samples were injected in splitless mode at an injection temperature of 260 °C and injection volume of 1 μl. Purge flow was 21.5 ml/min at 0.50 min with a total flow of 25.8 ml/min; gas saver mode was switched on. An oven was initially programmed at 60 °C (1 min) then ramped at 4 °C/min to 110 °C (3 min) then 8 °C/min to 260 °C (5 min) and 10 °C/min to 300 °C (12 min). Run time was 56.25 min with a 3 min solvent delay. The mass spectrometer was operated in electron-impact ionization mode at 70 eV with ion source temperature of 230 °C, quadrupole temperature of 150 °C, and transfer line temperature of 280 °C. Scanning of possible alkaloid compounds was from *m*/*z* 30 to 550 amu at 2.62 s/scan scan rate and was identified by comparing measured mass spectral data with those in *NIST 11 Mass Spectral Library* and literature.

Data analysis

The results of three (3) experiments were pooled and expressed as mean ± standard deviation (SD). Mean values were appropriately analyzed and compared using Student's *t* test (unpaired) and significance was accepted at $P \leq 0.05$. Also, IC_{50} (effective concentration of extract causing 50 % inhibition) values were calculated using nonlinear regression analysis. All statistical analyses were carried out using GraphPad Prism version 5.00 for Windows.

Result

Figure 1 presents the result of the modulatory effect of the alkaloid extracts from the leaf and fruit of Jimson weed on monoamine oxidase (MAO) activity. Both extracts inhibited MAO activity in a concentration-dependent manner (0–26.67 μg/ml). Considering the IC_{50} value presented in Table 1, the fruit extract had a higher significant ($P < 0.05$) inhibitory effect (13.70 ± 0.02 μg/ml) than the leaf extract (28.64 ± 0.05 μg/ml).

The result of the modulatory effect of the alkaloid extracts on acetylcholinesterase (AChE) activity is presented in Fig. 2. This showed that the extracts inhibited the activity of AChE in concentration-dependent manner (0–13.04 μg/ml). However, judging by the IC_{50} values (Table 1), the fruit extract had a more potent ($P < 0.05$) inhibitory effects (5.06 ± 0.02 μg/ml) than the leaf extract (9.32 ± 0.04 μg/ml).

Similarly, Fig. 3 presents the result of the modulatory effect of the alkaloid extracts on butyrylcholinesterase (BChE) activity. This revealed that BChE activity was also inhibited concentration dependently (0–13.04 μg/ml) by both extracts. However, as revealed by the IC_{50} value (Table 2), the fruit extract had more potent ($P < 0.05$) inhibitory effect (6.59 ± 0.02 μg/ml) than the leaf extract (14.70 ± 0.04 μg/ml).

The results of the GC-MS characterization of the phytoconstituents of the extracts are presented in Tables 2 and 3. The results revealed that 48 compounds were identified from the leaf extract while 56 compounds were identified from the fruit extracts. The compounds detected in the extracts include alkaloids such as atropine, scopolamine, amphetamine, 3-methoxyamphetamine, 3-ethoxyamhetamine cathine, spermine, phenylephrine, 3-piperidinemethanol, sarcosine, *N*-(cyclopentylcarbonyl)-, and decyl ester, among

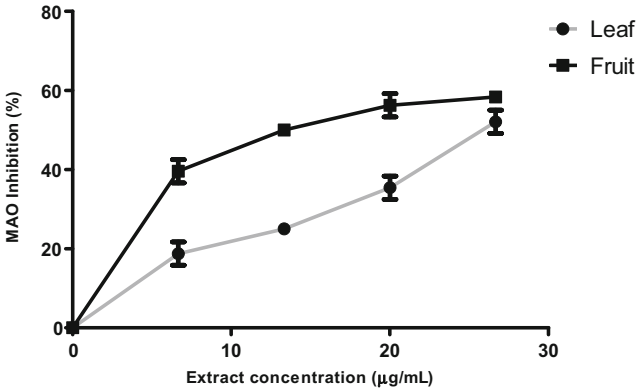

Fig. 1 Inhibitory effects (%) of alkaloid extracts from the leaf and fruit of Jimson weed (*D. stramonium* L.) on the activity of monoamine oxidase (MAO)

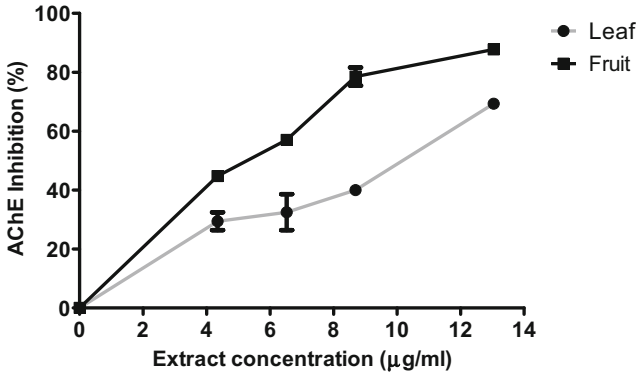

Fig. 2 Inhibitory effects (%) of alkaloid extracts from the leaf and fruit of Jimson weed (*D. stramonium* L.) on acetylcholinesterase (AChE) activity

others. The percentage distribution of the compounds also shows that the alkaloids are more abundant in the fruit extract.

Discussion

Jimson weed (*D. stramonium* L.) is gaining popularity for its several reported medicinal and pharmacological properties, including its effect on neuromodulation. These neuromodulatory effects which include hallucination, short-term memory loss, depression, and impaired cognitive function (Adegoke and Alo 2013) have been largely linked to the alkaloid constituents of the plant (Soni et al. 2012). In this study, the observed inhibitory effects of alkaloid extracts from the leaf and fruit of Jimson weed on brain MAO activity could contribute to the mechanism by which it induces neuromodulatory properties as reported in folklore, with the fruit producing more potent MAO inhibitory effect, but was less potent than that of previously reported standard nonselective MAO inhibitor iproniazid (IC_{50} = 0.72 μg/ml) (Zhi et al. 2016). This finding agrees with earlier studies in which neuromodulatory properties of plant alkaloids extracts have been linked to their MAO inhibitory effects. Plant extracts such as from Syrian rue (*P. harmala*) (Herraiz et al. 2010) and passionflower (*P. incarnata*) (Dhawan et al. 2004) contain a family of effective MAO inhibitors called beta-carbolines which have records of causing adverse health effects (Santillo et al. 2014). Furthermore, Herraiz and Chaparro (2005) reported that two beta-carboline alkaloids (norharman and harman) isolated from tobacco smoke exhibited significant MAO inhibition which could be associated with cigarette-induced addiction and depression.

Despite the well-reported therapeutic potentials of MAO inhibitors in the management of neurological disorders, there are several toxicological complications that arise from excessive MAO inhibition which can occur as a result of excessive intake of MAO inhibitors, adverse drug or dietary supplement reactions, or MAO inhibitors from plant sources. For example, serotonin toxicity is a serious pathological disorder resulting from hyperactivity of serotonin neurotransmitter as a result of excessive accumulation of serotonin due to excessive MAO inhibition (Boyer and Shannon 2005). Neurostimulants such as methylenedioxymethamphetamine (ecstasy) and tetrahydrocannabinol, which are commonly used by young people for their "mind-altering" properties has been reported to induce inhibitory effects on MAO activity which may contribute to their neuromodulatory properties especially in long-term exposure (White et al. 1996; Fisar 2012).

Table 1 IC_{50} values for the inhibitory effects of alkaloid extracts from the leaf and fruit of Jimson weed on monoamine oxidase (MAO), acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) activities

Sample	MAO IC_{50} values (μg/ml)	AChE	BChE
Leaf	28.64 ± 0.05^{b}	9.32 ± 0.04^{b}	14.70 ± 0.04^{b}
Fruit	13.70 ± 0.02^{a}	5.06 ± 0.02^{a}	6.57 ± 0.02^{a}

Values represent mean ± standard deviation of triplicate experiments. Values with different superscript letters along the same column are significantly different ($P < 0.05$)

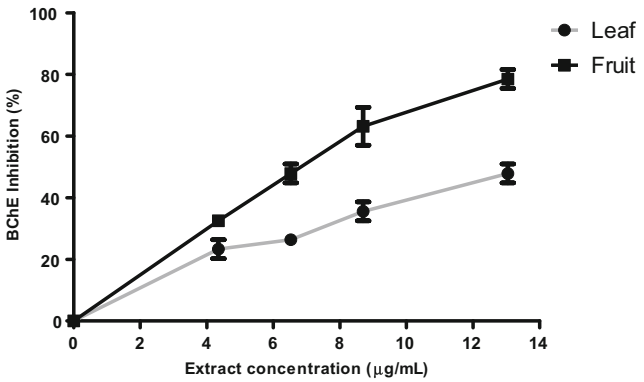

Fig. 3 Inhibitory effects (%) of alkaloid extracts from the leaf and fruit of Jimson weed (*D. stramonium* L.) on butyrylcholinesterase (BChE) activity

Table 2 GC-MS characterization of the alkaloid extract from the leaf of Jimson weed

S/S	RT (min)	Compound name	MW (amu)	% total	CAS number	Entry number in NIST11 Library
1	6.5	5-Aminoisoxazole	84.032	0.04	014678-05-8	1334
2	9.574	Chlorodifluoroacetamide	128.979	0.14	000354-28-9	12754
3	10.589	1-Heptadecanamine	255.293	0.16	004200-95-7	106613
4	11.842	1,3-Adamantanediacetamide	250.168	2.00	056432-73-6	102232
5	12.002	Cathine	151.1	0.14	000492-39-7	24935
6	12.601	3-Ethoxyamphetamine	179.131	0.04	135014-86-7	44803
7	13.284	Norpseudoephedrine	151.1	0.19	036393-56-3	24943
8	13.99	3-Methoxyamphetamine	165.115	0.23	017862-85-0	34315
9	18.228	Adipamide	144.09	0.40	000628-94-4	20535
10	19.623	*S*-[tri-*t*-butoxysilyl]-2-mercaptoethylamine	323.195	0.03	1000252-63-8	163194
11	20.038	3,3-Dimethyl-2-butanamine	101.12	0.10	003850-30-4	4167
12	20.175	*N*-Methyl-1,3-propanediamine	88.1	0.23	006291-84-5	1984
13	20.905	*N*-Methyl-1-octadecanamine	283.324	0.23	002439-55-6	130250
14	22.062	DL-Phenylephrine	167.095	0.05	001477-63-0	36227
15	22.395	Phenylephrine	167.095	0.03	000059-42-7	36217
16	22.881	Diethyl phthalate	222.089	0.81	000084-66-2	78782
17	23.101	*N*-Methylallylamine	71.073	0.17	000627-37-2	612
18	24.579	*N*-Methyl-2-phenyl-1-propylamine	149.12	0.32	1000379-96-9	23375
19	24.614	(−)-Norephedrine	151.1	0.05	000492-41-1	24941
20	26.003	Phenanthrene	178.078	0.20	000085-01-8	44142
21	26.294	*p*-Hydroxyamphetamine	151.1	0.06	000103-86-6	24953
22	27.576	Amphetamine-3-methyl	149.12	0.04	000588-06-7	23338
23	27.98	2-Cyano-acetamide	84.032	11.97	000107-91-5	1337
24	29.25	*N*-Methyl-benzeneethanamine	135.105	0.12	000589-08-2	15590
25	30.876	*o*-Veratramide	181.074	0.19	001521-39-7	46685
26	31.315	3-Piperidinemethanol	115.1	0.10	004606-65-9	7805
27	31.547	*R*-(−)-Cyclohexylethylamine	127.136	0.25	005913-13-3	11895
28	33.244	9-Octadecenamide	281.272	0.39	000301-02-0	128445
29	33.31	4-Methoxy-alpha-methyl-(+/−)-benzenethanamine	165.115	0.11	023239-32-9	34383
30	33.404	(*S*)-2-Butanamine	73.089	0.27	000513-49-5	756
31	33.482	Alpha-[(methylamino)methyl]-benzenemethanol	151.1	0.09	006589-55-5	25031
32	33.547	*P*,alpha-dimethyl-phenethylamine	149.12	0.25	000064-11-9	23388
33	33.618	Octodrine	129.152	0.36	000543-82-8	13020
34	33.832	(*S*)-(+)-1-Cyclohexylethylamine	127.136	0.13	017430-98-7	11910
35	34.099	*sec*-Butylamine	73.089	0.13	013952-84-6	755
36	34.283	2-Pentanamine	87.105	0.06	063493-28-7	1904
37	35.108	6-Amino-2-methyl-2-heptanol	145.147	0.04	000372-66-7	21449
38	35.322	2-Fluoro-beta,3-dihydroxy-*N*-methyl-benzeneethanamine	185.085	0.03	103439-04-9	49743
39	36.099	Tuaminoheptane	115.136	0.03	000123-82-0	7880
40	36.23	2,5-Dimethoxy-4-(methylsulfonyl)amphetamine	273.103	0.03	146724-75-6	121547
41	43.275	3-Propoxyamphetamine	193.147	0.06	1000124-04-4	55407
42	43.649	Alpha-(1-aminoethyl)-, [*R*-(*R**,*R**)]-benzenepropanoic acid	193.11	0.37	139344-69-7	55393
43	44.302	2-(Adamantan-1-yl)-1-methyl-ethylamine	193.183	0.58	1000310-59-0	55466
44	47.204	1,3-Dimethyl-8-[2-nitrophenethenyl]-purin-2,6-dione	327.097	3.17	1000128-96-1	166457
45	48.979	*N-sec*-Butyl-3-(2-hydroxy-3,4-dimethyl-phenyl)-3-phenyl-propionamide	325.204	2.20	1000296-11-4	165168
46	49.454	Carbamic acid, *N*-[10,11-dihydro-5-(2-methylamino-1-oxoethyl)-3-5*H*-dibenzo[b,f]azepinyl]-, ethyl ester	353.174	2.20	102821-92-1	185875
47	55.478	Sarcosine, *N*-(cyclopentylcarbonyl)-, decyl ester	325.262	11.75	1000321-34-1	165072
48	56.083	2-(Adamantan-1-yl)-*N*-(1-adamantan-1-ylethyl)-acetamide	355.288	2.90	1000311-05-4	187446

RT (*min*) retention time (min), *MW* (*amu*) molecular weight (atomic mass unit), % *total* percentage total of all compounds

Cholinergic toxicity occurs when there is accumulation of acetylcholine at synaptic cleft leading to overstimulation of postsynaptic neurons (Rodrigues et al. 2011). This is often due to excessive inhibition of AChE, resulting in accumulation of acetylcholine. If unchecked, cholinergic toxicity is capable of inducing severe neurological impairment, neurodegeneration, and even death of the organism (Nunes et al. 2003). Inhibition of brain AChE activity is often used as test for toxicity due to some environmental pollutants (Srivastava and Shivanandappa 2011). Therefore, in this study, the ability of alkaloid extracts from the leaf and fruit of Jimson weed to inhibit AChE activity could potentiate cholinergic toxicity and possibly neurodegeneration. Notably, the fruit extract showed a significantly higher AChE inhibitory effect compared to the leaf, but was less potent than that of previously reported standard AChE inhibitor prostigmine (IC_{50} = 0.046 μg/ml) (Oboh

Table 3 GC-MS characterization of the alkaloid extract from the fruit of Jimson weed

S/N	RT (min)	Compound name	MW (amu)	% total	CAS number	Entry number in NIST11 Library
1	29.0123	1-Dodecanamine	185.214	0.224	000124-22-1	49539.00
2	29.0954	*N*-(3-Methylbutyl)acetamide	129.115	0.588	013434-12-3	12958.00
3	29.309	4-Fluorohistamine	129.07	0.119	049872-60-8	12838.00
4	29.3624	1-Octanamine	129.152	0.298	000111-86-4	13025.00
5	29.4337	5-Methyl-2-hexanamine	115.136	0.199	028292-43-5	7889.00
6	29.7186	Spermine	202.216	0.081	000071-44-3	62363.00
7	30.7394	3-Methyl-1-butanamine	87.105	0.205	000107-85-7	1913.00
8	31.3508	Butanamide	87.068	1.202	000541-35-5	1866.00
9	31.422	1-Decanamine	157.183	0.525	002016-57-1	29174.00
10	31.7366	1-Aminomethyl-cyclododecanol	213.209	0.486	000832-29-1	71955.00
11	31.8553	Acetic acid, 2-(1-methyl-2-oxohydrazino)-, *N*′-[(*E*)-(2-hydroxyphenyl)methylidene]hydrazide, *N*-oxide	252.086	0.313	1000327-59-6	103357.00
12	31.974	Atropine	289.168	1.17	000051-55-8	135263.00
13	32.069	3-Methyl-3,5–(cyanoethyl)tetrahydro-4-thiopyranone	236.098	1.017	1000140-32-3	90373.00
14	32.1164	1-Heptadecanamine	255.293	0.745	004200-95-7	106613.00
15	32.1461	*o*-Veratramide	181.074	0.299	001521-39-7	46685.00
16	32.1758	Metaraminol	167.095	0.637	000054-49-9	36216.00
17	32.2351	5-Amino-pentanol	103.1	0.171	002508-29-4	4598.00
18	32.3954	(−)-Norephedrine	151.1	0.153	000492-41-1	24940.00
19	32.9118	Dihydro-5-hydroxy-2,4(1*H*,3*H*)-pyrimidinedione	130.038	0.306	001635-26-3	13168.00
20	33.1314	3-Chloro-*N*-methylpropylamine	107.05	0.184	065232-62-4	5134.00
21	33.256	(*Z*)-9-Octadecenamide	281.272	1.821	000301-02-0	128445.00
22	33.3629	Scopolamine	303.147	6.056	000051-34-3	147101.00
23	33.4994	1,3-Adamantanediacetamide	250.168	2.687	056432-73-6	102232.00
24	33.7961	*N*-(2-aminoethyl)-1,2-ethanediamine	103.111	0.844	000111-40-0	4529.00
25	33.9682	Atomoxetine	255.162	0.933	083015-26-3	106583
26	34.1166	*N*-Methyl-2-phenyl-1-propylamine	149.12	1.28	1000379-96-9	23375
27	34.2888	Phenylephrine	167.095	1.176	000059-42-7	36220
28	34.5321	Amphetamine	135.105	0.267	000300-62-9	15554
29	34.5558	1-Methyl-2-phenoxyethylamine	151.1	0.091	035205-54-0	24974
30	34.6983	2,2,2-Trichloro-acetamide	160.92	0.789	000594-65-0	31620
31	34.9238	3-Methoxyamphetamine	165.115	0.705	017862-85-0	34312
32	35.3334	*P*,alpha-dimethyl-phenethylamine	149.12	1.569	000064-11-9	23388
33	35.5708	Cathine	151.1	0.122	000492-39-7	24936
34	35.642	*N*-Methyl-benzeneethanamine	135.105	0.517	000589-08-2	15594
35	35.8676	1-(3,5-Dimethyl-1-adamantanoyl)semicarbazide	265.179	0.09	351327-47-4	114675
36	35.9031	2-Cyano-acetamide	84.032	0.804	000107-91-5	1335
37	36.1465	2-Butanamine, (*S*)-	73.089	0.241	000513-49-5	756
38	36.3542	*N*-(3,5-Dinitropyridine-2-yl)-L-aspartic acid	300.034	0.138	035899-60-6	145115
39	36.8291	2-Fluoro-beta,5-dihydroxy-*N*-methyl-benzeneethanamine	185.085	0.294	103439-07-2	49745
40	37.0071	*R*-(−)-Cyclohexylethylamine	127.136	0.217	005913-13-3	11895
41	37.2208	4-Methyl-2-pentanamine	101.12	0.106	000108-09-8	4161
42	37.3098	2-(Adamantan-1-yl)-1-methyl-ethylamine	193.183	0.353	1000310-59-0	55466
43	38.942	4-Methoxy-alpha-methyl-benzeneethanamine	165.115	0.074	000064-13-1	34373
44	39.4999	2-Octanamine	129.152	0.084	000693-16-3	13026
45	43.4766	(*S*)-(+)-1-Cyclohexylethylamine	127.136	0.251	017430-98-7	11910
46	43.7496	1,3-Dimethyl-8-[2-nitrophenethenyl]-purin-2,6-dione	327.29	0.589	1000128-96-1	166457
47	43.9752	2-(Adamantan-1-yl)-*N*-(1-adamantan-1-ylethyl)-acetamide	355.56	0.477	1000311-05-4	187446

Table 3 (continued)

S/N	RT (min)	Compound name	MW (amu)	% total	CAS number	Entry number in NIST11 Library
48	44.3135	4-Amino-*N*,*N*-dimethyl-furazan-3-carboxamide, oxime	171.076	0.608	129206-45-7	39157
49	44.4559	Benzaldehyde, 3,5-dichloro-2-hydroxy-, 2,2-dimethylhydrazone	233.0945	0.187	014490-83-6	87936
50	44.5509	Sarcosine, *N*-(cyclopentylcarbonyl)-, decyl ester	325.49	0.243	1000321-34-1	165072
51	44.6459	Carbamic acid, *N*-[10,11-dihydro-5-(2-methylamino-1-oxoethyl)-3-5*H*-dibenzo[b,f]azepinyl]-, ethyl ester	353.4149	0.453	102821-92-1	185875
52	45.002	2-Hydroxydesmethylimipramine	285.4	0.118	001977-15-7	129310
53	45.6074	2-Ethylacridine	207.27	3.255	055751-83-2	66996
54	45.8982	2-Acetylamino-3-(4-ethoxy-phenyl)-acrylic acid	249.262	0.958	325482-56-2	101272
55	46.652	Benzo[h]quinoline, 2,4-dimethyl-	207.27	1.011	000605-67-4	67018
56	48.8837	*N*-Methyl-1-adamantaneacetamide	207.3119	0.357	031897-93-5	66942

RT (*min*) retention time (min), *MW* (*amu*) molecular weight (atomic mass unit), % *total* percentage total of all compounds

et al. 2014). Previous studies on Jimson weed have shown that the fruit and especially the seed showed higher pharmacological and neuromodulatory effects than the leaf extracts (Soni et al. 2012). Actually, the most reported cases of *Datura* poisoning have been attributed to voluntary or accidental ingestion of the fruit or seed. Furthermore, previous studies have reported the anticholinesterase abilities of plant alkaloid extracts (Cometa et al. 2012). Therefore, the AChE inhibitory effect of Jimson weed observed in this study can be significantly attributed to its constituent alkaloids. Hence, consumption of Jimson weed leaf and fruit either as medicinal herb for its hallucinating effects or accidentally as food contaminants could possibly induce cholinergic toxicity as a result of inhibition of AChE activity.

We also observed, in this study that both leaf and fruit alkaloid extracts from Jimson weed inhibited BChE activity with the fruit showing significantly higher inhibitory effect, but was less potent than that of previously reported standard BChE inhibitor prostigmine (IC_{50} = 0.044 μg/ml) (Oboh et al. 2014). Although studies have shown that AChE is predominant over BChE in healthy human brain (Giacobini 2003), and while the precise function BChE still remains to be fully understood, it is believed to be involved in regulating cell proliferation and early stage of neuronal differentiation (Mack and Robitzki 2000). In addition, BChE can also hydrolyze acetylcholine in place of AChE but with less specificity (Çokuğraş 2003); therefore, its inhibition can also lead to impaired neuronal function and neurodegeneration. In addition, being another enzyme available to hydrolyze acetylcholine, inhibition of BChE by the extracts, especially in the situation of AChE inhibition could further potentiate cholinergic toxicity. Consequently, the inhibitory effect of Jimson weed alkaloid extracts on BChE activity could also contribute in part to their neuromodulatory properties.

The GC-MS characterization of the phytoconstituents in the alkaloid extracts revealed that in addition to the tropane alkaloids (atropine and scopolamine) which have been well characterized in *Datura* species, other psycho-active compounds such as amphetamine and its derivatives (3-methoxyamphetamine, 3-ethoxyamphetamine, and 3-methylamphetamine), cathine, spermine, 3-piperidinemethanol, and phenylephrine among others were also detected. These compounds which are more abundantly distributed in the fruit have been reported to produce varying degrees of neuromodulations. This is in agreement with earlier studies which have reported that alkaloids are more abundantly distributed in the fruit and especially the seed of Jimson weed compared to other parts (Miraldi et al. 2001; Soni et al. 2012). It is also believed that this inequality in distribution is responsible for the higher toxicity and neuromodulation experienced from ingestion of the fruit when compared to other parts of the plant. However, factors such as geographical location, time of harvest, and extraction methods have been reported to influence alkaloid contents of Jimson weed both quantitatively and qualitatively (Mairura and Setshogo 2008; Maheshwari et al. 2013). These factors might also be responsible for the uniqueness of the characterization in this study compared to previous studies. The tropane alkaloids (atropine and scopolamine) are central nervous system modulators, producing effects such as hallucination, depression, and memory impairment (Halpern 2004). Amphetamine and its derivatives have been reported to be MAO inhibitors and can greatly impair monoaminergic neurotransmission to elicit its psychostimulant-like effects (Funada et al. 2014; Santillo 2014). Cathine is identified in our Jimson weed extracts. And its more potent form cathinone has been reported for their neuromodulatory properties and is found present in plants such as khat (*Catha edulis*) leaves which are traditionally chewed in East African countries for their neurostimulating effects (Gashawa and Getachew 2015). Therefore, it is believed that these phytochemicals could also be responsible for the bioactivities observed in this study.

Conclusion

This study has revealed that the alkaloid extracts from the leaf and fruit of Jimson weed altered the activities of critical enzymes of the monoaminergic and cholinergic neurotransmission systems (in vitro). These modulatory effects could also be part of the mechanisms by which Jimson weed elicit its neuromodulatory effects as reported in folklore. However, the possible contribution of these findings to the physiological manifestations of Jimson weed-induced neuromodulation deserves to be investigated; hence, further in vivo studies are recommended.

Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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