NIPES

Journal of Science and Technology Research

Journal homepage: www.nipesjournals.org.ng

Phytoconstituents, Acute Toxicity and In-Vivo Anti-anxiety Activity of Chloroform and Ethylacetate Extracts of *Datura Stramonium* in Balb/C Mice

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Article Info

***Keywords*:**
Datura stramonium, phytoconstituents, toxicity, high performance liquid chromatography (HPLC), anti-anxiety activity.

Received 15 January 2023
Revised 28 January 2023
Accepted 28 January 2023
Available online 13 March 2023

https://doi.org/10.5281/zenodo.7729298

Abstract

Datura stramonium, family, Solanaceae, was investigated for its toxicity, phytochemical constituents and anti-anxiety activity in Balb/C mice.The plant leaves were extracted with a moderately polar solvents, chloroform and ethyl acetate respectively in a soxhlet extractor apparatus for eight hourly period and concentrated using a rotary evaporator (Model RE, 200, USA). Phytochemical constituents were identified according to standard methods and high performance liquid chromatographic (HPLC) technique was used to further characterize both extracts. The anti-anxiety activity was conducted by the hole board method to assess the level of anxiety in the mice. Saponins, phenolics, alkaloids, phytosterols, terpenoids, triterpenes, eugenols, and diterpenes were present in both extracts The results of the HPLC analysis showed that the ethyl acetate fraction contains the highest concentration of phytoconstituents for caffeic acid ((Retention time (RT), 5.466 min; peak 4), catechin (RT, 6.483 min; peak 5), scopolamine (RT, 11.050 min; peak 9), quercetin (RT, 12.166 min; peak 10) and atropine (RT, 13.700 min; peak 11),) than chloroform extract: caffeic acid (RT, 5.466 min; peak 1), catechin(RT, 6.466min; peak 5), scopolamine (RT, 11.050 min; peak 10), quercetin (RT, 12.166min; peak 11),) and atropine(RT, 13.700 min; peak12)). Group D of both extract which receive the 400 mg/kg dose showed a positive anti-anxiety activity (in a dose dependent manner) when compared with the standard control, diazepam. The study revealed that D. stramonium possess anti-anxiety activity in Balb/c mice.

1.0 Introduction

Datura stramonium, family *Solanaceae*, is a narcotic medicinal plant widely distributed in Edo and Delta State regions of Nigeria. It's locally called "apikan" or "apaka" in Yoruba, "zakami" in Hausa and "myaramuo" in Igbo [1]. Almost every part of the plants is narcotic, highly intoxicant, toxic, analgesic, aphrodisiac and antispasmodic [2]. In Delta state of Nigena, the leaves are burnt and the emerging smoke is inhaled for the relief of asthma and cough. The poultice of *D. Stramonium* or metel leaves is used as a very good remedy for rheumatic swellings of the joints, burns, in-growing toe nails, neuralgic pains, inflamed breasts as an anodyne and for controlling excessive flow of breast milk. The decoction of the leaves, fruits and flowers is a powerful intoxicant, sedative and generally prescribed by herbalists for mental illness, insanity and insomnia [1]. A drink made from

the seeds of *D. stramonium* is given as intoxicant to Fulani youths in northern Nigeria to incite them into "sharo contest" a festival defining attainment of manhood [3] while others drink it to carry out rigorous farm work, without feeling of pain or tiredness [4]. *Datura stramonium* is an, erect, branched, slightly hairy shrub measuring 0.5 to 2 meters high. The leaves are single, ovate to oblong-ovate measuring 9 to 18 centimeters long, with inequilateral base, pointed tip and irregularly and shallowly lobed marginswhile the flowers are white or nearly purple, axillary and solitary, with a large ovary. Meanwhile, the calyx is green, about 5-8 cm long, cleft at the apex, cylindric, and divided into linear teeth but seeds are numerous, finely pitted, closely packed, nearly smooth, and pale brown [5]. The plant is widely distributed in Africa, Asia, parts of Europe, India, and other tropical and subtropical regions of the world and it is widely cultivated for ornamental purposes [5]. The plant has been reported to contain hosts of phytochemicals such as flavonoids, terpenoids, alkaoids, phenolics and glycosides among others. The leaves, flower and seeds have also been reported to contain alkaloids, such as atropine, scopolamine, and hyoscyamine [6]. Consequently, due to its alkaloid content, this plant was primarily used as an intoxicant and hallucinogen [7] and [8]. In most developing countries of the world, the quest for a good life in search of food, shelter, security, money has mounted pressure to human health with anxiety challenges leading to high blood pressure, insomnia and restlessness among others. Presently, anxiety disorders are the most common psychiatric problems includng drug abuse [9] and it is characterized by a diffuse, unpleasant, vague sense of apprehension occurring in response to physiological and /or environmental factors. Anxiety is accompanied by muscular tension, restlessness, fatigue, inability to catch one's breath, tightness in the abdominal region, nausea, and problems in concentration and these degenerate the wellbeing of man. Synthetic organic chemists has adopted by design benzodiazepines (BZDs) [10] as major classes of chemical compounds used to treat anxiety and brings about calmness to the human body. However, these drugs are not without severe side effects [11]. The ethno medicinal importance of this plant and many other medicinal plants cannot be overstressed. Thus, the present study is aimed at determining the phyto-constituents of moderately polar extracts by high performance liquid chromatography (HPLC) and tests both extracts for *in-vivo* antianxiety activity in Balb/C mice.

Plate 1: *Datura stramonium*

2. Materials and Methods

2.1. Reagents

The reagents and solvents used for the extraction and HPLC profiling were Merck and HPLC grade respectively.

2.2 Collection of Plant Samples

Fresh leaves of *Datura stramonium* were collected from their natural habitat in Ovia North East Local Government Area of Edo State and were identified in the Department of plant Biology and Biotechnology, University of Benin with herbarium voucher number, UBHm 0258. The leaves were air dried at room temperature in the laboratory for twenty-eight days and pulverized into fine powder in preparation for extraction.

2.3 Extraction

Three hundred and ten grammes (310g) of powdered leaf samples were extracted with chloroform in a soxhlet apparatus for eight hours. The extract was collected dried over sodium sulphate (Na_2SO_4) and concentrated using a rotary evaporator (model, RE, 200) at 50°C to obtain a crude chloroform extract. The remnant of the powdered leaf was re-extracted following same procedure using ethyl acetate as solvent in a bid to obtain moderately polar phyto constituents of the plant leaves.

2.4 Phytochemical screening of hexane extract of *Datura metel*

The phytochemical screening of the chloroform and ethyl acetate extract of Datura metel were performed using standard procedures prescribed by Sofowora, [12]; Trease and Evans [13] and Rajasudha and Manikandan [14].

2.4.1. Test for glycosides (Modified Borntrager's Test)

1 mL of both extract were treated were dissolved in 1 mL of ferric chloride solution and immersed in boiling water for 5 minutes. Both mixtures were cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.

2.4.2. Test for saponins (Froth Test)

1 mL of both extracts were diluted with distilled water to 20 mL respectively and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

2.4.3. Test for flavonoid (Lead acetate Test)

2 mL of both extracts were treated with few drops of lead acetate solution respectively. Formation of yellow colour precipitate indicates the presence of flavonoids**.**

2.4.4. Test for phenolic compounds (Ferric Chloride Test)

1 mL of the chloroform and ethyl acetate extract were treated with 3-4 drops of ferric chloride solution. Formation of a bluish black colour indicates the presence of phenols.

2.4.5. Test for tannins (Gelatin Test)

To 2 mL of both plant extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

2.4.6. Test for Eugenols

2 mL of both plant extract were mixed with 5% KOH solution. The aqueous layers were separated and filtered. Few drops of dilute HCl were added to the filtrate. A pale yellow precipitate is indicative of a positive test

2.4.7. Test for phytosterols. (Libermann Burchard's test)

0.5 g of both plant extract were treated with 2mL chloroform and filtered. The filtrates were respectively treated with 2 mL of acetic anhydride, boiled and cooled. 2 mL Conc. Sulphuric acid was added to both solution. Formation of a brown ring at the junction indicates the presence of phytosterols.

2.4.8. Test for terpenoids (Salkowski test)
5 mL of both plant extract were respectively mixed in 2 mL of chloroform and 3mls of conc. H_2SO_4 was carefully added down the side of the inner wall of the test tubes to form a layer. A reddish brown colouration of the inter-phase is required for the presence of terpenoids.

2.4.9. Test for Triterpenes (Salkowski's Test)
5 mL of both plant extract were respectively mixed in 2 mL of chloroform and filtered. The filtrates were treated with 3mL of Conc. Sulphuric acid, shaken and allowed to stand. The appearance of golden yellow colour indicates the presence of triterpenes.

2.4.10. Test of Diterpenes (Copper acetate Test)
5 mL of both plant extract were respectively dissolved in 5 mL of distilled water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

2.4.11. Test for alkaloids (Hager's Test: Hager's reagent (Saturated picric acid solution)
2 mL of Picric acid was added to both plant extract. A yellowish precipitate test is a positive test.

2.5 HPLC Profile of chloroform and ethyl acetate extracts of *Datura metel*
2.5.1. Preparation of standard
1.2 mg of standard (Quercetin) was taken in 0.1% phosphoric acid in water (HPLC grade). From which 20µL were injected in HPLC system for making standard curve.

2.5.2. Preparation of sample
Ten grammes (10g) of both extract were extracted with acetonitrile and the extract stabilized with ethyl acetate in 25 mL standard flask and made up to the mark.

2.5.3. Procedure:
The two extracts (chloroform and ethyl acetate) and flavonoid standard were subjected to High-Performance Liquid Chromatography using 600 series HPLC pump and 2487 dual wavelength UV detector-254 and 360 nm of bioazyemes, Bangalore having Reprobond C_{18} column-4.6x250mm and 7725 Rheodyne injectors. The HPLC instrument was operated at room temperature (23 ± 2°C). Ten grammes each (10 g) of the extract samples were respectively extracted with acetonitrile, and the extracts stabilized with ethyl acetate in 25 mL standard flask. Five micro liters (5 µL) of both extracts were respectively injected in to the HPLC at a flow rate of 2.0 mL/min and the peak area were reported and used for quantification. The compounds eluted with two solvents such as acetonitrile and 0.1% phosphoric acid in water were used for the detection of coumaric acid and beta-caryophyllene as external standards. The total run time of the program was 20 minutes.

2.6 Sourcing of animals

Fifty (50) Swiss Balb/C albino mice were purchased from the Pharmacology animal house, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The animals were kept in clean cages and allowed to acclimatize for two weeks before experiment. They were maintained on standard animal

pellets and water ad libitum with approval for animal studies obtained from the Institutional Ethical Review Committee of the Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

2.7 Acute toxicity

The Balb/C mice were divided into 5 groups (1 control group (distilled water) and 4 test groups of extracts) with 3 mice per a group. Overnight fasted animals were kept in individual cages and were administered graded dose of chloroform and ethyl acetate extract of *D, stramonium*. The mice were observed for a period of 2 hours and after which at regular intervals for 24 hours for signs of acute toxicity of any kind like stupor, raised tail, salivation, convulsion, writhing and death. The acute toxicity test was conducted by new approach to Acute Toxicity Testing according to [15]. The median lethal dose (LD_{50}) value was estimated by the application of the equation below:

$LD_{50} = \sqrt{\{LD_0 \times LD_{100}\}}$

Where: LD_0 = Maximum dose without death;

LD_{100} = minimum dose with death

. Those that survived were used for the anti-anxiety experiment

2.8 Anti-anxiety Activity of *Datura stramonium* (Hole-board test model)

The *hole-board* apparatus consists of an enclosed arena with holes in the floor into which an animal can poke its head, referred to as *head-dipping* [16]. The frequency and duration of head-dipping are assumed to provide measures of neophilia as well as less anxiety. After weighing of the animal, acclimatization and the mice adaptation to its new environment, the animals were shared into 5 groups of five mice randomly and 5 mL of distilled water was administered to group I (control) while groups II, III, IV received 100, 200, 400 mg/kg of both extracts orally respectively. Group V received the standard drug, Diazepam at 2 mg/kg subcutaneously. Five (5) minutes after giving each extract, drug and control, the animals were placed on the hole board setup, and each head poke was recorded. The purpose of this head poke is to ascertain anti-anxiety effects of the extracts on the mice after administered orally. Some of the mice at first showed signs of fear when newly placed on the hole board apparatus and refused to move, the mice eventually began to move and frantically poked its head into the holes, at which point records were taken of the number of each poke into the hole. All this was done during five minutes duration for each mouse.

3. Results and Discussion

3.1 Phytochemical constituents

The phytochemical constituents detected in the chloroform and ethylacetate extract of *Datura stramonium* is shown in Table 1.

Table 1: Phytochemical constituents in chloroform and ethyl acetate extract of *Datura stramonium*

S/N	Phytochemical constituents	Chloroform extract (*D. stramonium*)	Ethyl acetate extract (*D. stramonium*)
1	Glycoside	-	+
2	Saponin	+	+
3	Flavonoid	+	+
4	Phenolics	+	+
5	Tannin	-	-
6	Eugenol	+	+
7	Phytosterols	+	+
8	Terpenoids	+	+

9	Triterpenes	+	+
10	Diterpenes	+	+
11	Alkaloids	+	+

Key: **-** = absent , + = present

Phyto constituents (Table 1) present in both extracts were saponins, phenolics, alkaloids, phytosterols, terpenoids, triterpenes, eugenols, and diterpenes while tannins were absent. This result obtained was similar to the findings of Ali and Endalew [17] who used chloroform solvents in their extraction process as well. However, from the work of Kankia [18]) who also adopted ethyl acetate for extraction, it was only alkaloids, saponins and tannins that were present in the extract. This may be due to the maceration method of extraction adopted which is different from the hot extraction method of soxhlet apparatus used in this research. The role of these phytochemicals for medicinal and pharmacological uses cannot be overemphasized. Flavonoid and saponins were also present in the ethyl acetate extract of *D. stramonium* [18].

3.2 Quantification of chemical constituents by HPLC

The HPLC chromatogram of the chloroform and ethyl acetate extract of *Datura stramonium* are indicated in Figures 1 and 2, while the chemical constituents are shown in Table 2 and 3 respectively.

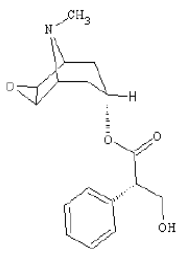
Table 2: HPLC profile of chloroform extract of *D. stramonium*

Peak No.	Retention time (Rt) (mins)	Name of compound	Peak area
1	1.266	Chlorogenic acid	650.85
2	2.516	Beta-caryophyllene	1516.48
3	4.450	Coumaric acid	403.01
4	5.466	Caffeic acid	166.75
5	6.466	Catechin	22.32
6	7.200	Epicatechin	21.23
7	7.216	Epicatechin	21.61
8	7.983	Metaloidin	24.68
9	9.316	Sinapic acid	30.95
10	11.050	Scopolamine	8221.26
11	12.166	Quercetin	57.054
12	13.700	Atropine	2017.79
13	14.916	Apigenin	177.92
14	17.616	Ferulic acid	201.66
15	19.416	Linalool	52.19
16	19.683	Limonene	22.97
17	20.500	Luteolin	21.41

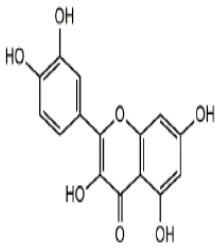
Table 3: HPLC profile of ethyl acetate extract of *D. stramonium*

Peak No.	Retention time (Rt) (mins)	Name of compound	Peak area
1	1.266	Chlorogenic acid	1037.48
2	2.516	Beta-caryophyllene	2358.57
3	4.450	Coumaric acid	605.10

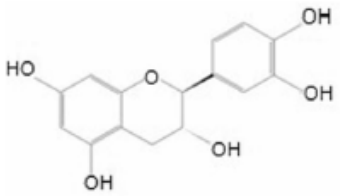
4	5.466	Caffeic acid	222.17
5	6.483	Catechin	128.91
6	7.333	Cinnamic acid	32.98
7	7.950	Metalodin	30.67
8	9.416	Sinapic acid	32.05
9	11.050	Scopolamine	9249.26
10	12.166	Quercetin	64.81
11	13.700	Atropine	2546.41
12	14.916	Apigenin	552.48
13	17.616	Ferulic acid	380.98
14	19.900	Sitosterol	40.89
15	19.416	Linalool	42.84
16	19.683	Limonene	66.95
17	20.500	Luteolin	34.55



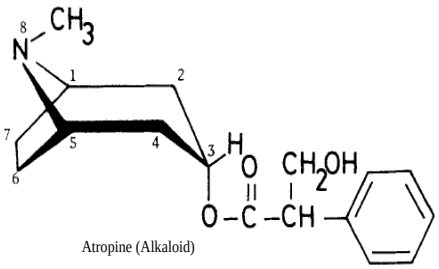
Scopolamine (Alkaloid)



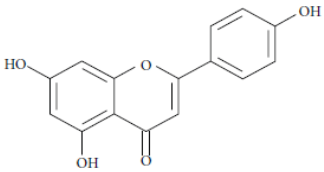
Quercetin (Flavonol)



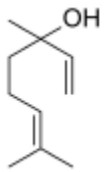
Catechin (Proanthocyanidins)



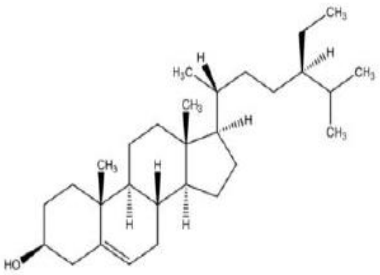
Atropine (Alkaloid)



Apigenin (Flavonoid)



Linalool (Terpenoid)



beta-Sitosterol (Sterol, triterpenoids)

Figure 3: Some of the chemical compounds detected from the chloroform and ethyl acetate extracts of D. *monostrium*

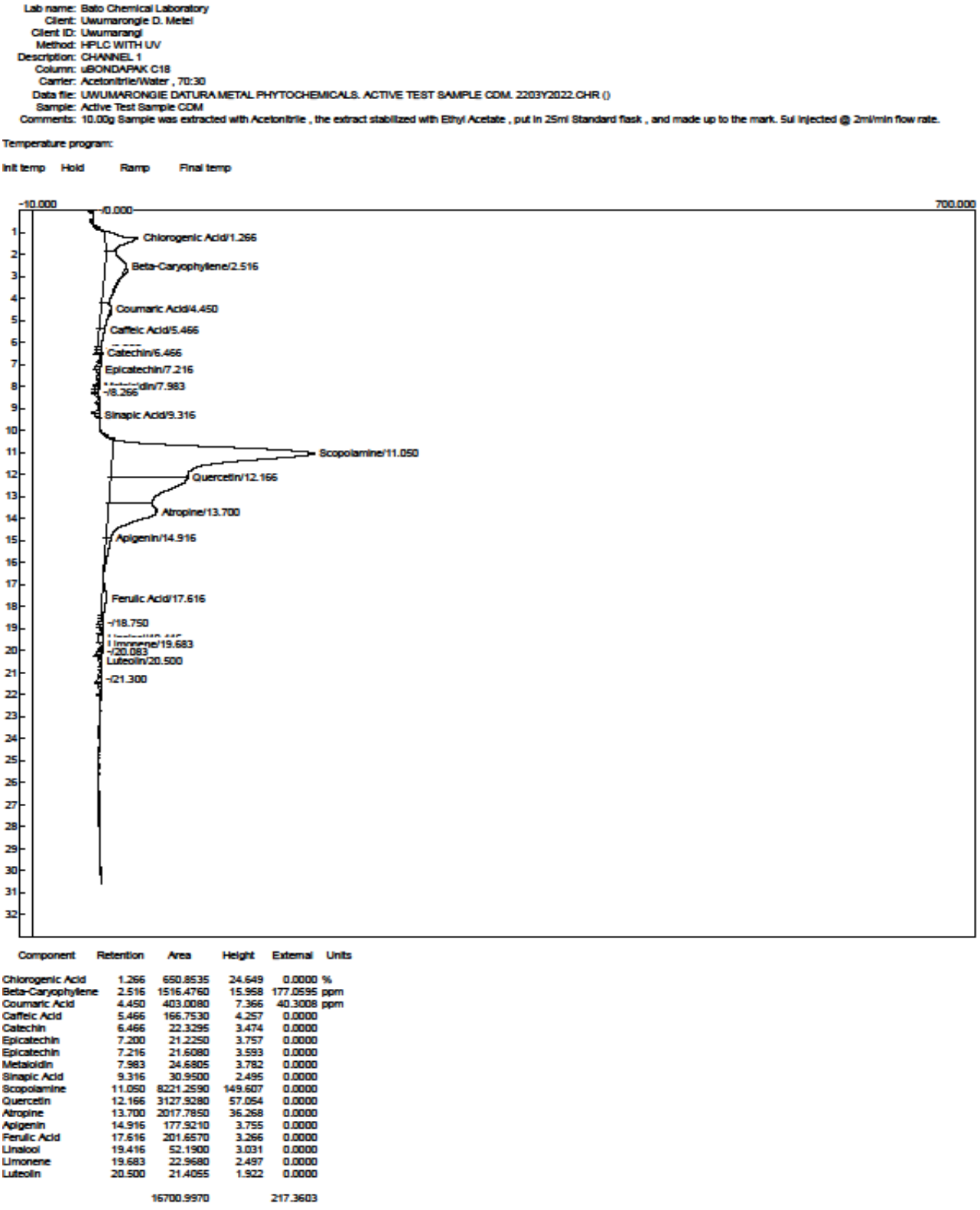
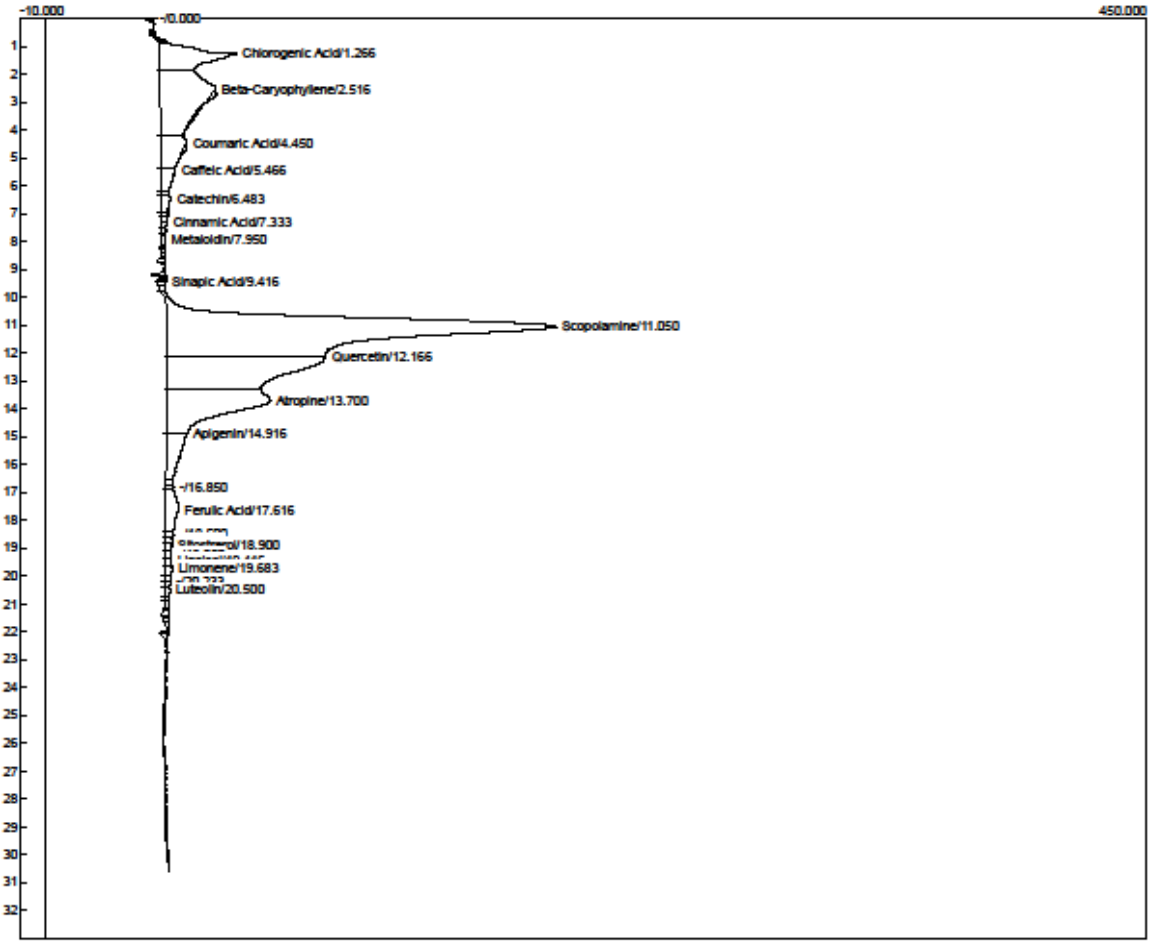


Figure 1: High performance liquid chromatography profile of chloroform extract of *D. stramonium*

Lab name: Bato Chemical Laboratory
Client: Uwumarongie D. Metel
Client ID: Uwumarongi
Method: HPLC WITH UV
Description: CHANNEL 1
Column: uBONDAPAK C18
Carrier: Acetonitrile/Water , 70:30
Data file: UWUMARONGIE DATURA METAL PHYTOCHEMICALS. ACTIVE TEST SAMPLE EADM . 2203Y2022.CHR ()
Sample: Active Test Sample EADM
Comments: 10.00g Sample was extracted with Acetonitrile , the extract stabilized with Ethyl Acetate , put in 25ml Standard flask , and made up to the mark. 5ul injected @ 2ml/min flow rate.

Temperature program:

Init temp	Hold	Ramp	Final temp



Component	Retention	Area	Height	External	Units
Chlorogenic Acid	1.266	1037.4810	31.319	0.0000	%
Beta-Caryophyllene	2.516	2358.5760	22.714	282.3220	ppm
Coumaric Acid	4.450	605.1000	10.824	63.1375	ppm
Caffeic Acid	5.466	222.1690	5.980	0.0000	
Catechin	6.483	128.9075	4.214	0.0000	
Cinnamic Acid	7.333	32.9790	2.361	0.0000	
Metaloidin	7.950	30.6745	1.700	0.0000	
Sinapic Acid	9.416	32.0500	4.223	0.0000	
Scopolamine	11.050	9249.2560	159.115	0.0000	
Quercetin	12.166	3634.6195	64.806	0.0000	
Atropine	13.700	2546.4125	42.277	0.0000	
Apigenin	14.916	552.4770	8.382	0.0000	
Ferulic Acid	17.616	380.9820	5.293	0.0000	
Sitostrerol	18.900	40.8910	2.861	0.0000	
Linalool	19.416	42.8440	2.791	0.0000	
Limonene	19.683	66.9520	3.082	0.0000	
Luteolin	20.500	34.5465	2.291	0.0000	
		20996.9175		345.4595	

Figure 2: High performance liquid chromatography profile of ethyl acetate extract of *D. stramonium*

The results of the HPLC profile of the chloroform and ethyl acetate extract of *D. stramonium* respectively revealed their highest peaks for the presence of scopolamine (Figure 1 and 2).

Chlorogenic acid, caffeic acid, catechin, sinapic acid, scopolamine, quercetin, atropine were detected in both extract at varying percentage. The results of the HPLC analysis showed that the ethyl acetate fraction contains the highest concentration of phytoconstituents for caffeic acid ((Retention time (Rt), 5.466 min; peak 4), catechin(Rt, 6.483 min; peak 5), scopolamine (Rt, 11.050 min; peak 9), quercetin (Rt, 12.166 min; peak 10) and atropine (Rt, 13.700 min; peak 11),) than chloroform extract: caffeic acid (Rt, 5.466 min; peak 1), catechin(Rt, 6.466min; peak 5), scopolamine (Rt, 11.050 min; peak 10), quercetin (Rt, 12.166min; peak 11),) and atropine(Rt, 13.700 min; peak12)). More so, sitosterol (Rt, 19.900 min; peak 14), a phyto sterol detected in the ethyl acetate extract was absent in chloroform extract. Beta sitosterol are widely found in most medicinal plants, vegetables, tomatoes, seeds, nuts and wheats. They function as antioxidants, analgesic and anti-inflammatory chemical agents [19]. The results further revealed scopolamine as the most dominant flavonoid in both extract of *D. stramonium*

Scopolamine is a tropane alkaloid and the constituent responsible for the toxicity and poison in *Datura stramonium* are tropane alkaloids, thus explaining its abundance. It was formally used as a medication for treating motion sickness and postoperative nausea and vomiting [20]. It is also reported that cocaine and hyoscyamine/scopolamine are able to pass the blood-brain barrier and commit dose-dependent hallucination and psychoactive effects and in Russia and China, *Datura* extracts was added to enhance the thrilling effect of beer [21]. The presence of atropine has been linked to the intoxicating effect of *Datura* species. In fact, Atropine was derived from the Greek name, "Atropa" after the Greek goddess of fate and the goddess of the kingdom of the dead, Atropos. Quercetin has been described as versatile molecule with many pharmacological potentials including antioxidant, neurological, antiviral, anticancer, cardiovascular, antimicrobial, anti-inflammatory, analgesic and sedative effects [22]. Benabderrahim *et al.* [23] also reported the presence of catechin and epicatechin, antioxidant agents, in *D. innoxia* in their research while in this research our identification of both constituents was on *D. stramonium.* Apigenin and caffeic acid have also been reported by Rahmoune *et al.* [24] to be present in *D. stramonium* and *D. innoxia*. Apigenin is classified as phenypropanoid, a secondary metabolite involved in several plant functions like fertility, pigmentation, woodiness and protection against biotic and abiotic agents.Among other chemical constituents identified were quercetin, luteolin and sinapic acid [24].

3.3 Acute toxicity study

The oral administration of crude extracts of *D. stramonium* (chloroform and ethyl acetate extracts) at graded doses of 1000, 1600, 2900 and 5000 mg/kg body weight showed no indication of acute toxicity (Tables 5 and 6) except the highest dose of chloroform extract at 5000 mg/kg after 24 hours . There were no effect, toxic signs/symptoms and mortality even after 72 hours (3 days) of treatment and cautious observation for other groups of chloroform extract and all dose groups of ethyl acetate extract.. More so, there were no variation in behavioural pattern or physiological responses like depression, shallow breathing, raised tails, salivation, paw licking and restlessness.

Table 4: Oral acute toxicity results of chloroform extract of *D. stramonium* in Balb/C mice

Group	Doses (mg/kg)	Number of lethality	Percentage mortality
Control	DW (5mL/kg)	0/3	0
Chloroform	1000	0/3	0
Chloroform	1600	0/3	0
Chloroform	2900	0/3	0
Chloroform	5000	1/3	33

DW = Distilled water

$LD_{50} \leq 1$ mg/kg (Extremely toxic); 1 mg/kg $\leq LD_{50} \leq 50$ mg/kg (Highly toxic);

50 mg/kg ≤ LD_{50} ≤ 500 mg/kg (Moderately toxic);
500 mg/kg ≤ LD_{50} ≤ 5000 mg/kg (Slightly toxic)
5000 mg/kg ≤ LD_{50} ≤ 15000 mg/kg (Non-toxic or harmless). (Hodge and Sterner scale in [25]

Table 5: Oral acute toxicity results of ethyl acetate extract of *D. stramonium* in Balb/C mice

Group	Doses (mg/kg)	Number of lethality	Percentage mortality
Control	DW (5mL/kg)	0/3	0
Chloroform	1000	0/3	0
Chloroform	1600	0/3	0
Chloroform	2900	0/3	0
Chloroform	5000	0/3	0

DW = Distilled water
LD_{50} ≤ 1 mg/kg (Extremely toxic); 1 mg/kg ≤ LD_{50} ≤ 50 mg/kg (Highly toxic);
50 mg/kg ≤ LD_{50} ≤ 500 mg/kg (Moderately toxic);
500 mg/kg ≤ LD_{50} ≤ 5000 mg/kg (Slightly toxic)
5000 mg/kg ≤ LD_{50} ≤ 15000 mg/kg (Non-toxic or harmless). (Hodge and Sterner scale in [25].
From the Hodge and Sterner scale, both extract used in this study can be considered as relatively non-toxic even with the highest dose except the chloroform extract which gave 33% mortality after the 24 hours for the acute toxicity test.

3.4 Anti-anxiety activity

The number of head dipping of Balb/c mice for the anti-anxiety studies is shown in Tables 6 and 7.

Table 6: anti-anxiety activity of chloroform extract of *D. stramonium* in Balb/c mice

Time (minutes)	**Group A**	**Group B**	**Group C**	**Group D**	**Group E**
	Control (5mL)	**100 mg/kg**	**200 mg/kg**	**400 mg/kg**	**2 mg/kg**
	Number of head dipping				
1	19	34	43	33	35
2	24	31	46	35	39
3	20	37	44	37	40
4	23	38	47	40	44
5	25	25	32	41	49

Table 7: anti-anxiety activity of ethyl acetate extract of *D. stramonium* in Balb/c mice

Time (minutes)	**Group A**	**Group B**	**Group C**	**Group D**	**Group E**
	Control (5 mL)	**100 mg/kg**	**200 mg/kg**	**400 mg/kg**	**2 mg/kg**
	Number of head dipping				
1	18	26	42	33	35
2	22	30	47	35	35
3	21	31	46	37	38
4	25	38	49	41	44
5	25	35	31	60	49

The results chloroform extract (Table 6) above explain the increasing boldness, the mice experienced in head-dipping as the extract was given and this increased across the group. From the control group, the mice showed anxiety in dipping his head into the hole thus maintaining a decreasing dips or fewer head dips during the five minutes duration. Only Group D (400 mg/kg) dose showed a dose dependent anti-anxiety activity for chloroform extract when compared down the group with the standard drug, diazepam, where there were less anxiety, due to increase in the head dips. Similarly, all the three doses of the ethyl acetate extracts maintain steady increases of the head dips with dose dependent activity for group D (400mg/kg) when compared with the diazepam group (positive control). This experiment indicated a positive anti-anxiety activity for all the extract groups (B, C and D) for both extract since the frequency and duration of head-dipping are assumed to provide measures of neophilia as well as less anxiety [3].

4. Conclusion

The study indicated that both moderately polar extract of *D. stramonium* are non-toxic and contain phyto constituents with medicinal potentials and this was supported by the anti-anxiety findings.

Conflict of Interest

The authors declare no conflict of interest in this work.

5. References

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