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High-Performance Liquid Chromatography (HPLC) Profile and Anxiolytic Activity of Hexane Extract of *Datura Metel* Leaves Extract in Balb/c Mice

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Abstract

Datura metel. family- Solanaceae, is a narcotic medicinal plant with various physiological effects. In this study, the anxiolytic activity in Balb/c mice and high performance liquid chromatography (HPLC) profile of the hexane extract of the leaves were investigated. The plant leaves were extracted in with hexane solvent in a soxhlet extractor apparatus for eight hourly period and concentrated using a rotary evaporator (Model RE, 200, USA). Phytochemical screening were done according to standard methods, flavonoid profile was assessed using HPLC analysis while anxiolytic activity conducted by the hole board method. Steroids, flavonoids, terpenoids were present in the extract except tannins. Scopolamine was detected as the highest flavonoid constituents in the extract. Group C which received the 200 mg/kg dose showed a positive anxiolytic activity (more head dippings) when compared with the standard control, diazepam. However, high anxiolytic activities were observed for groups B (100 mg/kg) and D (400 mg/kg) though not dose dependent while group A (negative control, distilled water) showed anxiogenic activity of increase in anxiety. The study revealed that D. Metel possess anxiolytic activity in Balb/c mice.

1.0 Introduction

Since time immemorial, plants have played a huge role in humanity. Plants doesn't just stop at being the main source of our food, they transcend to being a huge source of our medicine and raw materials for other human purposes diseases [1]. Medicinal plants are a gift to mankind from nature as they provide a lot of health benefits. In Africa and Asia, medicinal plants have been used for thousands of years down as cure for many illnesses and diseases. Nigeria is one of the countries in the world with unique wealth of medicinal and vast knowledge of use of herbal medicine for treatment of various [2]. *Datura metel*, family: *Solanacea*, is a shrub which comprises of various species of poisonous, vespertine-flowering plants. It grows to roughly 1.5-1.8 meters in height and largely distributed in warmer regions of the world. Its flowers are shaped like trumpets and commonly called Devil's trumpet, Angel's trumpet, Indian thornapple and Hindu datura. The flowers are white or nearly purple, axillary and solitary, with a large ovary while calyx is green, measuring from 5 to 8 cm long and the cleft at the apex, cylindric, and divided into linear teeth [3]. The leaves have bitter taste with anesthetic, anti-asthmatic, anti-spasmodic, anti-tussive, hallucinogenic, hypnotic properties. More so, the plant seeds and fresh leaves have abortifacient. Narcotic, anodyne and antispasmodic properties [3]. Datura metel leaves has long been used as an intoxicating and narcotic agent especially during some African traditional ceremonies. In Nigeria, rural people used the smoked leaves against insomnia and anxiety. In Ayurvedic medicine, *Datura metel* seeds are used to treat skin rashes, hand and leg ulcers, bronchitis, jaundice and high blood sugarwhile in Brazil,

the leaves are used for making tea for its sedative effect. While the flowers are dried and smoked as cigarettes [3]. In south-east Asia, the seeds are usually mixed with food or other medicinal plant to create aphrodisiacs. While the Malaysian people collect fifty seeds and prepare them as tincture for psychotropic dosage [4]. Reported pharmacological studies have revealed that *Datura metel* possess sedative effect [5], anaesthetic activity in dogs [6], spasmogenic activity [7], anti-depressant activity [8], anticancer potency [9] and hallucinogenic effect [10]. It is also used in preparing anxiolytic drugs for the treatment of anxiety [3]. *Datura metel* is a plant with an abundance of phytochemicals like tropane, alkaloids responsible for the toxicity of the plant and steroids; for its hallucinating effects. Other constituents such as scopolamine and atropine alkaloids are also reported [3]. Anxiety has many definitions and all points to the same thing which is an unwholesome and unpleasant state of inner disorder and an uneasy dread of anticipated events. It is a feeling of uneasiness and worry, usually generalized and unfocused as an overreaction to a situation that is only subjectively seen as menacing. It is often accompanied by muscular tension, restlessness, fatigue, inability to catch one's breath, tightness in the abdominal region, nausea, and problems in concentration. Anxiety is closely related to fear, which is a response to a real or perceived immediate threat (fight or flight response) [11]. Synthetic drugs like benzodiazepines (BZDs) [12] are the major classes of compounds used to treat anxiety and they remained the most commonly prescribed treatment for anxiety [13]. However, these drugs possess significant potential side effect [14] and this lends urgency for the development of new anxiolytic drugs or drug precursor from medicinal plants with less side effects Thus, the study is aimed to evaluate the anxiolytic activity by hole board test and characterize by high performance liquid chromatography the hexane extract of *Datura metel* leaves.

2.0 Materials and Methods

2.1 Collection of Plant Samples

Fresh leaves of *Datura metel* 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.

2.2 Extraction

Two hundred and sixty-seven grammes (267g) of powdered leaf samplewere extracted with hexane in a soxhlet apparatus for eight hours. The extract was dried over sodium sulphate (Na_2SO_4) and concentrated using a rotary evaporator (model, RE, 200) at 50°C to obtain a crude solid mass (79.3g ; 29.7%)

2.3 Phytochemical screening of hexane extract of *Datura metel*

The phytochemical screening of the Datura metel plant extract was performed using standard procedures prescribed by Sofowora, [15]; and Trease and Evans [16]

2.3.1. Test for glycosides

1 mL of the plant extract was dissolved in 1 mL of glacial acetic acid containing one drop of ferric chloride solution. This was under-layered with 1ml of conc. H_2SO_4. A brown ring is required for the presence of glycoside.

2.3.2. Test for saponins

0.5 g of plant extract was shaken with water in a test-tube and observed for frothing. Saponin rein Weiss (supplied by Merck) was used as a standard.

2.3.3. Test for flavonoid

2 mL of the plant extract was boiled with distilled water and filtered. 5 mL of 20% NaOH and few drops of dilute HCl were added to the solution. Formation of a colourless solution is indicative of a positive test.

2.3.4. Test for phenolic compounds

1 mL of the plant extract was added to 5 mL of 90% ethanol. In addition, 1 drop of 10% $FeCl_3$ was added. A pale yellow colouration of indicative of positive test.

2.3.5. Test for tannins

To 2 mL of the plant extract, 10 mL of distilled water was added and boiled for 5 minutes and then filtered into halves. To about 2 drops of the filtrate, ferric ($FeCl_3$) solution was added; formation of a bluish precipitate is required for hydrolysable tannin.

2.3.6. Test for Eugenols

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

2.3.7. Test for steroids

2 mL of acetic anhydride was added to 0.5 g plant extract in 2 mL of dilute H_2SO_4. A colour change from violet to blue or green is required for the presence of steroids.

2.3.8. Test for terpenoids (Salkowski test)

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

2.3.9. Test for alkaloids

2 mL of Picric acid was added to the plant extract. A yellowish precipitate test is a positive test.

2.4 HPLC Profile of hexane extracts of *Datura metel*

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.

Preparation of sample

Ten grammes (10g) of the hexane extract was extracted with acetonitrile and the extract stabilized with ethyl acetate in 25 mL standard flask and made up to the mark.

Procedure:

The hexane extract 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). The diluted extract sample (20 µL) was injected in to the HPLC at a flow rate of 2.0 mL/min and the peak area was reported and used for quantification. The compounds were eluted with two solvents such as acetonitrile and 0.1% phosphoric acid in water was used for the detection of quercetin as standard (Mradu *et al,* 2012). The total run time of the program was 30 minutes.

2.5 Sourcing of animals

Twenty-five (25) 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.

2.6 Acute toxicity Test

The acute toxicity was conducted with the hexane extract of *D. metel* according to guidelines prescribed by the Organisation for Economic Cooperation and Development [17] A group of Balb/c mice (n = 6) were injected with extract orally at a dose of 500, 1000 and 2000 mg/kg. The doses were increased as the mice survived at the smaller doses. Distilled water was used as a control and the mice were observed carefully during 24 hour for any effect, toxic signs/symptoms and mortality. Those that survived were used for the anxiolytic experiment

2.7. Anxiolytic Analysis of *Datura Metel* (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* [18]. The frequency and duration of head-dipping are assumed to provide measures of neophilia as well as anxiolysis. After weighing of the animal, acclimatization and the mice adaptation to its new environment, the animal was shared into 5 groups of five mice randomly and 0.2 mL of distilled water was administered to group I (control) while groups II, III, IV received 100, 200, 400 mg/kg of the extract orally. Group V received the standard drug, Diazepam at 2 mg/kg subcutaneously. 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 (anxiolytic) 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 record was 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 constiuents detected in the hexane extract of *Datura metel* is shown in Table 1

Table 1: Phytochemical constituents in hexane etract of *Datura metel*

S/N	Phytochemical constituents	Hexane extract (*Datura metel*)
1	Glycoside	+
2	Saponin	+
3	Phenolics	+
4	Tannins	-
5	Eugenol	+
6	Steroid	+
7	Terpenoids	+
8	Alkaloids	+
9	Flavonoids	+

Key: - = absent , + = present

Biochemical constituents are responsible for plants activities such as antioxidant, antimicrobial, anticancer, antifungal and anti-viral properties. The phytochemical screening of the hexane extract revealed the presence of glycosides, saponins, phenolics, alkaloids, steroids, eugenols, while tannins was absent. According to Firdaus in [3], total alkaloid content reported for *D. metel* leaves extract was 0.426% with mainly atropine and a small amount of hyoscyamine. While the Seeds contain 0.426% alkaloid (mainly hyoscyamine) and the roots contain 0.35% hyoscyamine. More so, total alkaloid content reported for *D. metel* leaves extract was 0.426% with mainly atropine and a small amount of hyoscyamine. While the Seeds contain 0.426% alkaloid (mainly hyoscyamine) and the roots contain 0.35% hyoscyamine. The phytochemical study of the seeds by Ratan in [19], yielded alkaloids, tannins, phlorotannins, cardiac glycosides, carbohydrates, flavonoids, amino acids, and phenolic compounds while the flower extract gave Scopolamine (0.5%); hyoscyamine (0.04%) and atropine (0.01%). However, when compared to moderately polar extract by ethyl acetate in the research of Sakthi [20], the phyto constituents were similar except with the presence of tannins.

3.2. Quantification of flavonoids by HPLC

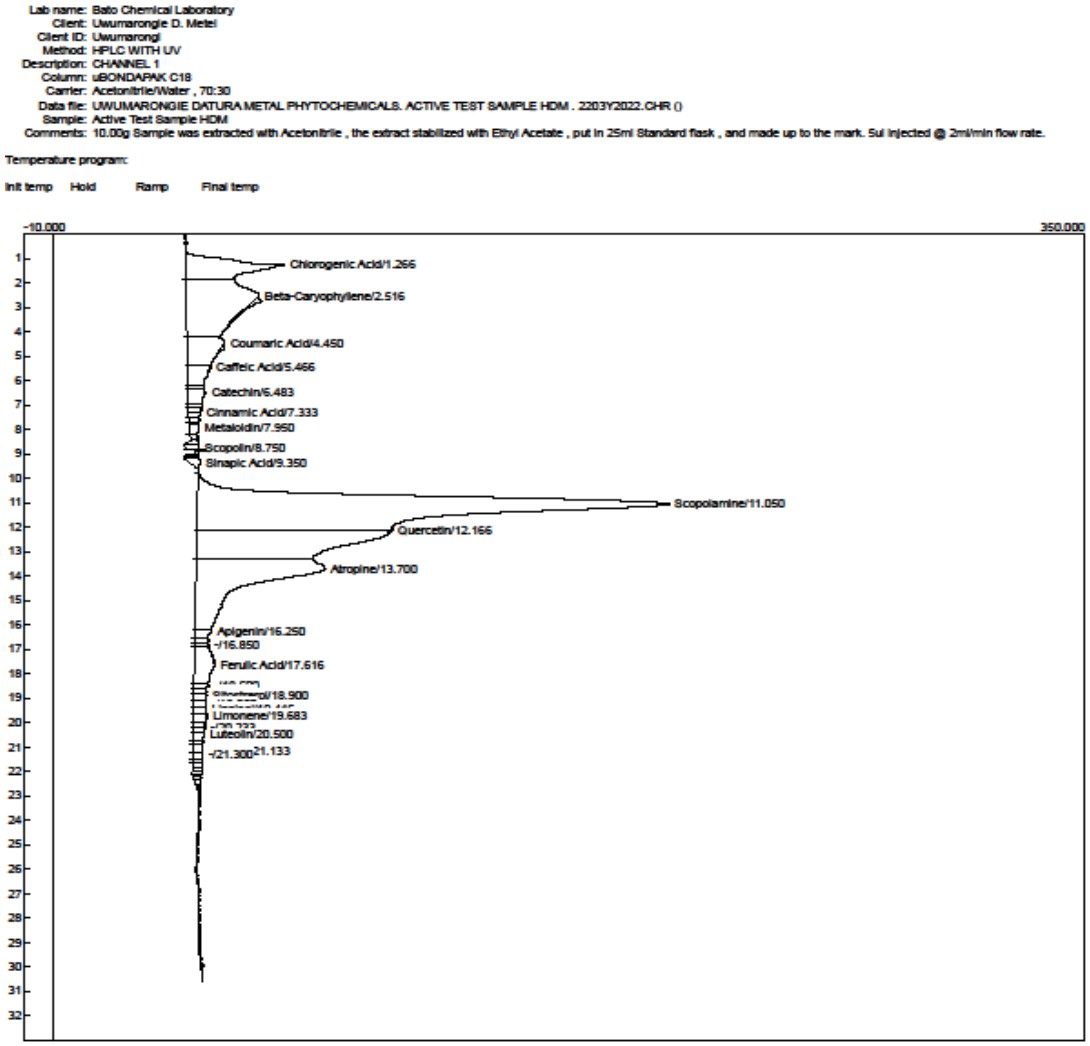
The HPLC chromatogram of the hexane extract of *Datura metel* given in Figure 1, indicated some chemical constituents as shown in Table 2 and the chemical profile of the standard Quercetin is shown in Table 3

Table 2: HPLC profile of hexane extract of *D. metel*

Peak No.	Retention time (Rt) (mins)	Name of compound	Area Percent (%)
1	1.266	Chlorogenic acid	1181.3855
2	2.56	Beta-caryophyllene	2658.3150
3	4.450	Coumaric acid	744.0795
4	5.466	Caffeic acid	312.4090
5	6.483	Catechin	199.6535
6	7.333	Cinnamic acid	61.0590
7	7.950	Metalodin	72.2160
8	8.750	Scopolin	50.2930
9	9.350	Sinapic	67.7070
10	11.050	Scopolamine	9380.0370
11	12.166	Quercetin	3738.9540
12	13.700	Atropine	3294.9320
13	16.250	Apigenin	108.4480
14	17.616	Ferulic acid	521.3370
15	18.900	sitosterol	66.2210
16	19.416	linalool	66.3240
17	19.683	limonene	105.3160
18	20.500	luteolin	65.9505
19	21.133	Solanine	69.2865

Table 3: HPLC and profile of the standard

Compound	Quercetin
Classification	Flavonoid
Retention time	12.166
Quantity (mg/ml)	1.2
Molecular formula	$C_{15}H_{10}O_7$
Molecular weight (g/mol)	302



Component	Retention	Area	Height	External	Units
Chlorogenic Acid	1.266	1181.3855	33.553	0.0000	%
Beta-Caryophyllene	2.516	2658.3150	24.849	319.7894	ppm
Coumaric Acid	4.450	744.0795	12.809	80.5099	ppm
Caffeic Acid	5.466	312.4090	7.886	0.0000	
Catechin	6.483	199.6535	6.040	0.0000	
Cinnamic Acid	7.333	61.0590	4.121	0.0000	
Metaloidin	7.950	72.2160	3.412	0.0000	
Scopolin	8.750	50.2930	4.510	0.0000	
Sinapic Acid	9.350	67.7070	3.474	0.0000	
Scopolamine	11.050	9380.0370	160.067	0.0000	
Quercetin	12.166	3738.9540	66.266	0.0000	
Atropine	13.700	3294.9320	43.765	0.0000	
Apigenin	16.250	108.4480	5.606	0.0000	
Ferulic Acid	17.616	521.3370	6.852	0.0000	
Sitostreroi	18.900	66.2110	4.442	0.0000	
Linalool	19.416	68.3240	4.382	0.0000	
Limonene	19.683	105.3160	4.678	0.0000	
Luteolin	20.500	61.9505	3.901	0.0000	
Solanin	21.133	69.2865	3.500	0.0000	
		22761.9135		400.2993	

Figure 1: High performance liquid chromatography profile of hexane extract of *D. metel*

HPLC profile of hexane extract of *D. metel*t revealed the presence of chlorogenic acid acid (Retention time (RT), 1.266 min; peak 1), catechin (RT,6.483 min; peak 5), cinnamic aciD(RT, 7.333min; peak 6), scopolamine (RT =, 11.050min; peak 10), quercetin (RT,12.166 min; peak 11) and linalool (RT,19.416 min; peak 16) (Figure 1 and Table 2). The results showed that scopolamine is the most dominant flavonoid in the *D. metel* HPLC analysis when compared to the standard quercetin. The re-extraction of the hexane extract by acetonitrile gave the possibility of the presence of this tropane alkaloid, scopolamine. This study also corroborates the identification of atropine and scopolamine alkaloids from the leaves and seeds of *D. metel* by Sayegh [21].

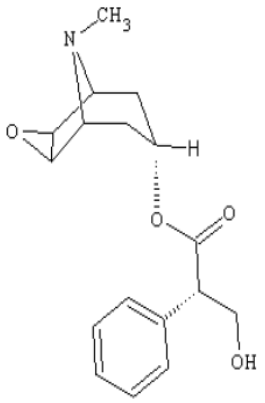


Figure 2: Scopolamine

Scopolamine is a tropane alkaloid. The constituent responsible for the toxicity and poison in *Datura metel* are tropane alkaloids, thus explaining its abundance. It was formally used as a medication for treating motion sickness and postoperative nausea and vomiting [22]. It is also sometimes used before surgery to decrease saliva. When used by injection, effects begin after about 20 minutes and last for up to 8 hours. It may also be used orally and as a transdermal patch. Common side effects include sleepiness, blurred vision, dilated pupils, and dry mouth, increased thirst.

3.3. Anxiolitic activity

The number of head dipping of Balb/c mice for the anti-anxiety studies is shown in Table 4.

Table 4: Anxiolytic activity of hexane extract of *D. metel* in Balb/c mice

Time (minutes)	Group A	Group B	Group C	Group D	Group E
	Control (0.2mL)	100mg/kg	200 mg/kg	400mg/kg	2mg/kg
	Number of head dipping				
1	22	26	40	38	38
2	32	31	49	33	49
3	21	41	47	48	35
4	26	38	50	61	44
5	25	35	32	33	32

The results (Table 4) above explain the increasing boldness, the mice experienced in head-dipping as the extract was given and 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. From Group B (100 mg/ml) down to the standard drug, diazepam, there were less anxiety, due to increase in the head dips thus indicating slightly dose dependent activity for group C (200mg/kg) and the diazepam group (positive control). This experiment indicated a positive anxiolytic activity for all the extract groups (B, C and D) since the frequency and duration of head-dipping are assumed to provide measures of neophilia as well as anxiolysis [3].

4. Conclusion

The study indicated the presence of flavonoids from phytochemical screening and this was supported by the flavonoid constituents analyzed by the high performance liquid. All the doses

investigated had anxiolytic activity in Balb/c mice and this corroborates the traditional use of t5he plant as sedatives.

Conflict of Interest

The authors declare no conflict of interest in this work.

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