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Phytochemical Analysis of *Datura stramonium* (Solanacaeae) Leaves using Aqueous and Ethanolic Extracts

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Abstract

Datura stramonium is known to elicit several pharmacological activities like anti-inflammatory activity, anti-asthmatic activity, anticholinergic activity, alopecia, and anti-carcinogenic activity, amongst others. However, reports have shown that excessive intake of this plant could affect the brain (hallucination) and even lead to death. The present study conducted a qualitative and quantitative analysis of the phytochemical constituents of D. *stramonium*. Standard procedures were used to extract the phytochemical constituents from leaves using ethanolic and aqueous extracts. Results indicated that in the ethanolic extract of the leaves, alkaloids, saponin, and phenols were present, while in the aqueous extract, alkaloids and phenols were present with saponin absent. The quantitative analysis showed that the extract of leaves of the Datura stramonium plant contains more alkaloids than saponin, with a percentage alkaloid concentration of 6.67±0.21 and saponin concentration of 0.75±0.07. In conclusion, in contrast to water, ethanol appears to be a suitable solvent for extracting various active phytochemicals from D. *stramonium* leaves. Also, the high concentration of alkaloids in D. *stramonium* leaves may cause acute psychosis experienced when ethanolic or aqueous extract of the leaves is consumed.

Keywords: *Datura stramonium*; Phytochemicals; Pharmacological; Psychosis

Introduction

Plants have continuously played a significant role in treating human traumas and diseases worldwide. The use of herbal curative products and supplements has vastly increased over the decades, with not less than 80% of people worldwide relying on them for some part of primary health care in both developed and developing countries due to the growing recognition of natural products [1]. These plants have been used as remedies for humans because they contain chemical components of therapeutic value [2]. According to the World Health Organization, medicinal plants are the best source to obtain a variety of newer herbal drugs, which have been fast-growing worldwide during the last few decades [3]. Plants' most critical bioactive constituents are alkaloids, tannin, flavonoids, and phenolic compounds [4] other secondary metabolites are terpenoids and alkaloids. Steroids, glycosides, and volatile oils [5].

Datura is a member of the Solanaceae family (nightshade family), including edible plants like tomatoes, potatoes, eggplant, and peppers (i.e., fruits of the genus *Capsicum*). The genus is widely distributed in the warm and tropical regions of the world and consists of potent hallucinogenic plants such as D. *inoxia*, D. *stramonium*, D. *metel*, D. *wrightii*, D. *ceratocaula*, D. *quercifolia*, D. *tatula*, D. *discolour*, and D. *fastuosa* [6]. The most common species within this family, *Datura stramonium*, is native to Asia but is also found in the United States, Canada, and the West Indies. It is widespread in temperate, tropical, and subtropical regions. Traditionally, D. *stramonium* has been used for mystic and religious purposes [7] and as a herbal medicine for its narcotic potentials and also, in the treatment of asthma [8]. The seed of D. *stramonium* is smoked to achieve hallucinogenic experiences [9]. About ten species of Datura are found, of which Datura anoxia and

D. *stramonium* are the most critical drug plants [10]. Taxonomic evaluation of the variability in the Datura genus has scarcely been carried out using genetic markers, suggesting a potentially vast inherent variation among its population [11]. Hence it is expected that the genetic makeup of Datura population continues to mutate, hence affecting the resultant effects of the secondary metabolites present in the plant.

In Ayurvedic medicine, *Datura stramonium* is a valuable remedy for various human ailments, including ulcers, wounds, inflammation, rheumatism and gout, sciatica, bruises and swellings, fever, asthma and bronchitis, toothache [12] Many folk medicine remedies use D. *stramonium* therapeutically. In the Hindu religion, the seed of D. *stramonium* is associated with the God Shiva, which can promote misuse of the plant on religious occasions, such as Shivaratri and Swasthani Puja [13]. In recent drugs, the therapeutic uses of D. *stramonium* are overshadowed by its toxic effects. The administration of vast amounts of D. *stramonium* affects the central nervous system nervosa with symptoms like confusion, flaky behaviour, hallucinations, and future memory loss. Death by D. *stramonium* poisoning is rare; recovery could take many days [14]. Therefore, an intensive understanding of the potential pharmacologic and toxicological effects of D. *stramonium* is required. This study focuses on botany, phytochemistry, pharmacology, toxicology, and ethnomedicinal uses of D. *stramonium*.

Materials and Method

Collection and identification of plant material

Fresh leaves of *Datura stramonium* were collected from disturbed vegetation sites (7.0856 °N, 6.3015 °E) in the Elele, Uzairue, Edo state, Nigeria. Specimens were taxonomically authenticated at the Department of Plant Biology and Biotechnology herbarium, Edo state University Uzairue and assigned a voucher number: EUI/00022.

Sample preparation and extraction of phytochemicals:

Following authentication, leaves were washed severally and sun-dried for five days before grinding to smooth powder grinding and packed in an air-tight bottle. The powdered leaves of D. *stramonium* were extracted using a soxhlet extractor following the method outlined by Adebayo and Sofowora (1978). Prepared leave samples weighing 30g were wrapped in a thimble and placed inside the extraction compartment. A known quantity of Ethanol was measured using the measuring cylinder, poured into a flat bottom flask, and placed in the heating mantle to the boiling point of 85 °C. As the Ethanol is heated, the vapour enters the extraction compartment and soaks the thimble; the condenser condenses the vapour back into the flat bottom flask that contains the solvent. The process continued for 2 hrs. The extract was filtered using Whatman no. 1 filter paper and concentrated using a rotary evaporator at 40 °C to yield crude extract, kept in a refrigerator at 4 °C for further analysis. The same procedure was followed for aqueous extraction.

Qualitative phytochemical screening of the crude extract

Qualitative tests were done on the crude extracts of the plant to identify the presence of phytochemicals such as alkaloids, phenols, flavonoids, saponins, glycosides, and tannin in the plant extract.

a) Test for alkaloids: Presence of alkaloids was determined using the method of (Odebiyi & Sofowora, 1978). 2ml of the extract was stirred with 5ml of 1% aqueous HCl on a steam bath, filtered while hot. Distilled water was added to the residue. 2ml of the filtrate was then treated with a few drops of Wagner's reagent (solution of iodine in Potassium iodide). The appearance of reddish-brown precipitate gives a positive test for alkaloids.

b) Test for Phenols: Presence of phenols was determined using the method of Sofowora (1993). The procedure uses 2ml of the plant extract and 2ml of ferric chloride solution in a test tube. A deep bluish-green solution indicates the presence of phenols.

c) Test for Flavonoids: Test for Flavonoids was performed using the method of [15]. a gram powdered sample was heated with 10ml ethyl acetate over a steam bath (40 to 50°C) for 2mins and was filtered hot. The filtrate was treated with 1ml dilute ammonia. A yellow colouration demonstrates a positive test for flavonoids.

d) Test for glycosides: (Kellar-Kiliani test) Presence of glycosides was determined using the method of [16]. Two ml of filtrate was added to 1ml of glacial acetic acid. 1 ml of ferric chloride was added to1ml of concentrated sulfuric acid. The green blue colouration of the solution indicates the presence of glycoside.

e) Test for tannin: It was performed using the method of [17]. 2 ml of extract was boiled in 20 ml of water in a test and then filtered. A few drops of 0.1% ferric chloride were added. The appearance of green or a blue-black colouration indicates the presence of tannin.

f) Test for saponin: Test was performed using Dahwan and Gupta's (2017) method. 2ml of each extract was taken and diluted with 2ml of distilled water. The test tubes were then agitated for 15 minutes by hand. The formation of foam on top of the test tube indicates the presence of saponins in the plant extract.

g) Test for terpenoid (Salkowski test) Test for terpenoid was performed using the method of Sofowora (1993). 2ml of each extract was added to 1 ml of chloroform, and Two ml of concentrated H_2SO_4 was added to form a layer. The formation of reddish-brown colouration indicates the presence of terpenoids.

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Quantitative Analysis

Determination of total alkaloids

This test was determined using the method of [18] 5g of the sample was weighed into a 250ml beaker, and 10% acetic acid in Ethanol was added, covered, and allowed to stand for four hours. It was filtered, and the extract was concentrated in a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed [18]. The residue was dried in an oven, and the weight arrived using an electronic weighing balance Model B-218. The percentage of the alkaloid is expressed mathematically as

% Alkaloid= (Weight of Alkaloid)/(Weight of Sample) ×100

Determination of total saponin

Plant extract weighing 20g was mixed into a conical flask with 100cm^3 of 20% aqueous ethanol. The mixture was heated over a hot water bath for four h with continuous stirring at about 55 °C. The mixture was filtered, and the residue was re-extracted with another 100ml of 20% ethanol. The combined extracts were reduced to 40ml over a water bath at about 90 °C. The concentrate was transferred into a 250ml separator funnel, and 20ml of diethyl ether was added and agitated. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated by adding 60ml of n-butanol. The combined n-butanol extract was washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to constant weight the saponin content was calculated [19].

% Saponin= (Weight of Saponin)/(Weight of Sample) ×100

Results and Discussion

(Table 1) & (Figure 1)

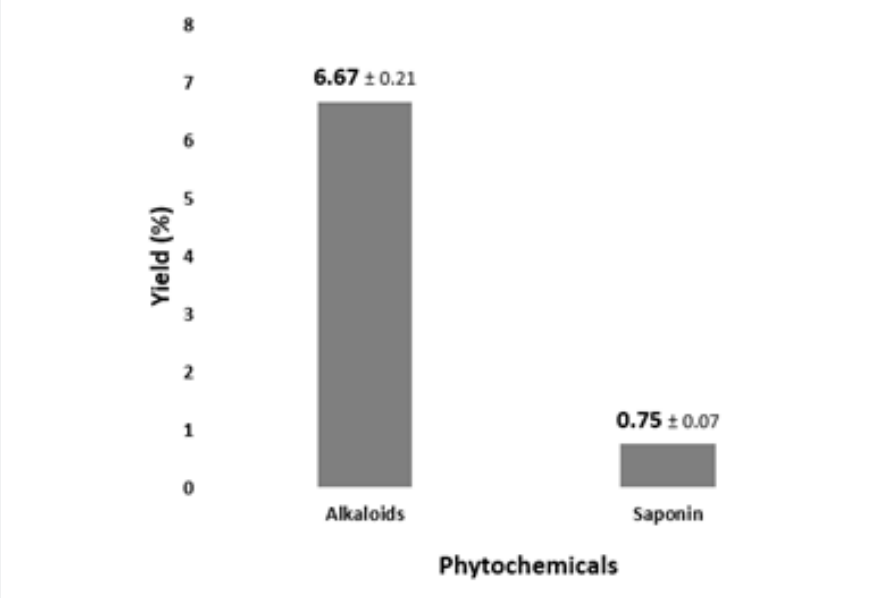

Figure 1: Concentration of selected phytochemicals in *Datura stramonium*.

Table 1: Selected phytochemicals in *Datura stramonium*.

Phytochemicals	Ethanol	Water
Alkaloids	++	+
Flavonoids	-	-
Saponin	+	-
Tannin	-	-
Phenols	++	+
Glycosides	-	-
Terpenoids	-	-

++ (highly abundant), + (minute), - (absent).

Datura stramonium is a famous medicinal plant that has health benefits against many diseases. These health advantages are mainly attributed to many active phytochemicals in various parts of this plant [20]. This study was conducted to ascertain the best extraction solvent to extract the maximum number of phytochemicals from the *Datura stramonium* leaves.

In qualitative analysis, the ethanol extract of *Datura stramonium* exhibits positive results for three phytochemicals, while aqueous extract exhibited positive results for two phytochemicals. The phytochemicals are shown in Table 1. The qualitative analysis detected the presence of different phytochemicals in the dried

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leaves extract of the *Datura stramonium* plant obtained by using other solvents, i.e., ethanol and distilled water. The results showed that all the extracts formed using ethanol solvents from *Datura stramonium* plant leaves contain phytochemicals like alkaloids, saponin, and phenols. Alkaloids have been associated with medicinal use for centuries. One of their biological properties is their cytotoxicity ability which has a wide range of pharmacological activities, including antimalarial (e.g., quinine), antiasthma, anticancer, cholinomimetic [21], vasodilatory, antiarrhythmic, analgesic, antibacterial and anti-hyperglycemic activities [22]. Saponins have many health benefits like anti-tumour, anti-mutagenic, and antioxidant activities that reduce the risk of cancer and heart diseases. Studies have illustrated the beneficial effects of saponin on blood cholesterol reduction, reducing cancer risk by preventing cancer cells from growing, stimulating the immune system, and protecting against viruses and bacteria [23].

Alkaloids and phenols were present in the water extract but minute quantities. However, flavonoids, glycosides, terpenoids, and tannins were absent in the section using ethanol and water. This may be due to the poor solubility of these phytochemicals in water. This could signify the inefficiency of water as a phytochemical extraction solvent from *Datura stramonium* plant leaves.

A quantitative study was further conducted to determine the amount of the total alkaloids and total saponin in these plant extracts for direct comparison, shown in Figure 1 below. It was demonstrated that ethanolic extract contains the maximum number of alkaloids and saponin. The observation made in this study was similar to the findings of [24], which reported higher antioxidant and antimicrobial activities in the ethanolic extracts of their samples.

Previous studies have shown that Datura stramonium plant extract made with ethanol effectively treats gout by inhibiting xanthine oxidase [25] and also possesses anti-proliferative activity against cancerous cell lines [26] Reports have even shown that the ethanolic extract of the *Datura stramonium* plant contains anthelmintic activity and can suppress gastrointestinal nematodiasis in sheep and goats [27]. This highlights that the abundance of various phytochemicals in the Datura stramonium ethanolic plant extract holds great potential to treat various human diseases and has profound medical applicability. Distilled water which results in the little extract of various phytochemicals, may be due to the poor solubility of these phytochemicals and should not be the solvent of choice for extraction. Several reports have indicated the activities of these phytochemicals present in Datura stramonium to possess Anticholinergic activity [9], Antimicrobial Activity, Anticancer activity, Antiinflammatory activity. Larvicidal and mosquito repellent activities. Notwithstanding, D. *stramonium* use in medicine should be with caution. For instance, its use by expectant mothers for asthma treatment has resulted in a continuous release of acetylcholine and desensitization of nicotinic receptors, which could harm the foetus [28-30].

Conclusion

These investigations have revealed that the leaves of *Datura stramonium* are very medicinal. In qualitative analysis, the ethanol extract of *Datura stramonium* exhibits positive results for three phytochemicals. In comparison, the aqueous extract exhibited positive results for two phytochemicals. Phytochemicals identified in the leaves showed that the plant could serve as an anti-inflammatory, antioxidant, anti-tumour, and antimicrobial agent. The health benefits of these leaves have proved the plant to be a potential source of the helpful drug. Therefore, it is believed that the presence of these bioactive compounds could be beneficial in developing novel medicines.

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