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Brief Report

# Assessment of Vitamin Composition of Ethanol Leaf and Seed Extracts of *Datura Stramonium*

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## Abstract

**Background:** Herbs have gained recognition as highly efficient tools in the treatment and management of diseases both in modern and traditional medicine. *Datura stramonium* is a good example of such a medicinal herb. *D. stramonium* is popularly called thorn apple or Jimson weed in the family of Solanaceae. It has both toxic as well as medicinal potentials. *D. stramonium* leaves, seeds, and stems have been extensively studied for various pharmacological properties. *D. stramonium* seed is among the top plants commonly abused as a drug by Nigerian youths. Chemical constituents are responsible for the medicinal potential of plants.

**Objectives:** This study was planned to investigate and compare the vitamin contents of ethanol extract of *D. stramonium* leaf and seed.

**Methods:** The determination of vitamin levels was carried out using the Association of Official Analytical Chemists (AOAC) and other standard methods.

**Results:** The order of vitamin composition in both leaves and seeds was $E > C > A > B_6 > B_9 > B_{12} > B_2 > B_1 > B_3$. Moreover, the concentration of vitamins E, C, and A, being the most abundant, was 6.65:1.64 mg/100 g, 3.65:1.05 mg/100 g, 2.38:1.82 mg/100 g, for leaf and seed extracts, respectively. Further, there were significant differences ($P < 0.05$) in the contents of vitamins A, C, E, and $B_2$ of the leaves and seeds, with the leaves having higher vitamin levels than the seed.

**Conclusion:** The number of vitamins present in the samples may be responsible for the highly nutritious and medicinal properties of *D. stramonium*. From the results of this study, it is obvious that *D. stramonium* leaves and seeds can serve as good nutritional supplements, which may also provide the users with adequate nutrients that help with the management of various health challenges. However, further studies are required to ascertain appropriate doses that could lead to toxicity.

**Keywords:** Vitamins, *Datura stramonium,* Medicinal herbs, Chemical constituents, Pharmacological properties

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## Background

In recent years, there has been a growing rise in the use of medicinal plants because they are useful natural resources and are regarded as potentially safe drugs (1). These plants have been analyzed for various pharmacological roles, including biological, antimicrobial (2), and hypoglycemic functions (3). The medicinal properties of plants depend on chemical compounds that generate specific physiological actions (4-6). About 60% of the total global population relies on medicinal plant use for healthcare maintenance (7).

*Datura* has been popular among traditional Chinese and Indian systems of Medicare for ages (8). It is used for the management of pain, fever, asthma, ulcer, and bronchitis (9). Excessive intake of *Datura stramonium* can cause hallucinations and death due to its high alkaloid content (2,10,11). Several pharmacological and ethnomedicinal uses of this plant abound. It has anti-inflammatory and antioxidant properties (12) and stimulatory effects on the central nervous system, the enhancement of the respiratory tract, and also the maintenance of healthy teeth and skin (13). *D. stramonium* is rich in compounds with anticholinergic effects and hence can be used in treating symptoms related to organophosphate intoxication (14). Every part of *D. stramonium* is toxic; however, *D. stramonium* ripe seeds are the most toxic part (2,10,15). The unpalatable effects of its toxicity can be extremely deleterious. Thus, prior knowledge of its toxicity and dosage is essential.

Vitamins are organic molecules that perform an array of

functions within the body system. They serve as cofactors for enzyme-catalyzed reactions, and this is the most prominent role of vitamins. Vitamins cannot be produced by mammalian cells and, hence, must be supplied through food (16). Inadequate intake or absorption, excess loss, or enhanced utilization of vitamins can lead to vitamin deficiencies, which sometimes may manifest as disease conditions (16). Oxidative stress was found to have a pathological role in some types of chronic disorders and syndromes such as rheumatoid arthritis (17), diabetes mellitus, inflammatory disorders, cancer, and hypertension (18). It also arises when the generation of free radicals surpasses and out-weighs the intracellular biological defense system (19). Oxidative stress damages proteins, lipids, and nucleic acids, culminating in various diseases (20). Vitamins C, vitamin A, and vitamin E are antioxidant vitamins and hence good scavengers of free radicals. Other vitamins play a myriad of functions, especially in intermediary metabolism. Plant parts such as leaves, seeds, and fruits are good sources of vitamins. The pharmacological effects of plant products are accredited to their bioactive components such as phytochemicals, minerals, and vitamins. Phytochemical screening and pharmacological effects of *D. stramonium* are widely reported. However, to the best of our knowledge, information on vitamin composition quantification of the leaves and seeds of *D. stramonium* is scarce. Hence, the present study was planned to determine and compare the vitamin constituents of the leaves and seeds of *D. stramonium* and their relationship with traditional medicine and health benefits.

## Materials and Methods

### *Chemicals and Reagents*

All used chemicals and reagents were of analytical grade.

### *Materials*

*Datura stramonium* leaves and seeds were the materials used in this research. Fresh leaves and seeds of fully grown *D. stramonium* were collected from Amaozara Ozizza in Afikpo North Local Government Area of Ebonyi State in May 2022 and identified by Mr. Nwankwo Onyebuchi, a plant taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria (Voucher number: EBSU-H-397).

### *Methods*

#### *Preparation of the Crude Ethanol Leaf Extract of Datura stramonium*

The method described by Abubakar and Haque (21) was used for the extraction. Fresh leaves and seeds of *D. stramonium* were washed and shade dried for two weeks and later pulverized and sifted. Then, 400 g of each powdered sample were soaked in 2000 mL of 98% ethanol for 72 hours at room temperature with intermittent rocking. Thereafter, it was filtered, the solvent was removed through heating on a water bath, and the dried filtrate was stored and used for the analysis.

#### *Determination of Vitamin Composition of Datura stramonium Ethanol Leaf and Seed Extracts*

Vitamin A was assessed by the Association of Official Analytical Chemist (AOAC) method (22), and vitamins $B_1$, $B_2$, $B_3$, $B_6$, $B_{9,}$ and $B_{12}$ and C content were determined through the Nollet Leo (23), while vitamin E concentration was determined via the method described by Desai (24).

#### *Determination of Vitamin A*

Vitamin A was determined by the AOAC (22) method.

#### *Procedure*

Four grams of the sample were weighed into a beaker, and then 5 mL of 50 % potassium hydroxide solution and 50 mL of ethyl alcohol were added and refluxed in a water condenser for an hour. The solution was then cooled and transferred to a 500 mL separator, to which 50 mL of hexane was added and shaken vigorously for 5 minutes, resulting in the formation of two separate layers. Afterward, the organic layer was passed through anhydrous sodium sulfate into a 200 mL volumetric flask while the aqueous layer was shaken 3 times, and 30 mL of hexane was collected each time. All the organic layers were pooled together and diluted to 200 mL with hexane. The absorbance was recorded in an ultraviolet (UV) spectrophotometer at 325 nm.

$$\text{Vitamin A}\frac{\text{IU}}{100\text{g}}\text{sample} = \frac{absorbance \times 200 \times 1830}{Weight\ of\ the\ Sample} \times 100$$

#### *Determination of Thiamine ($B_1$)*

Thiamine content was determined through the Nollet Leo method (23).

#### *Procedure*

Exactly 2 g of the extract and working standard solution were weighed into two different dry-separating funnels. Thereafter, 10 mL of chloroform and 10 mL of dye solution were introduced into both solutions, then shaken continuously for about 2 minutes, and allowed to stand for 5 minutes. The chloroform layer was collected by being passed through anhydrous sodium sulfate. Then, the absorbance in a UV spectrophotometer was read at 420 nm using chloroform as blank.

$$\text{Conc.}(\text{mg}) = \frac{SA \times StW \times 1 \times \times 10 \times 10 \times 1 \times SP \times 100 \times 1000}{\text{StA} \times 100 \times 100 \times 1 \times 1010}$$

where *SA* stands for sample absorbance, *StW* indicates standard weight, *SP* is standard purity,

*StA* depicts standard absorbance, and W indicates the weight.

#### *Determination of Riboflavin ($B_2$)*

Riboflavin content was determined through the Nollet Leo method (23).

*Procedure*

Two grams of the extract were weighed into a conical flask and dissolved with 100 mL of distilled water, and 2 mL of glacial acetic acid was added. The solution was boiled for 5 minutes and then cooled. Then, 20 mL of 1.0 M sodium hydroxide solution was added and diluted with 350 mL water. The solution was filtered, and absorbance was read at 444 nm using a spectrophotometer (water was used as blank).

$$Conc.(mg) = \frac{A \times DF \times volume\ of\ cuvette}{E}$$

where A is the absorbance at 444 nm, E is the extinction coefficient, and DF is the dilution factor.

*Determination of Niacin ($B_3$)*

Niacin (vitamin $B_3$) content was determined via the Leo and Nollet method (23).

*Procedure*

Precisely 1 grgam of sample was weighed into a test tube, macerated with 50 mL of distilled water, and filtered. Then, 1 mL of filtrate was pipette into another test tube, and 6.5 mL of distilled water, 0.5 mL of 1:5 ammonium, and 1 mL of cyanogen bromide were added and shaken. One milliliter of sulfanilic acid was added and allowed to stand for 5 minutes. Further, 0.5 mL of concentrated hydrochloric acid was added and then diluted with 10 mL of distilled water, and absorbance was measured at 430 nm.

*Determination of Vitamin C (Ascorbic Acid)*

Ascorbic acid was determined via the method described by Nollet Leo (23).

*Procedure*

Exactly 1 mL of each sample was put in a 25 mL conical flask. Then, 10 mL of oxalic acid (0.05 M)-EDTA (0.02 M) solution was added, and the entire mixture was left for 24 hours to provide the required reaction time. After 24 hours, the sample was filtered through 0.45 µm filter paper. Then, 2.5 mL of each sample was transferred to a separate 25 mL volumetric brown flask, after which 2.5 mL of the oxalic acid (0.05 M)-EDTA (0.02 M) solution was then added. Subsequently, metaphosphoric acetic acid solution (0.5 mL) was added, followed by sulphuric acid (5% v/v) solution (1 mL) and 5 % (m/v) ammonium molybdate solution (2 mL) in each volumetric brown flask, and the volume was made up to 25 mL distilled water. Afterward, a known concentration of ascorbic acid (0.1 % m/v) standard concentration was used as the standard, and the same process was carried out on the test sample. The absorbance of the test sample and the standard sample was read at 760 nm against the blank test on a UV/visible spectrophotometer.

The concentration (Cx) of vitamin C (%w/v or mg/mL) in the test sample was calculated using the formula:

$Cx=(Ax\times Cs)/As$

Cs is known concentration of ascorbic acid; Ax is absorbance of test sample; As is absorbance of standard sample; Cx is concentration of ascorbic acid

*Determination Tocopherol (Vitamin E)*

Tocopherol content was determined via the method described by Desai (24).

*Procedure*

One milliliter of the test sample and 1 mL of ethanol were mixed, and 3 mL of petroleum ether was introduced to this mixture, shaken intensely, and then centrifuged. Then, 2 mL of the supernatant was collected and allowed to evaporate until drying. Thereafter, 0.2 mL of bathophenanthroline reagent was added. Tubes containing $\propto$-tocopherol standard were also treated exactly in the same way as the test sample. Care was taken to reduce unnecessary exposure to direct light. After one minute, 0.2 mL of 0.001 M orthophosphoric acid reagent was introduced and mixed thoroughly, and the total volume of all the tubes was topped up to 3 mL with ethanol. The absorbance was taken at 536 nm against the reagent blank containing ethanol, and the level of α-tocopherol in plasma was expressed as mg/dL.

***Statistical Analysis***

Data were expressed as mean ± standard deviation from triplicate (n = 3) determinations. Mean values were appropriately checked and compared using a one-way analysis of variance followed by Turkey's post hoc test, and significance was set at $P < 0.05$. Graph Pad Prism software version 5.00 for Windows was used for data analysis.

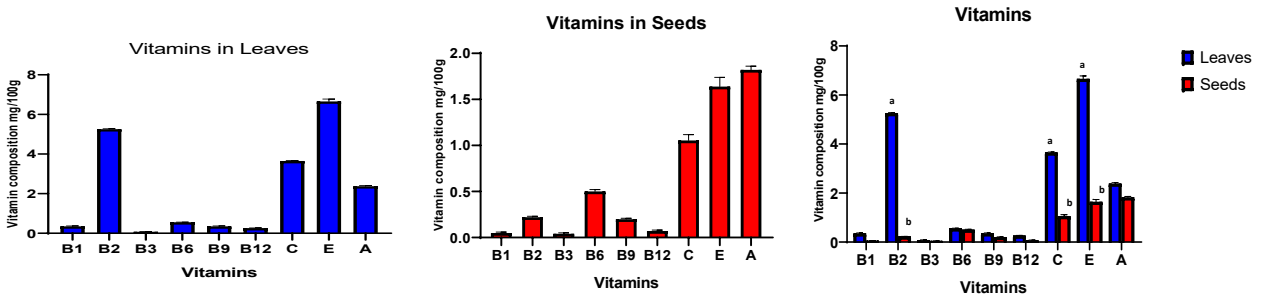
## Results

***Vitamin Composition of Datura stramonium Ethanol Leaf and Seed Extracts***

The order of vitamin constituents in both leaves and seeds is $E > C > A > B_6 > B_9 > B_{12} > B_2 > B_1 > B_3$. Further, there were significant differences ($P < 0.05$) in the contents of vitamins A, C, E, and $B_2$ of the leaves and seeds, with the leaves having higher vitamin levels than the seed (Figures 1a-c). The concentration of vitamins E, C, and A, being the most abundant, was 6.65:1.64 mg/100 g, 3.65:1.05 mg/100 g, and 2.38:1.82 mg/100 g for leaf and seed extracts, respectively.

## Discussion

Medicinal plants are of immense value, especially for the maintenance of good health and drug discovery (25). Datura possesses analgesic, antioxidant, anticancer, and antimicrobial potentials (26,27). All these effects are accredited to its bioactive component. Several authors have reported various pharmacological effects of this plant, but there is limited information about its vitamin




**Figures 1.** (**a and b**) Vitamin Composition of *Datura stramonium* Ethanolic Leaf and Seed Extracts Expressed in mg/100g of Sample. **(c)** Comparative Vitamin Composition of *D. stramonium* Ethanolic Leaf and Seed Extracts Expressed in mg/100 g of Sample. *Note. D. stramonium*: *Datura stramonium*. The values represented in the chart are mean ± standard deviation from triplicate readings (n = 3). Bars with no alphabets are significantly the same at $P > 0.05$, while bars with different alphabets are significantly different at $P < 0.05$ across the rows

composition. In this study, we examined the vitamin composition of ethanol seed and leaf extracts of *D. stramonium* and its relationship with the acclaimed health benefits.

The order of vitamin composition in both leaves and seeds was E > C > A > $B_6$ > $B_9$ > $B_{12}$ > $B_2$ > $B_1$ > $B_3$. Further, the leaves had higher vitamin levels than the seed. Vitamins are organic molecules that perform several functions in the body (16). In this study, vitamins were in appreciable concentration, especially the antioxidant vitamins. Vitamins C, A, and E are antioxidant vitamins and hence good scavengers of free radicals. Other vitamins play a myriad of functions, especially in intermediary metabolism. A deficiency of vitamin can cause some abnormalities such as scurvy from the lack of vitamin C, eye damage from the lack of vitamin A, and the like. It is worth noting that many activated carriers in intermediary metabolism are vitamin derivatives, including electron carriers that are needed for redox reactions such as electron transport (flavin adenine dinucleotide) and carbon dioxide transfer (biotin). Although animals require vitamins in small quantities, the study of vitamins is an integral part of health science due to the indispensable roles vitamins play in the well-being of the human body.

The result of this study uncovered *D. stramonium* as a good source of vitamins A, C, and E (Figure 1), which corroborates the finding of Aja et al (28) who reported *Phoenix dactylifera* fruits as a good source of vitamin C and other vitamins. The concentration of obtained vitamin C was consistent with the result of Offor et al (29) and Ibiam et al (30) regarding the leaves of *pumpkin* and ethanol leaf extract of *Buchholzia coriacea*, respectively.

Vitamin C is a good antioxidant that inhibits infection and reduces toxicity. An adequate intake of vitamin C prevents scurvy and maintains healthy skin (31). Vitamin C takes part in protein metabolism and the production of collagen. In this study, vitamin C level was similar to values reported by Blessing et al (32) for pumpkin (3.47–4.39 mg/100 g).

Vitamin E is an antioxidant that is mainly utilized for the prevention and treatment of heart disorders (33). As an antioxidant, it helps prevent different types of cancer and other diseases ranging from Alzheimer's disease to other types of neurological disorders. Vitamin E contributes to the protection of cells from oxidative stress by decreasing the release of reactive oxygen species via monocytes and hence possesses anti-inflammatory potential.

The high level of vitamin A portends that the leaves could aid growth and development, the maintenance of the immune system, and good vision (34).

The concentration of obtained vitamin $B_1$ (thiamine) was consistent with the result of Uraku et al (35) for the leaves of *Culcasia scandens*. Thiamine (vitamin $B_1$) plays a significant role in energy production, carbohydrate metabolism, and optimum nerve cell function.

Riboflavin (vitamin $B_2$) content was 5.26 and 0.22 mg/100 g in leaves and seeds, respectively. Therefore, vitamin $B_2$ level was higher in leaves than in seeds. This corroborates the finding of Blessing et al (32) for pumpkins. Riboflavin is a cofactor of enzymes needed for energy metabolism (36). It is also part of the mitochondrial respiratory chain as an electron carrier and aids normal vision and skin health.

The quantity of obtained vitamin $B_3$ (niacin) was in tandem with that of Offor (37) for *Dissotis rotundifolia* leaves. Vitamin $B_3$ plays a crucial role in DNA repair and metabolism, and it is a precursor of nicotinamide adenine nucleotide and nicotinamide adenine dinucleotide phosphate, which are electron and proton acceptors, respectively (38).

Vitamin $B_6$ (pyridoxine) contents in both samples were similar. Vitamin $B_6$ enhances the metabolism of proteins, fatty acids, and hematopoietic cell production (39).

Folic acid ($B_9$) is essential in the metabolism and repair of DNA. Its deficiency is common among pregnant mothers, elderly, and malnourished children. Folic acid is needed for the production of purines and pyrimidines, which are needed for DNA production and red blood cells (38). Tetrahydrofolate is the coenzyme form of folic acid and acts as a coenzyme in the transfer and utilization of one carbon moiety (40). In our recent studies, we observed that ethanol leaf extract of *D. stramonium* attenuates oxidative stress and hepatic damage in albino rats that received methotrexate injection (12,41). Methotrexate is a folate antagonist; therefore, the attenuation of the biochemical dysregulations triggered by methotrexate

could be accredited to the appreciable concentration of folate present in the *D. stramonium* extract, as seen in the present study.

## Conclusion

According to the obtained results, leaves and seeds of *D. stramonium* contain a considerable amount of vitamins and hence are a good source for the discovery of therapeutic agents. However, further studies are needed to understand the mechanism of action by utilizing various biological models.

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### Authors' Contribution

**Conceptualization:** Esther Ugo Alum.
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**Methodology:** Wilfred Aja.
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**Validation:** Emmanuel I. Obeagu.
**Visualization:** Michael Ben Okon.
**Writing–original draft:** Esther Ugo Alum.
**Writing–review & editing:** Esther Ugo Alum.

### Competing Interests

Authors have no conflict of interests to declare.

### Ethical Approval

The study was approved on May 15, 2022 by the Ethical Committee of the Biochemistry Department, Ebonyi State University, Abakaliki, Nigeria (Ethical Approval number: EBSU/BCH/ET/22/017). The guidelines agree with the world standards for the care and use of animal models in research (NIH Publication No. 85-23, revised 1996).

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