Phytochemical Profiling and Nutraceutical Benefits of Methanol Leaf Extracts from *Datura alba ness*

Abstract

Introduction: Species of Datura plant has provided substantial pharmacological benefits against several diseases in the last decade. However, not much has been reported on *Datura alba ness* (DAN), no report has been able to the proximate, characterized its phytochemical components. Hence, the present study evaluated the proximate, phytochemical and bioactive profile *Datura alba ness*.

Materials and Methods: Fresh leaf of *Datura alba ness* was collected from Abua Odua local government area of Rivers State, Nigeria and properly identified and authenticated. The fresh leaves were subjected to proximate analysis using standard protocols while the dried leaves were extracted using methanol by method of cold maceration to obtain methanolic extract of *Datura alba ness* (MEDAN). The MEDAN was further subjected to phytochemical screening to obtained the various phytochemicals and Gas chromatography and mass spectrometry (GC-MS) analysis to identify and characterize the various bioactive compounds.

Results: Proximate analysis of fresh leaves of DAN showed the presence of moisture (60.4%), crude protein (21.9%), carbohydrate (7.6%), crude fiber (4.77%), ash (4.17%) and fat (1.19%) while the phytochemical screening showed the presence of flavonoids (49.14%), alkaloids (19.16%), tannins 1(12.19%), saponins (11.72%), phenols (5.33%), cardiacglycosides (1.91%), oxalates (0.43%) and phytates (0.43%). Also, the GC-MS analysis of MEDAN identified twenty-nine (29) active compounds with four (4) observed as the most abundant (with area concentration >10%). They include:Bis (2-ethylhexyl) phthalate (15.97%), 13-hexyloxacyclotridec-10-en-2-one (14.26%), n-hexadecanoic acid (12.87%) and Gamma.-Sitosterol (10.77).

Conclusion: The identification of various nutrients, compounds, and bioactive substances through proximate, phytochemical, and GC-MS analysis of fresh leaves and methanol extract of *Datura alba ness* highlights its potential as a supplement and therapeutic agent. These findings suggest that *Datura alba ness* contains numerous bioactive compounds that may offer effective treatments for conditions related to oxidative stress, nervous system disorders, tissue damage, inflammation, and bacterial infections.

Keywords:*Datura alba ness*, Proximate, Phytochemical, GC-MS, Bioactive Compound,

UNDER PEER REVIEW

INTRODUCTION

Traditional medicine originated from the natural resources of the environment, which communities adopted out of necessity to survive diseases and remained the primary healthcare system until the emergence of modern medicine[1, 2].Phytopharmaceuticals are natural or formulated herbal remedies that derive their effectiveness from one or more plant-based compounds, commonly known as phytochemicals [3, 4]. Natural compounds from plants have attracted considerable attention due to their therapeutic potential against several disease

conditions[5, 6]. Due to their availability, affordability, and minimal side effects, nearly half of the world's population relies on herbal products for their primary healthcare needs [7]. A key focus in the study of plant-derived products is the identification and characterization of the biologically active compounds they contain, which paves the way for further pharmacological, biological, and scientific research [8]. Although, the potential potency and efficacy of some of these products have been documented; however, further studies are needed to effectively screen, identify, and characterize their various bioactive ingredients [7].

The Datura genus is typically described as consisting of annual or perennial herbs or shrubs with glandular or sometimes simple hairs that belong to the Solanaceae family. The leaves are petiolate, with a simple blade that is either sinuate or entirely toothed. Solitary flowers are found in the inflorescences, located in branch forks or leaf axils. In general, the plants lack bracts, peduncles, and bracteoles. The large, usually actinomorphic flowers often have stout pedicels. The long, cylindrical or tubular calyx is circumscissile near the base, while the funnel-shaped, elongated corolla has cuspidate lobes [9, 10]. The seeds are dark, flat, and kidney-shaped, while the fruits resemble walnuts, covered in spines, earning them the name 'thorn apple'". They are also commonly known as Jimsonweed or the Devil's trumpet [10, 11].

Datura has been widely used in traditional medicine. It is a curious twist of nature that a plant which is primarily known for its toxic effects also has medicinal applications. It has been used as an anaesthetic for setting bones, as well as for treating bruises, wounds, and skin ulcers. They have also been employed in the treatment of neurological disorders, cardiovascular conditions, fevers, catarrh, pain, diarrhoea, and respiratory issues [9-12]. *Datura alba ness* (DAN) is one of the most common species found in Nigeria. Commonly referred to as *Zakami* while the Ogoni and Ndokwa tribes call it *Jegemi* and *Gegemu* respectively. Although several other Datura species have been scientifically characterized, there is a lack of data on the phytochemical profiling and nutraceutical properties of *Datura alba Ness*. Therefore, this study aims to fill that knowledge gap by providing a comprehensive analysis of the nutritional and phytochemical aspects of *Datura alba ness*, a common species found in Nigeria.

MATERIALS AND METHODS

Collection, Identification and preparation of plant material

Fresh leaves of Datura alba ness were gathered from a garden in the Abua/Odua Local Government Area of Rivers State, Nigeria. The plant was identified and authenticated by a taxonomist from the Department of Plant Science and Biotechnology at the University of Port Harcourt, receiving the voucher number UPH/295.

Proximate Analysis of Plant Material

Fresh leaves Datura alba ness were used for the proximate analysis following standard protocols. Moisture content, crude protein, crude fibre, fat, ash, carbohydrate and energy value were as per the Association of Official Analytical Chemists (AOAC) method [13, 14].

UNDER PEER REVIEW

Moisture Content: To determine moisture content, fresh leaf samples of DAN were weighed in Petri dishes and then dried in an oven at 105°C for 24 hours. The percentage of weight loss was calculated as the moisture content.

Ash Content: To determine the ash content, leaf samples of DAN were dried in an oven to remove moisture and then weighed. They were then placed in a muffle furnace at 500–600°C for 4 to 6 hours until all organic matter was burned off, leaving only the ash. After cooling in a desiccator, they were weighed with the ash, and ash content was calculated as a percentage of the sample's original weight.

Crude fibre content: This was determined using a dried sample of DAN. About 10g of dried DAN was boiled 1.25% H_2SO_4 for 30 minutes to remove soluble components. The remaining residue was then filtered and washed in warm distilled water to remove the acid. The resultant residue was then boiled in 1.25% NaOH for 30 minutes and then filtered and washed in warm water to remove the base. The residue was then dried in an oven at 105°C and weighed. The dried residue was then burned in a furnace at 550°C to remove any remaining organic matter. The fibre content was determined by subtracting the ash weight from the fibre residue. For the determination of the

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Nitrogen content: Here, the Kjeldahl method was used. About 10g of DAN was placed in a Kjeldahl flask and concentrated H_2SO_4 and copper sulfate were added as catalysts. The mixture was heated, converting the organic nitrogen in the sample to ammonium sulfate. The solution was then cooled and NaOH added to neutralize the H_2SO_4 which converts ammonium ions into ammonia gas. The ammonia is then distilled and absorbed in a boric acid solution. The ammonia-boric acid solution was titrated with H_2SO_4 until the endpoint was reached. The nitrogen content was calculated based on the amount of titrant used, and the protein content was determined by multiplying the nitrogen content by a conversion factor (typically 6.25 for plant samples).

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Lipid content: This was done using the Soxhlet extraction method. Dried leave samples of DAN were placed in an oven at 105°C to remove moisture. About 10g of moisture-free sample of DAN was placed in a Soxhlet extractor with petroleum ether added to the extraction flask. The apparatus was heated to reflux the solvent after dissolving and extracting lipids from the sample. The solution was evaporated to remove the solvent, leaving only the lipids. The resultant lipids were weighed and calculated as the percentage of crude lipids.

Carbohydrate content: This was determined using the 'estimation by difference' method, where the sum of the moisture, ash, protein, and lipid contents is subtracted from one hundred (100)

Preparation and Extraction of Plant Material

The fresh leaves of Datura alba ness were washed, and air dried at room temperature for two (2) weeks to obtain crispy dry leaves leaves which were ground into coarse particles. Extraction of the leaves was done by cold maceration according to previously described procedures [15, 16].

UNDER PEER REVIEW

About 400 g of coarsely ground particles of *Datura alba ness* 400g were soaked in 2000 ml of methanol and allowed to stand for 72hrs with intermittent stirring. The macerated mixture was then filtered using filter paper. The filtrate was dried in a water bath at a temperature of 50°C to obtain the methanol extract of *Datura alba ness* (MEDAN).

Qualitative phytochemical analysis

Qualitative screening and detection of MEDAN were carried out to identify various phytoconstituents following the standard protocols outlined by Trease and Evans [17]

Alkaloids: Mayer's test was used to detect alkaloids in MEDAN. In this process, 2 ml of the extract was combined with 1 ml of Mayer's reagent. The appearance of a pale-yellow precipitate confirmed the presence of alkaloids.

Flavonoids: The Lead acetate test was used to detect the presence of flavonoids. A few drops of Lead acetate solution to MEDAN. The formation of a yellow precipitate indicated the presence of flavonoids.

Tannin/polyphenol; the MEDAN was diluted and 3-4 drops of 10% $FeCl_3$ were added. A blue colour indicated the presence of gallic tannins, while a green colour signified catechol tannins.

Saponins: About 2 g of the MEDAN were boiled in 20 ml of distilled water. Then, 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously. The formation of froth indicated the presence of saponins

Glycosides: The Molisch's Reagent Test was used to detect glycosides. Approximately 5 ml of Molisch's reagent was added to 1 g of the extract, followed by the addition of concentrated H2SO4. The appearance of a violet colour indicated the presence of glycosides.

Steroids: Steroids were detected by adding a few drops of acetic anhydride to the MEDAN extract. A colour change from violet to blue to green indicated the presence of steroids.

Quantitative Phytochemical Analysis

The quantitative chemical evaluation was carried out to detect the amount of the various phytochemicals following standard protocols by Harborne, J.B [18]

Flavonoids: About 100 g of MEDAN was with aluminium chloride solution and ethanol which produced a yellow-colored complex with flavonoids. The absorbance was measured at 420 nm using a spectrophotometer. The concentration was calculated using a standard curve of quercetin.

Alkaloids: The MEDAN was treated with bromocresol green, which forms a yellow-coloured complex with alkaloids. The absorbance was measured at 470 nm and a standard curve based on a known alkaloid standard (quinine) was used to determine the concentration.

UNDER PEER REVIEW

Tannins: The extract was mixed with Folin-Denis reagent, which reacts with tannins to yield a blue colour. The absorbance was measured at 760 nm and a standard curve based on tannic acid was used to determine the concentration.

Saponins: The extract was reacted with Vanillin and Sulfuric acid to form a red colour. The absorbance was measured at 560 nm and a standard curve based on digitonin was used to determine the concentration.

Phenols: The extract was mixed with Folin-Ciocalteu reagent to produce a blue colour. The absorbance was measured at 760 nm and a standard curve based on gallic acid was used to determine the concentration.

Cardiac Glycosides: The extract was hydrolyzed with concentrated H_2SO_4 to release aglycones, which are then reacted with acetic anhydride to yield coloured complexes, mostly reddish-brown or blue. The absorbance was measured at 620 nm and a standard curve based on digoxin was used to determine the concentration.

Oxalates: The extract was added to calcium chloride solution to precipitate calcium oxalate and then filtered to collect the precipitate (calcium oxalate). The calcium oxalate was dissolved in concentrated sulfuric acid and then allowed to cool at room temperature. The dissolved oxalate solution was now titrated with a standard potassium permanganate solution until a permanent pink which indicated the endpoint. The volume of potassium permanganate used for the titration was noted and used in the calculation of the amount of oxalate.

Phytates: The extract was added to Wade's reagent (ferric chloride-sulfuric acid)to form a coloured complex with phytates. The absorbance was read at 500 nm and a standard curve based on sodium phytate was used to determine the concentration.

Chemical characterization Gas chromatography-mass separation (GC-MS)

The phytochemicals in MEDAN were characterized using a GC-MS QP2010 Plus (Shimadzu, Japan). Identification of the phytochemicals in the sample was performed with a QP2010 gas chromatography system equipped with a Thermal Desorption System (TD 20) linked to Mass Spectroscopy (Shimadzu, Japan). The ionization voltage was set to 70 eV. Gas chromatography was conducted in temperature programming mode using a Restek column (0.25 mm, 60 m, XTI-5). The initial column temperature was set at 80ºC for 1 minute, then increased linearly at 70ºC per minute to 220ºC, held for 3 minutes, followed by a linear increase of 10ºC per minute to 290ºC for 10 minutes. The injection port temperature was maintained at 290ºC, and the GC-MS interface was also kept at 290ºC. The sample was introduced through an all-glass injector operating in split mode, with a helium carrier gas flow rate of 1.2 mL per minute.

Compounds were identified by comparing their retention times and fragmentation patterns with the mass spectra obtained from the GC-MS. The identity of the active components in the extract

UNDER PEER REVIEW

was verified by comparing their retention indices, peak area percentages, and mass spectral fragmentation patterns against data stored in the National Institute of Standards and Technology (NIST) digital library to confirm the names, molecular weights, formulas, structures, and bioactivities of the compounds.

RESULTS

Table 1: Proximate analysis of fresh leaves of *Datura alba ness*

S/N	Constituents	Content (%)
1.	Crude fibre	4.77±0.01
2.	Fat	1.19 ± 0.01
3.	Ash	4.17 ± 0.01
4.	Carbohydrate	7.60 ± 0.01
5.	Moisture content	60.4 ± 0.01
6.	Crude protein	21.9 ± 0.01

Table 1 presents the results of the proximate analysis of fresh *Datura alba ness* leaves. The data reveal that the leaves contain 60.4% moisture. Protein (21.9%) and carbohydrates (7.60%) are identified as the most and second most abundant nutritional components, respectively, while fats (4.17%) are the least abundant dietary component.

Table 2: Qualitative and quantitative phytoconstituents of methanol extract of *Datura alba nes*

Phytochemical Constituent	Qualitative	Quantitative (mg/100g)	Quantitative (%)
Flavonoids	+++	12.62 ± 0.01	49.14
Alkaloids	+++	4.92 ± 0.02	19.16
Tannins	+++	3.13 ± 0.02	12.19
Saponins	++	3.01 ± 0.01	11.72
Phenols	++	1.37 ± 0.01	5.33
Cardiac glycosides	+	0.49 ± 0.02	1.91
Oxalates	+	0.11 ±0.11	0.43
Phytates	+	0.03± 0.01	0.12

+ present
++ moderately present
+++ highly present

Table 2 presents the qualitative and quantitative analysis of the phytoconstituents in the methanol extract of fresh Datura alba Ness leaves. The data indicate that the extract contains a significant amount of valuable phytoconstituents. Flavonoids were found to have the highest concentration (12.62 mg/100g, 49.14%), followed by alkaloids (4.92 mg/100g, 19.16%). Oxalates and phytates had the lowest concentrations, at 0.11 mg/100g and 0.03 mg/100g, respectively.

UNDER PEER REVIEW

Table 3: The Gas Chromatographic-Mass Separation (GS-MS) spectral analysis of methanol extract of *Datura alba ness*.

Peak number	Retention time	Area time	Area %	Name of compound
1	10.737	8328159	3.65	Hydrocinnamic acid
2	12.275	755279	0.33	beta-Phenylethyl butyrate
3	17.867	3075029	1.35	1-Tetradecanol
4	19.101	29367983	12.87	n-Hexadecanoic acid
5	20.391	2957377	1.30	9,12,15-Octadecatrien-1-ol
6	20.492	3684843	1.61	13-Hexyloxacyclotridec-10-en-2-one
7	21.190	816331	0.36	Methyl stearate
8	21.278	9140328	4.00	9,12-Octadecadienoic acid
9	21.350	13824365	6.06	7-Hexadecenal
10	21.611	2081058	0.91	Octadecanoic acid
11	22.045	1947820	0.85	Tetracosane
12	22.209	9660346	4.23	Dodecanoic acid,1,2,3-propanetriylest
13	22.941	2437672	1.07	cis-11-Tetradecen-1-ol
14	23.359	5424929	2.38	Ricinoleic acid
15	24.036	1512491	0.66	Tetracosane
16	25.202	36461427	15.97	Bis(2-ethylhexyl)phthalate
17	25.264	7109481	3.11	Stigmasterol
18	26.329	4768528	2.09	9-Tetradecenal, (Z)-
19	26.470	2986245	1.31	Linoleic acid ethyl ester
20	26.536	3831073	1.68	Dotriacontane
21	26.748	24577804	10.77	Gamma.-Sitosterol
22	27.001	4965656	2.18	Cholest-5-en-3-ol, 24-propylidene-(3.
23	27.395	2128723	0.93	Squalene
24	27.633	996996	0.44	1-Monooleoylglycerol
25	28.003	32551778	14.26	13-Hexyloxacyclotridec-10-en-2-one
26	28.090	6254316	2.74	9,19-Cyclolanost-24-en-3-ol
27	28.367	2383390	1.04	Delta.-Tocopherol
28	28.731	1641813	0.72	Tetrapentacontane
29	29.204	2590497	1.13	Gamma.-Tocopherol

UNDER PEER REVIEW

Table 3 lists the biomolecular compounds identified in the methanol leaf extract of *Datura alba ness,* with a total of twenty-nine compounds detected. The five most abundant compounds are Bis(2-ethylhexyl) phthalate (15.97%), 13-Hexyloxacyclotridec-10-en-2-one (14.26%), n-Hexadecanoic acid (12.87%), γ-Sitosterol (10.77%), and 7-Hexadecenal (6.06%).

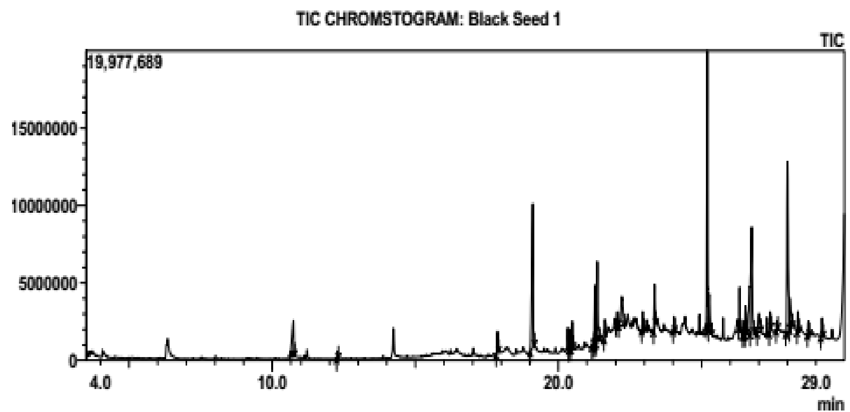

Figure 1: Total ionic chromatogram analysis of methanol leaf extract of *Datura alba ness* extract.

Figure 1 displays the total ion chromatogram analysis of the methanol leaf extract of *Datura alba ness.* The analysis shows that the extract reaches its peak height of 19,977,689 between 25 and 26 minutes."

DISCUSSION

Medicinal plants serve as a crucial source of novel herbal medicines, with the global demand for herbal drugs increasing significantly in recent decades [5]. The World Health Organization estimates that 80% of the global population relies on herbal and folk medicines as sources of therapeutic agents and active chemical constituents, fueling the rapid growth of related healthcare sciences[19]. Numerous active phytochemicals—such as terpenoids, tannins, alkaloids, phenols, flavonoids, steroids, glycosides, and volatile oils—have been extracted from medicinal plants, including their crude extracts and essential oils. These secondary metabolites have been linked to the treatment of both curable and incurable diseases[9, 20]. The present study provides a comprehensive analysis of the nutritional and phytochemical properties of *Datura alba Ness*, a common speciesof Datura found in Nigeria.

Proximate analysis of fresh leaves of DAN revealed the presence of moisture (60.4%), crude protein (21.9%), carbohydrate (7.6%), crude fibre (4.77%), ash (4.17%) and fat (1.19%) with protein and carbohydrates being the most abundant dietary components (Table 1). Leaves are among the most abundant and affordable sources of protein. The nutritional quality of protein depends on the balance of essential amino acids, which humans cannot produce and therefore must obtain through their diet [21]. The present study reveals that DAN is a good source of protein. This is higher than what was observed for *Datura metel*[22] and *Datura stramonium*

UNDER PEER REVIEW

leaves [23]. Carbohydrates form a major class of naturally occurring organic compounds that are essential for the maintenance and sustenance of life in both plants and animals, while also serving as key raw materials for various industries. [23]. Data from the study suggests that DAN is equally a good source of carbohydrates, providing lower carbohydrate content when compared with *Datura stramonium* leaves [23]. Also, DAN was shown here as a good source of dietary fibre although it had lower fibre content compared to *Datura stramonium* leaves [23]. Dietary fibres are the indigestible parts of plant-based foods, primarily found in fruits, leaves, vegetables, and grains. Unlike other carbohydrates, they cannot be broken down by the body's digestive enzymes. Instead, they pass through the digestive system relatively intact, providing several health benefits such as reduced body weight and reduced risk of cardiovascular diseases [24, 25].The study also showed that *Datura alba ness* (DAN) is a good source of ash (4.17%), indicating the presence of various mineral elements in the leaves. Additionally, the high moisture content (60.4%) suggests that it could accelerate the growth of microorganisms, potentially shortening the shelf life of stored fresh leaf samples[26]. The low-fat content (1.19%) might imply that the leaves are less palatable as dietary fats are known to enhance the taste of food by absorbing and retaining flavor.

The result of phytochemical analysis shows that DAN contains large quantities of flavonoids, alkaloids, tannins, saponin and phenols with small quantities of cardiac glycosides, oxalates and phytates (Table 2). The presence of this array of phytochemicals confers on DAN very pharmacological properties. The study shows that flavonoids (49.14%) were the most abundant phytochemical constituting half of the active constituent of the leaf. Flavonoids are one of the most diverse and widespread groups of natural compounds. The presence of hydroxyl groups grants them scavenging properties and plays a crucial role in preventing lipid peroxidation[27, 28]. They have been reported to exhibit anti-inflammatory effects, and hypoglycemic properties, and may reduce glucagon levels, enhancing glucose utilization and lowering blood glucose, while also stimulating insulin release from the pancreas[29].Extracts from seeds and leaves of other *Datura* species have been demonstrated to contain a significant quantity of flavonoids [30] with potent antidiabetic, hypoglycaemic and anti-inflammatory activities [30-33]. Alkaloids were the second most abundant phytochemical found in MEDAN (14.92%). Alkaloids are natural compounds containing heterocyclic nitrogen atoms, historically used in the preparation of spices, medicines, and poisons[28, 34].They exhibit a range of pharmacological effects, including anti-inflammatory, antibacterial, antimitotic, analgesic, local anaesthetic, hypnotic, psychotropic, and antitumor activities, among others[35, 36]. Extracts from the seed and leaves of other *Datura* species have been demonstrated to exhibit analgesic, antibacterial and psychotropic activities [37-39]. Saponins (12.19%) and tannins (11.72%) which were found in almost equal concentration are also important phytoconstituents which have been shown to possess potent anti-microbial, hypocholesterolemic and anti-diabetic properties[40, 41]. Extracts from the seed and leaves of other *Datura* species have been demonstrated to exhibit potent anti-microbial and hypocholesterolemicactivities [30, 31, 42]. The low concentrations of cardiac glycosides (1.91%), oxalates (0.43%) and phytates (0.12%) in the MEDAN show that it less likely to be

toxic as these phytochemicals are known to be toxic at high concentrations [43, 44]. However, some extracts from seeds and leaves of other *Datura* species have been shown to exhibit toxicity [10, 45, 46].

Furthermore, the results from Gas chromatography and mass spectrometry (GC-MS) analysis of MEDAN identified twenty-nine (29) active compounds with four (4) identified as the most abundant (with area concentration >10%). These abundant compounds include Bis(2-ethylhexyl)phthalate (15.97%), 13-hexyloxacyclotridec-10-en-2-one (14.26%), n-hexadecanoic acid (12.87%) and Gamma.-Sitosterol (10.77). Both long- and short-term exposure to Bis(2-ethylhexyl)phthalateis toxic to humans and animals [47-49]. However, 13-hexyloxacyclotridec-10-en-2-one is said to possess antimicrobial and anti-inflammatory effects and may protect against liver damage [50]. On the other hand, n-hexadecanoic acid acts as an antioxidant, helping to alleviate oxidative stress and protect cells from damage. Also, other studies suggest that studies suggest that n-hexadecanoic acid may have anti-inflammatory properties, potentially helping to manage conditions like rheumatoid arthritis and asthma[51, 52]. Gamma-sitosterolcan help lower cholesterol levels by competing with cholesterol for absorption in the intestines, potentially reducing the risk of heart disease. Also is said to possess anti-inflammatory properties while reducing the risk of cancer and boosting the immune system [53-55].

CONCLUSION

The presence of various nutrients, compounds, and bioactive substances identified through proximate, phytochemical, and GC-MS analysis of fresh leaves and methanol extract of *Datura alba ness* supports its potential use as a supplement and therapeutic agent. Based on these findings, it can be concluded that *Datura albaness* contains several bioactive compounds that could serve as effective treatments for diseases related to oxidative stress, nervous system disorders, tissue damage, inflammation, and bacterial infections.

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UNDER PEER REVIEW

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UNDER PEER REVIEW

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UNDER PEER REVIEW

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UNDER PEER REVIEW

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