Title: Chemical analysis and *in-vitro* antioxidant potential of *Datura stramonium* seed-oil grown in Ogbomoso Southwest Nigeria

Abstract:

***Objective*:** The present study analyzed the chemical components and the antioxidant potentials of and antimicrobial activities of the oil isolated from *Datura stramonium* seeds that were grown in Ogbomoso, Southwest, Nigeria.

***Methods*:** Fresh seeds of *Datura stramonium* were collected in the premises of LAUTECH Teaching Hospital. Ogbomoso, South West Nigeria around February 2018. The seed oil was thereafter extracted with 70% methanol and concentrated with a rotary evaporator. The iodine, saponification, acid, peroxide values were determined titrimetrically. An *in-vitro* 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, total phenol content, reductive potential, ferric reducing antioxidant assay, and hydroxyl radical scavenging activity was used to determine the antioxidants activity of the oil spectrophotometrically. Gas chromatography-mass spectrophotometry (GC-MS) and high-performance liquid chromatography (HPLC) were used to identify the chemical constituents of the oil.

***Results*:** The iodine, saponification, acid, and peroxide values of the oil were 86.15 ± 1,01 mg/g oil, 253.85 ± 0.98 MgKOH/g, 5.27 ± 0.032 mgKOH/g, and 2.80 ± 0.79 milli – equivalentO_2/kg respectively. A concentration-dependent increase in *in-vitro* antioxidant activities characterized the data from all the antioxidant parameters tested. While results obtained from GC-MS analysis reveals that the *Datura stramonium* seed oil contained phenolic compounds, alkene compounds, aliphatic compounds, amide compounds, and fatty acid, HPLC identified the presence of catechin, rutin, and kaempferol

***Conclusion*:** Oil from *Datura stramonium* seed has potent antioxidant potentials and can therefore be looked into for possible utilization.

Keywords: antioxidants, chromatography, *Datura stramonium,* free radicals, seed oil.

Introduction:

Datura Stramonium is also known as Jimson weed, Locoweed, Angel's trumpet, Devil's trumpet. Its common name is Africa Thorn apple and Gegemu in Yoruba. It is a hallucinogenic plant found in the urban and rural areas, in cornfields, and along roads sides (Bouziri *et al*., 2011). Datura genus is widely distributed across the tropical and warm temperate regions of the world (Parashuram, 2011). *Datura Stramonium* is native to North America, Europe, Asia, and Africa (Khare *et al*., 2007). It is germinated through seeds. The germination period of the seed is usually 3 to 6 weeks. When properly stored, the seeds will remain viable even after several years (Joshi, 2000). *D. Stramonium* is an annual plant. It herbaceous stem, with branched, and glabrous or only lightly hairy. When cultivated, the *Datura Stramonium* plant can grow up to a height of about 1 meter (Norton *et al*., 2008). The stems of Datura Stramonium are highly branched and are spreading; the stems are also stout, leafy, smooth, erect, with pale yellowish-green in appearance. Leaves are simple dentate, slightly hairy, oval glabrous, bid, and about 4 to 6 inches long. *Datura Stramonium* bears funnel-shaped, white, or purple-colored flowers. The average length of the flower is about 3 inches. Seeds are large and full of thorns (Gaire *et al*., 2008).

About ten species of Datura are known, out of which *Datura anoxia* and *Datura Stramonium* are mostly researched into with potent phytochemical of pharmacologic importance. It had been reported that every part of the *Datura Stramonium* plant, such as the leaves, stems, flowers, seeds, seeds, and even the root are all capable of producing secondary metabolites that are of medical importance (Attama *et al*., 2009).

Researchers are lately showing more and more interest in the effective biological potentials of oils, especially those of plant origin. These biological activities include but are not limited to their antioxidant capacities (Al-Aamri *et al*., 2018; Elgohary *et al*., 2018; Asari *et al*., 2019). Furthermore, increasing data from previous studies have suggested the usage of oils in the food and drug industries as a source of natural antioxidants due to their potential antioxidant capacities and extremely low toxic potentials (Amorati *et al*., 2013; Badekova *et al.,* 2021). The antioxidants and other biological activities of many of these oils from plant origin can be attributed to the presence of the plant's secondary metabolites such as phenol, flavonoids, and others (Al-Aamri *et al*., 2018).

Consequently, the present study aimed to assess the quality and the antioxidant potentials of *Datura Stramonium* seed oil.

Materials and Methods:

Plant material

Fresh seeds of *Datura Stramonium* were collected in the premises of LAUTECH Teaching Hospital. Ogbomoso, South West Nigeria around February and were authenticated by Prof. A.J. Ogunkunle, of the Department of Pure and Applied Biology, LAUTECH, Ogbomoso, Oyo State, Nigeria.

Collection and extraction procedure

Datura Stramonium seeds were washed with clean water, cut into small pieces, and were allowed to air-dried under room temperature after which the dried seed was ground to a very fine powder with the aid of an electric blender. The powdered seeds of *Datura Stramonium* (2 kg) were exhaustively extracted with 6 liters of 70% methanol. The methanolic filtrate was then concentrated with the aid of rotary evaporator at 60°C to obtain a dark-brown oily substance.

Determination of free fatty acid value

10 g of the sample was weighed into a conical flask containing 50 ml ethanol that had been previously neutralized by the addition of 2 ml phenolphthalein solution. The mixture was thoroughly vortexed and placed in a water bath at 40°C. The mixture under this condition is gently titrated with 0.25 N Sodium hydroxide (NaOH) with vigorous shaking until a permanent faint pink color that persists for at least 1 minute is developed to obtain the titer value. The free fatty acid value of the sample was calculated from the titer value using the following formula:

$$FFA = \frac{28.2 \times N \times V}{W}$$

Where:

N = normality

V = volume NAOH used (titre value)

W = weight of the sample

Determination of saponification value

25 mL of potassium hydroxide/ethanol was added to 2 g of the test sample in a conical flask with an attached reflux condenser. The mixture was heated continuously in a boiling water bath for 30 minutes to allow all the fat to saponify completely. 1 mL of phenolphthalein/ethanol was added to the mixture immediately after boiling and the excess of alkali was titrated with 0.5 N hydrochloric acid and the titer value was noted. A blank test containing the same quantities of reagents but without the test sample was equally carried out and the titer value was also noted.

The saponification value of the sample was calculated from the titer values using the following formula:

$$\text{Saponification value} = \frac{(V2 - V1) \times 0.028805 \times 1000}{weight\ (g) of\ the\ test\ sample}$$

Where:

V_1 = titer value of the sample

V_2 = titer value of the blank

Determination of iodine value

The iodine value of the sample was done using the method of Wijs (Pearson, 1970). The Wijs'reagent was prepared by dissolving 8.0 g iodine trichloride (ICl_3) in 200 mL glacial acetic acid and 9.0 g iodine in 300 mL CCl_4. The two solutions were mixed and diluted to 1 L with glacial acetic acid. 0.3 g of the test sample was weighed and dissolved in carbon tetrachloride (CCl_4) and 25 mL of Wijs' reagent was added. The mixtures were thoroughly mixed and allowed to stand for 30 minutes in the dark. 20 ml of 10% potassium iodide solution and 100 mL distilled water were added. The iodine liberated was titrated with 0.1 M sodium thiosulphate solution using starch as the indicator just before the endpoint. A blank test containing the same quantities of reagents but without the test sample was equally carried out. The iodine value of the sample was calculated from the titer values using the following formula:

Iodine value (mg I_2/100 g sample) = $\frac{(V1 - V2) \times 1.269}{W}$.

Where:

V_1 = volume (cm^3) of thiosulphate used to titrate blank

V_2 = volume (cm^3) of thiosulphate used to titrate test sample

W = weight (g) of the test sample.

Determination of peroxide value

10 ml of acetic acid chloroform solution (ratio 4/6) was added to 0.3g of the test sample in a 250 ml flask with a stopper shaking vigorously to allow the test sample to dissolve after which 1 ml

of potassium iodide was added and the entire mixture was allowed to stand in the dark for 5 minutes at room temperature. 20 ml of distilled water was thereafter added with continuous shaking. The liberated iodine was titrated with 0.01 N sodium thiosulphate solution and once a light yellow coloration was observed 1 ml of 1.5% starch solution was added as an indicator and titrated till the mixture turned colorless. A blank test containing the same quantities of reagents but without the test sample was equally carried out. The peroxide value of the sample was calculated from the titer values using the following formula:

Peroxide value (meq/kg) = $\frac{(V1-V2)\times C \times 1000 \times N}{W}$

Where:

V_1 = titer value of the sample

V_2 = titer value of the blank

C = molar concentration of sodium thiosulphate

N = normality of sodium thiosulphate

W = weight of the test sample.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity

DPPH free radical scavenging activity of the oil was assayed using the DPPH photometric method of Mensor *et al*. (2001). When an antioxidant compound that is capable of donating hydrogen reacts with DPPH, the DPPH is reduced with a concomitant change of color from deep violet to golden/light yellow can be monitored at 518 nm. Briefly, 1 mL of 0.3 mM of DPPH solution was added to 1 ml of the oil sample, the resultant mixture was incubated at room temperature in the dark for 30 min. The absorbance values were read at a wavelength of 518 nm.

The percentage antioxidant activity was then calculated from the absorbance, using the formula below:

$$DPPH\ scavenging\ effect\ (\%)\ = \left(\frac{Acontrol - Asample}{Acontrol}\right) X\ 100$$

Total phenol content

This was estimated as described by McDonald *et al*. (2001). The assay is based on the reduction of Folinn-Ciocalteu reagent by the phenolic compounds. The reduced Folinn-Ciocalteu reagent is blue and the absorbance can be measured at the wavelength of 500-750 nm. Briefly, 1 ml of the oil sample was added to 0.2 mL each of Folin-Ciocalteu reagent and 2 mL of distilled water. One mL of 15% Na_2CO_3 was mixed with the solution. The mixture was incubated for 30 minutes at 40°C and the absorbance was thereafter read at 760 nm. The total phenol content of the sample was expressed as μg/mg of gallic acid equivalent (GAE).

Reductive potential

The method of Oyiazu (1986) was employed for the assessment of the reductive potential of the oil sample. 150 μL of the oil sample in 1 mL of distilled water was mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. 2.5 mL of 10% trichloroacetic acid (TCA) was added and the solution was centrifuged at 1000 X g for 10 min. 2.5 mL of the upper layer of solution was thereafter mixed with 2.5 mL of distilled water and 0.5 mL of 1% $FeCl_3$. The absorbance was read at 700 nm.

Ferric reducing antioxidant power assay (FRAP)

FRAP assay was done according to the method described by Tsai and co-workers (2011), with slight modification. FRAP uses antioxidants as reductants in a redox-linked colorimetric method to test the total antioxidant power directly. A reagent containing 0.83 mmol L1 TPTZ and 1.67

mmol L1 ferric chlorides at 0.1 mol L1 acetate buffer (pH 3.6). The sample was mixed with 0.9 ml of reagent and incubated at 25°C for 10 min Formation of a blue-colored ferrous tripyridyltriazine (Fe2+ -TPTZ) from a yellow ferric tripyridyltriazine (Fe3+ -TPTZ) was observed at an absorbance of 593 nm. Values were expressed in µmole Fe^{2+}/mg dry weight of the test sample. Gallic acid was used as the standard reference.

Hydroxyl radical ($OH^{\cdot}$) scavenging activities

All solutions were freshly prepared before the assay. One mL of the reaction mixture contained 100 µL of 2.8 mM 2-deoxyribose (dissolved in phosphate buffer (10 mM), pH 7.4), 500 µL solution of various concentrations of the extract (25, 50, 75 and 100%), 200 µL of 200 µM $FeCl_3$ and 1.04 µM EDTA (1:1 v/v), 100 µL of H_2O_2 (1.0 mM) and 100 µL of ascorbic acid (1.0 mM). After an incubation period of 1 hour at 37°C, the extent of deoxyribose degradation was measured by TBA reaction (Halliwell *et al*., 1987). The % inhibition of hydroxyl radical was calculated and calculation of results as described above.

Gas Chromatography-Mass Spectroscopy

The GC-MS analysis was carried out according to the method described by Ajayi *et al*. (2011) using a Hewlett Packard Gas Chromatograph (Model 6890 series) furnished with a flame ionization detector and Hewlett Packard 7683 series injector, MS transfer line temperature of 250°C. The GC is equipped with a fused silica capillary column- HP-5MS (30 x 0.25 mm), film thickness 1.0 µm. The oven temperature was held at 50°C for 5 mins holding time and raised from 50 to 250°C at a rate of 2°C /min, using helium (99.99%) as carrier gas at a constant flow of 22 cm / s. One micron extract (1 mg dissolved in 1 ml of absolute alcohol) in a divided ratio of 1:30 was injected. The MS analysis was performed on an Agilent Technology Network mass model (model 5973) coupled to a Hewlett Packard gas chromatograph (model 6890) equipped

with a NIST08 library software database. Mass spectra were recorded at 70 eV/200°C; Scan speed of 1 scan / s. Compounds were identified using the NIST library database. The mass spectrum of a single unknown compound was compared with the known compounds stored in the software database of the NIST library.

High-Performance Liquid Chromatography (HPLC)

HPLC was carried out using an apparatus consisting of a pump (Model LC10AT and FCV-10al) and DGU-14A degasser (Shimadzu, Tokyo, Japan), equipped with a UV-VIS detector model L-4250 (Merch Hitachi, LTD Tokyo, Japan). Data acquisition was performed by an interface Model Data Apex CSW32 (Prague, Czech Republic) with a PC. A reversed-phase Supelcosil column LC18 (15 cm x 4.6mm, ID 3 mm particle size), and a Supelguardcartridge column (Supelco, Bellafonte, PA) were used for the chromatographic analysis. The absorbance of the eluted compounds was monitored at 242 nm. The mobile phase was acetonitrile-water 85 –15 (v/v) at a flow rate of 1 mLmin. The peaks were identified by their retention times in comparison to the external standards. The concentrations of individual compounds were determined using the calibration curve of the oil.

Results

Table 1 showed the iodine value, saponification value, free fatty acid value, and the peroxide value of *Datura Stramonium* seed oil. As presented value for each of the parameter determined were 86.15 ± 1,01 mg/g oil, 253.85 ± 0.98 MgKOH/g, 5.27 ± 0.032 mgKOH/g, and 2.80 ± 0.79 milli – equivalentO_2/kg respectively.

Table 2 depicts the mean value of percentage DPPH scavenging activities of *Datura Stramonium* seed oil. The seed oil caused a concentration-dependent increase in the percentage of DPPH radical scavenging activities. For instance, the lowest concentration of 25% led to 28.37 ±

0.021% DPPH radical scavenging activity, while the highest concentration of 100% induced 82.57 ± 0.19% DPPH radical scavenging activities.

The mean value of total phenol contents of *Datura Stramonium* seed oil using garlic acid as the standard was presented in Table 3. As showed, the seed oil contained an appreciable quantity of phenolic compounds. The data indicated that the contents of the total phenol in the seed oil increased as the concentration of *Datura Stramonium* seed oil tested increased. That is the lowest concentration of 25% contained the lowest amount of phenolic compounds (0.170 ± 0.003 µg/mg GAE), while the highest concentration of 100% contained the highest amount of total phenols (0.238 ± 0.009 µg/mg GAE)

The data obtained from the assessment of reductive potentials of *Datura Stramonium* seed oil are presented in Table 4 expressed as mean value ± SEM. The data presented showed that the *Datura Stramonium* seed oil possessed reductive potentials when tested *in vitro*. As shown, 25, 50, 75, and 100% *Datura Stramonium* seed oil when tested *in vitro* potential power in the order 0.129 ± 0.03 µg/Ml, 0.150 ± 0.02 µg/Ml, .0170 ± 0.05 µg/Ml and 1.110 ± 0.008 µg/Ml respectively.

The mean value of ferric reducing antioxidant power of *Datura Stramonium* seed oil using garlic acid as reference antioxidant is depicted in Table 5. As presented, *Datura Stramonium* seed oil at 25% concentration showed 66.78 ± 0.18 mg/ml ferric reducing antioxidant power, while at 50, 75 and 100%, the antioxidant power increased to 81.32 ± 0.79 mg/ml, 89.26 ± 0.35 mg/ml and 121.33 ± 0.48 mg/ml respectively.

The percentage hydroxyl radical scavenging activity of various concentrations of *Datura Stramonium* seed oil was presented in Table 6. The seed oil when tested *in vitro* showed 65.08 ±

0.0034 percentage hydroxyl radical scavenging activities at 25%, 76.07 ± 0.009 percentage hydroxyl radical scavenging activities at 50% and 83.14 ± 0.006, 98.78 ± 0.005 percentage hydroxyl radical scavenging activities at 75% and 100% respectively indicating a concentration-dependent increase hydroxyl radical scavenging activities of the *Datura Stramonium* seed oil.

As presented in Table 7, GC-MS identified phenolic compound, alkene compound, aliphatic compound, amide compound, and fatty acids in *Datura Stramonium* seed oil

As showed, Table 8 indicated that three different compounds were identified in the *Datura Stramonium* seed oil with the aid of high-performance liquid chromatography. The three compounds as identified by HPLC are: catechin, rutin, and kaempferol

Discussion

Previous researches have shown that consumption of oils has diverse effects on cellular lipid homeostasis and the development of chronic disease, and well-being (Kaufman and Wiesman, 2007). Since oil from various sources generally have different composition and none of the oil is suitable for all purposes (Ramadan *et al*., 2006), Researchers have therefore beamed their searchlight on new sources of edible oils especially from plant seeds which are known to be a good source of healthy oils. In this study, chemical characteristics, phenolic content, and antioxidant activity of *Datura Stramonium* seeds oil were determined.

Meanwhile, despite the acclaimed health benefits of oil from plant origin, it has been suggested that consumption of some of these oils may not be suitable for consumption as their consumption by a human may lead to the onset of cardiovascular diseases (AlKhattaf *et al*., 2020). In the present research, we did a preliminary assessment of the quality of *Datura Stramonium* seeds oil by the determination of its iodine value, saponification value, acid value, and peroxide value.

Iodine value is the most often quoted value of oil. It is a measure of the degree of unsaturation of oil. It is defined as the amount of iodine (in g) added to 100g of oil (Dijkstra, 2016). Since the oxidative stability and melting point are related to the degree of unsaturation, the iodine value, therefore, gives an estimation of these qualities. The greater the iodine value, the more the degree of unsaturation and the higher the susceptibility of the oil to oxidation. That is, oils with high iodine values contain more of the highly unsaturated fatty acids that are prone to fast degradation reactions such as autoxidation or polymerization. (Beyene *et al*., 2011). The observed high iodine value in the present study (86.15 ± 1,01 mg/g oil), depicted that *Datura Stramonium* oil has a high concentration of unsaturated fatty acids and possibly a low degree of saturation. Meanwhile, previous studies have recommended a switch from saturated fats to unsaturated fats because of the high risk of a cardiovascular disorder that is often associated with high consumption of saturated fatty-acids (De Souza *et al*., 2015; Li *et al*., 2015; German and Dillard, 2004; Nettleton, 1995). Meanwhile, highly unsaturated oils can, however, undertake oxidative degradation as a result of their double bonds (Adetola *et al*., 2016) except sufficient antioxidant is added (Xiu-Qin *et al*., 2009; Cheung *et al*., 2007; Dziedzic and Hudson, 1983).

The saponification value indicates the molecular weights of triacylglycerol in an oil sample. The saponification value has an inverse relationship with the average chain length or weight of the fatty acids in the oil. That is, a higher Saponification value depicts a high amount of fatty acids with a lower carbon chain (Muhammad *et al*., 2011). Therefore, the shorter the average carbon length (C_4-C_{12}) the higher is the saponification value (Tamzid et al., 2007). The high value obtained for *Datura Stramonium* oil in the present study (253.85 ± 0.98 MgKOH/g) depicts that *Datura Stramonium* oil extract contains high amounts of short-chain fatty acids (< C_{12}). According to Codex Alimentarius (2005) and the Asian and Pacific Coconut Community

(APCC) Standards, the standard permissibility level of saponification values ranges between 250 – 260 mg KOH/g oil and 248 – 268 mg KOH/g oil respectively. This means that the saponification value of *Datura Stramonium* obtained in the present study fell within the Codex and APCC standard permissible level and thus meets up with the required standard. The high saponification value indicates that *Datura Stramonium* oil has the potential to be used in the cosmetic industry (Akbar *et al*., 2009), and may also be suitable for soap making (Akinhanmi and Atasie, 2008).

The fatty acid value is the number of milligrams of potassium hydroxide (KOH) that is required to neutralize the fatty acids in 1 gram of sample. It is a measure of the amount of free fatty acids in the oil. Normally, fatty acids are found in the triacylglycerol form, however, during extraction/processing the fatty acids may get hydrolyzed into free fatty acid and glycerol in a reaction catalyzed by lipase. The higher the value of fatty acid of oil, the higher the level of free fatty acids which is an indication of decreased oil quality. According to the American Oil Chemist Society, the acceptable levels for all oil samples should be $\leq$ 0.6 mg KOH/g (AOCS, 2003; Mozaffarian and Clarke, 2009). The acid values obtained in this study for *Datura Stramonium* were 5.27 mg KOH/g, which is above the permissible level of 0.6 mg KOH/g (AOCS, 2003; Mozaffarian and Clarke, 2009). The high acid value displayed by *Datura Stramonium* oil depicted that most of the triacylglycerol contained in the oil had been hydrolyzed to free fatty acids which suggest reduced oil quality

The peroxide value of any oil determines its oxidative state. It is equally an important marker in determining the quality of the oil. Since peroxide is the early product of oxidation, the peroxide value of oil provides information on how fresh is the oil. Elevated peroxide value is an indication that lipid oxidation had taken place (Dijkstra, 2016). A peroxide value that is more than 9

meqO_2/kg is an indication of a bad oxidation state of the oil (Ozcan, 2009). As reported in this study, a peroxide value of 2.8 – equivalentO_2/kg indicates that the *Datura Stramonium* oil used in this study is in a good state and has not been oxidized which represents good extraction and maintenance conditions. This means that *Datura Stramonium* seeds oil can be stored for a long time without becoming rancid as a result of oxidation. Meanwhile, a peroxide value of between 20 and 40 mill-equivalentO_2/Kg indicates that the oil is rancid and oxidatively damaged (Nehdi, 2011). Furthermore, the Peroxide value of *Datura Stramonium* oil is even lower than that of pomegranate seeds oil (3.42 mill-equivalentO_2/Kg), linseed oil (11.28 mill-equivalentO_2/Kg), and sunflower oil (12.87 mill-equivalentO_2/Kg) (Cerchiara *et al*., 2010; Amri *et al*., 2017).

Antioxidant generally refers to a compound that is capable of neutralizing free radicals typically by donating an electron to the free radicals to make the free radical to be more a stable compound. In other words, when an antioxidant compound donated an electron to the reactive radicals (free radicals), it will convert the radicals into a more stable and passive condition and thereby aborting the chain reaction of the free radical (Ganu *et al*., 2010). The ability of *Datura Stramonium* to function as an antioxidant was in this study access through its percentage DPPH scavenging activities, reductive potential, ferric reducing antioxidant power, percentage hydroxyl radical scavenging activity, and total phenol contents.

DPPH is a free radical that is stable at room temperature; it produces a purple coloration in methanol. In the presence of an antioxidant molecule, it is reduced and gives rise to a yellowish methanol solution (Badmus *et al*., 2010). The ability of a molecule to donate a hydrogen atom to a free radical is one of the mechanisms involved in the evaluation of antioxidant activity (Miliauskas *et* al., 2004). As shown, the DPPH radical scavenging activities of *Datura Stramonium* seed oil (Table 2) showed that the *Datura Stramonium* possesses high antioxidant

activity. This activity increases with increasing concentration in a dose-dependent manner. This observed antioxidant potential may be due to the presence of phenolic compounds in the *Datura Stramonium* oil as observed in this study.

The reducing power of a biological compound is related to its ability to transfer electrons, and it may therefore be used as an important marker of its likely antioxidant potential (Sanchez-Moreno, 2002). As presented in Table 4, the methanolic extract of *Datura Stramonium* showed a significant reductive capability. This reducing capability increased with increasing concentration of the *Datura Stramonium* seed oil. This finding implies that extract of *Datura Stramonium* possesses hydrogen ion (H^+) donating potential and as such has the potential to serve as an antioxidant. This finding is in total agreement with every other obtained in the present study.

The total antioxidant power of a plant extract can be assessed by Ferric Reducing Antioxidant Power assay (FRAP) (Szollosi and Varga, 2002). FRAP assay depends upon the ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reductant at low pH. Fe (II)-TPTZ is a substance with an intensive blue coloration that can be monitored spectrophotometrically at 593 nm. Data from previous studies reveal that FRAP assay is highly sensitive for the determination of the total antioxidant potential of the various biological substances, such as plant extracts. (Szollosi and Varga, 2002; Rattanachitthawat*et al*., 2010). In the FRAP method, the antioxidants in the biological compounds serve as a reductant in a redox-linked colorimetric reaction (Guo *et al*., 2003). In this study, the antioxidant capacity of the *Datura Stramonium* oil was measured as ferric reducing antioxidant power (FRAP). Data obtained revealed that oil extracted from the plant possesses great antioxidant capacity. This is not surprising as it is in total agreement with other data obtained in the present study

The hydroxyl radical is one of the most dangerous free radicals produced in cells (Medda *et al*., 2021). It is the most reactive product of reactive oxygen species generated from successive single electron reductions of molecular oxygen (O_2) during metabolic processes in the cell and is primarily responsible for the cytotoxic effects that are observed in aerobic organisms (Halliwell and Gutteridge, 1992). It is generally assumed that OH is generated in biological systems from H_2O_2 by the Fenton reaction (Halliwell and Gutteridge, 1992). It is considered to be one of the rapid initiators of the lipid peroxidation process; they do this by abstracting hydrogen atoms from polyunsaturated fatty acid (PUFA), which causes the peroxidation of membrane lipids. Hydroxyl radicals also abstract hydrogen atoms from each of the carbon atoms of the sugar moiety of DNA leading to oxidative damage of DNA (Badmus *et al*., 2011), and these effects have been implicated in mutagenesis, carcinogenesis, and aging (Halliwell and Gutteridge, 1999).

In this study, methanolic extract of *Datura Stramonium* was found to cause concentration-dependent hydroxyl radical scavenging activities indicated as percentage inhibition (Table 6). These data imply that methanolic extract of *Datura Stramonium* is a potent scavenger of hydroxyl radical and may therefore prevent the various hydroxyl radical-induced pathological conditions. These powerful hydroxyl radical scavenging activities exhibited by *Datura Stramonium* extract may be due to the presence of phytochemicals such as flavonoids and phenol as revealed by other data from the present study.

There is an established direct correlation between phenolic compounds and antioxidant activity (Sato, 1996). Phenolic compounds are generally regarded as the most important antioxidative compound in the plant. As a result of this, the total antioxidant ability of a plant largely depends on its phenolic contents (Moskovitz *et al*., 2002). Data obtained in this study that *Datura Stramonium* oil extract is rich in phenolic compounds. The concentration of these phenolic

compounds increases as the concentration of the oil increases from 25 to 100%. The result is further confirmed by the data obtained from both GC-MS and HPLC analysis of the *Datura Stramonium* oil extract. The high antioxidant activity of *Datura Stramonium* oil and its rich phenol contents further confirm the strong correlation between antioxidant activities and the concentration of phenolic compounds.

Data obtained from GC-MS analysis of the oil extract showed that *Datura Stramonium* oil extract contained phenolic compounds, fatty acids, aliphatic, amide, and alkene compounds. The presence of phenolic and other compounds in the oil extract further lend credence to the excellent antioxidant capacity of the oil extract and showed in the present study.

Furthermore, HPLC analysis confirmed the presence of catechin, rutin, and kaempferol in the *Datura Stramonium* oil extract. Catechins are flavonols that belong to polyphenolic compounds. The complex of Catechin, epicatechin, and epigallocatechin gallate, are the major flavonoids found in green tea (Li et al., 2018). Data from many previous studies revealed that catechin plays a vital role in protection against degenerative diseases owing to its strong antioxidant power (Ide et al., 2018). Many other researchers have also established an inverse relationship between catechin intake and the risk of cardiovascular diseases (Ikeda et al., 2018). Similarly, Cosarco and co-workers (2019) observed that catechin and its derivatives represent a class of phenolic compounds with a very wide therapeutic potential which includes; antiallergic properties, immunostimulatory, antidiabetic, antibacterial, antiviral, antioxidant, antitumor, lipid-lowering, and cardiovascular effects. Meanwhile, Rutin also referred to as rutoside, quercetin-3-rutinoside, and sophorin is a flavonol and is abundant in many plants, such as apples and tea. It is a very important nutritional component of food substances (Ganeshpurkar and Saluja, 2017; Harborne, 1986). Chemically rutin is a glycoside that is made up of flavonolic aglycone quercetin and

disaccharide rutinose. Previous studies have implicated rutin in several pharmacological activities such as antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective, and cardioprotective activities (Javed et al., 2012; Richetti et al., 2011; Calderón-Montaño *et al*., 2011). Similarly, Kaempferol is a yellow compound and one of the most encountered aglycone flavonoids in the form of a glycoside. It is a tetrahydroxyflavone in which the four hydroxy groups are positioned on carbon 3, 5, 7, and 40 (Li *et al*., 2015). It is a natural flavonol-type flavonoid that can be found in various parts (seeds, leaves, seeds, and flowers) of plants such as tea and many common vegetables. (Bhagwat *et al*., 2014; Rajendran *et al*., 2014; Sharifi-Rad *et al*., 2018). Kaempferol and its glycosylated derivatives have been identified in different medicinal plants and have been shown to exhibit cardioprotective, neuroprotective, anti-inflammatory, antidiabetic, antioxidant, antimicrobial, antitumor, and anticancer properties (Calderon-Montano *et al*., 2011).

Conclusion

In conclusion, our results indicated that *Datura Stramonium* seed oil possesses potent antioxidant potentials as evident in its ability to scavenged DPPH free radical, good total phenol contents, potent reductive potential, good reducing antioxidant power, ability to scavenge hydroxyl radicals, and presence of potent secondary metabolites as revealed by GC-MS and HPLC analysis of the seed oil.

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Table 1. Chemical parameter of *Datura Stramonium* seed oil

S/N	Parameters	Results
1	Iodine Value	86.15 ± 1,01 mg/g oil
2	Saponification Value	253.85 ± 0.98 MgKOH/g
3	Free Fatty Acid Value	5.27 ± 0.032 mgKOH/g
4	Peroxide Value	2.80 ± 0.79 milli – equivalentO_2/kg

Values are expressed as the mean of triplicate measurements ± standard error of the mean (SEM)

Table 2. Percentage DPPH scavenging activities of *Datura Stramonium* seed oil

Concentration	% DPPH scavenging activities
25%	28.37 ± 0.021
50%	50.48 ± 0.017
75%	68.84 ± 0.09
100%	82.57 ± 0.19

Values are expressed as the mean of triplicate measurements ± standard error of the mean (SEM)

Table 3. Total phenol contents of *Datura Stramonium* seed oil

Concentration	Total phenol content (µg/mg GAE)
25%	0.170 ± 0.003
50%	0.197 ± 0.001
75%	0.210 ± 0.017
100%	0.238 ± 0.009

Values are expressed as the mean of triplicate measurements ± standard error of the mean (SEM)

Table 4. The reductive potential of *Datura Stramonium* seed oil

Concentration	Reductive potential (µg/ml)
25%	0.129 ± 0.03
50%	0.150 ± 0.02
75%	.0170 ± 0.05
100%	1.110 ± 0.008

Values are expressed as the mean of triplicate measurements ± standard error of the mean (SEM)

Table 5. Ferric Reducing Antioxidant Power (FRAP) of *Datura Stramonium* seed oil

Concentration	Mean reducing antioxidant power (mg/ml)	Reference antioxidant
25%	66.78 ± 0.18	Gallic acid
50%	81.32 ± 0.79	Gallic acid
75%	89.26 ± 0.35	Gallic acid
100%	121.33 ± 0.48	Garlic acid

Values are expressed as the mean of triplicate measurements ± standard error of the mean (SEM)

Table 6. Percentage hydroxyl radical scavenging activity of *Datura Stramonium* seed oil

Concentration	% hydroxyl radical scavenging activity
25%	65.08 ± 0.0034
50%	76.07 ± 0.009
75%	83.14 ± 0.006
100%	98.78 ± 0.005

Values are expressed as the mean of triplicate measurements ± standard error of the mean (SEM)

Table 7. Chemical compounds identified by GC-MS in *Datura Stramonium* seed oil

Peak Number	Retention time (min)	Area (%)	Group of a compound identified
16	9.861	1.18	Phenolic compound
17	10.104	40.89	Phenolic compound
18	10.208	13.28	Phenolic compound
22	10.756	9.08	Alkene compound
23	11.080	2.54	Alkene compound
27	11.842	12.89	Phenolic compound
31	12.414	1.13	Alkene compound
35	13.095	2.27	Aliphatic compound
52	17.063	1.19	Fatty acid
57	18.703	2.42	Amide compound

Table 8. Compounds identified by HPLC in *Datura Stramonium* seed oil

Retention time (min)	Amount (PPM)	Identified compound
1.935	402.73788	Catechin
2.502	241.10211	Rutin
9.503	2.38724	Kaempferol

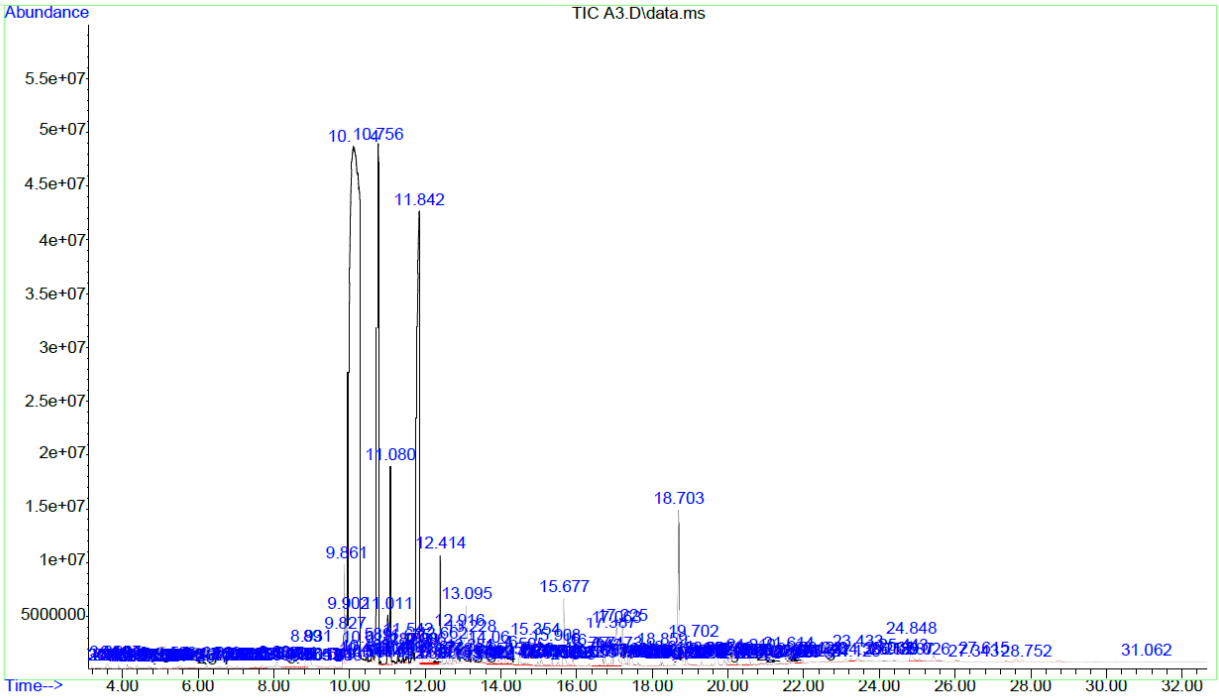

Figure 1. Gas chromatogram of *Datura Stramonium* seed oil

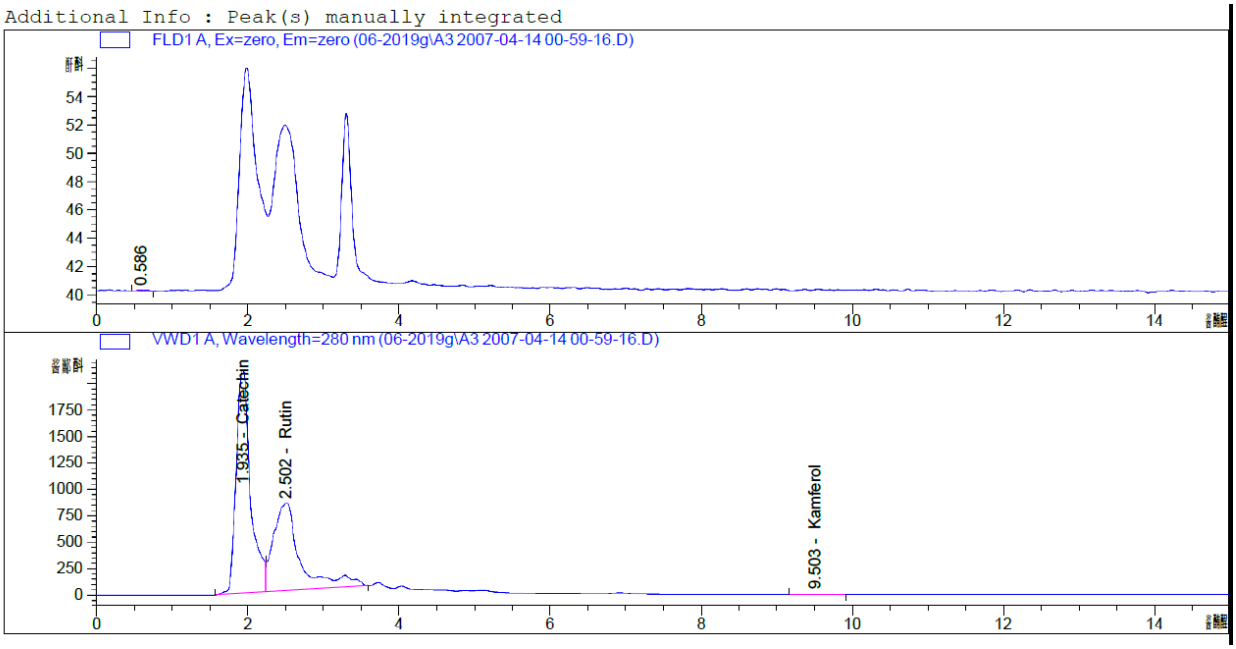

Figure 2. HPLC chromatogram of *Datura Stramonium* seed oil