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Chintua Ephraim Igara
Chemistry Research Unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic Unwana, P.M.B. 1007, Afikpo Ebonyi, Nigeria

Mecha Kalu Ndukwe
Chemistry Research Unit, Department of Science Laboratory Technology, AkanuIbiam Federal Polytechnic Unwana, P.M.B. 1007, Afikpo Ebonyi, Nigeria

Ahamefula Anslem Ahuchaogu
Department of Pure and Industrial Chemistry, Abia State University, Uturu, P.M.B. 2000, Uturu, Abia, Nigeria

Ignatius Uchenna Nwali
Chemistry Research Unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic Unwana, P.M.B. 1007, Afikpo Ebonyi, Nigeria

Chinweotito Glory Okpani
Chemistry Research Unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic Unwana, P.M.B. 1007, Afikpo Ebonyi, Nigeria

Corresponding Author:
Chintua Ephraim Igara
Chemistry Research Unit, Department of Science Laboratory Technology, AkanuIbiam Federal Polytechnic Unwana, P.M.B. 1007, Afikpo Ebonyi, Nigeria

Chemical composition by gas chromatography and mass spectrometry and antioxidant activity of *Cassia alata* plant leaf

Chintua Ephraim Igara, Mecha Kalu Ndukwe, Ahamefula Anslem Ahuchaogu, Ignatius Uchenna Nwali and Chinweotito Glory Okpani

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Abstract
Nature has provided humans with a diversity of medicinal plants employed in curing and taking care of many ailments. These medicinal plants contain many phytochemicals and bioactive compounds, which have been reported to possess some pharmacological properties. The study investigates the phytoconstituents and antioxidant activity shown by the *Cassia alata* plant *leaf*- a known medicinal plant. Gas chromatography-mass spectrometry (GC-MS) technique was applied in evaluating the chemical composition while Ferric reducing activity power (FRAP) and 2, 2-Dipheny l-1-picryl hydrazyl radical activity inhibition techniques were utilized to evaluate the antioxidant activity of methanol extract of the plant leaf. Ascorbic acid served as the standard. For different amounts of 50, 100, 200, and 400 µg/ml, the result of inhibitions of DPPH were revealed to be 4.14±0.00%, 12.99±0.55%, 19.55±0.06% and 25.73±0.17% respectively while that of the standard ranged from 18.41±0.17% -88.39±0.09%. Also, the FRAP results at different amounts of 50, 100, 200 and 400 µg/ml are detected to be 0.00±0.00% , 2.72±0.02%, 5.66±0.06% and 11.5±0.34% while the standard ranged from 5.09±0.16% - 52.99±0.21%.The GC-MS chemical assay of *C. alata* extracts afforded ten compounds which include 1-Butanol-3- methyl acetate, Pentadecane, 2, 6, 10, 14-tetramethyl., β-D-Glucopyranose-4-o-β-galactopyranosyl, Hexadecanoicacid- methyl ester, n-Hexadecanoic acid, 9, 12, 15-Octadecatrienoic acid methyl ester [ZZZ], Phytol, 9, 12, 15-Octadecatrienoic -2, 3- dihydroxyl propyl ester, Ethyl -iso-allocholate and Vitamin E. Hexadecanoic acid- methyl ester was found to the highest 77.1% in composition while 9, 12, 15-Octadecatrienoic -2- 3- dihydroxyl propyl ester 8.6% was detected to be the least. Some of these bioactive compounds have been observed to have pharmacological and biological activities which are desirable in pharmaceutical formulations.

Keywords: Antioxidant, phytochemicals, FRAP, DPPH, GC-MS, *Cassia alata*

1. Introduction
Mankind has for a long period of time benefited from plant resources in areas of food, shelter, clothing and health sustenance. Natural products from plants are used for food, fragrance, flavoring, spices, and medicine. Nature has provided humans with a diversity of useful plants with the potentials for curing and managing many ailments. For primary healthcare systems, about 75-85% of the world population mainly in developing countries rely on herbal and alternative medicines for their well-being (Cragg *et al.*, 1999; Parekh*et al.*,2005) [11, 34]. This is a result of their accessibility, affordability, lesser side effects and contra-indications. The clamor for medicinal plants is based on the chemical constituents of plants which have shown many biological and pharmacological activities.
Since the ancient period, extracts from plants and plant-related drugs have vastly played great roles to the general health of humans **(**Anyanwu and Nwosu, 2014) [5]. Naturally, plants have the capacity to undergo a biosynthesis of various phytochemicals which have the potential of carrying out some physiological and pharmacological activities (Jackson 1989) [22], thus necessitating their use in herbal medicine and pharmaceutical formulations.
Today, medicinal plants, their phytoconstituents, and knowledge about them with respect to their use as medications make up the field of phytomedicine (Subramanian *et al.*, 2019) [40]. Recently, scientist interests in medicinal plants, as a result of the increased efficacy of phytoconstituent preparations and challenges of side effects of modern medicine have received attention. Several studies have validated the scientific knowledge and application of medicinal plants in phytomedicine (Okwu and Igara, 2011) [30].

According to Bader *et al. (*2016) [9], many medicinal plants produce anti-inflammatory and antioxidant activities which protect them from cellular oxidation reactions and other microbes highlighting the importance of searching for natural medicine. The pharmacological properties of these plant products such as antimicrobial, antioxidant, antiviral, anti-tussive, anti-cancer, anti-inflammatory, anti-diabetic, memory enhancing, cholesterol-lowering, and hepato-protective are exploited in the pharmaceutical industries for synthetic and modern drug formulations (Newman and Cragg, 2020) [29]. The application of plant extracts in traditional medicine has attracted global interest. Alternative and herbal medicines have been promoted as serving as sources of affordable and comprehensive medicare, especially in developing nations (Igara *et al*., 2016) [20].

Free radicals are considered as causative agents of some organ-damaging health challenges such as inflammation, diabetes, cancer, liver disease, cardiovascular disease, Alzheimer's disease, aging, and memory loss (Sajeeth *et al*.,2011; Harsha *et al*.,2012; Pankaj *et al*., 2007) [37, 16, 33]. These free radicals are species with unpaired electrons and are very unstable. Examples are reactive oxygen species (ROS) which are in hydroxyl radical ($^{-}$OH), hydrogen peroxide (H_2O_2) superoxide anions (O^{2-}), singlet oxygen, and nitrogen oxide (Atta *et al*., 2017; Tiwari 2001) [8, 41]. These ROS disrupt enzyme reactions and cause damage to some cellular components through covalent binding and lipid peroxidation.

Free radicals are responsible for oxidative stress which results due to differentials between pro-oxidant species and oxidant inhibitors in the body system. Oxidative stress and inflammation are seen as major causative agents that are involved in the pathology of some chronic diseases and degenerative disorders (Harsha *et al*., 2012; Pankay *et al*., 2007) [16, 33]. Plants produce some antioxidant molecules that come with a wide range of mechanisms to counteract the actions of free radicals which have caused serious health impairments **(**Pankay*et al*., 2007) [33]. Synthetic oxidants inhibitors like butylhydroxyanisole (BHA) and butyl hydroxyl toluene (BHT) are used in food industries as preservatives and have been found to show some side effects in humans. Plants have been found to possess some natural antioxidant phytochemicals which the body accepts with fewer side effects; hence the use of herbal medicine is widely accepted and has increased world over (Arulpriya *et al*., 2010) [7].

Cassia alata, a plant of immense medicinal value belongs to the family Fabaceae. The name "candle bush" was given to it due to its framework of inflorescences as a flowering shrub (Abo, *et al.*, 2008.) [1]. It has the property of being an annual or bi-annual herb. The height of the plant is 1 to 4 m and it survives in temperate and humid zones. The shape of the leaves is oblong; leaflets are 5 to 14 with robust petioles and dense flowers. Zygomorphic flowers like that of *C. alata* have yellow flowers, 7 stamens and a pubertal ovary. *C. alata* plant is cultivated by seeds and dispersed by winds and insects 1500m above sea level (Farnsworth *et al.*, 1992; Hennebelle *et al.*, 2009) [14, 18].

Cassia alata (L.) is generally cultivated for its medicinal and ornamental purposes in tropical, subtropical, and humid zones of the world (Irwin and Barneby, 1982) [19]. *Cassia alata* grows in humid and temperate climates in Africa, Asia, Mexico, Australia, South America and different parts of India (Ross, 1999) [36]. Huge medicinal potentials have been detected in some parts of the *C. alata* plant supporting its use in many cultural health systems such as Ayurvedic, Chinese and African traditional medicines. Adedayo *et al*, (2001) [2] and El-Mahmood *et al.*, (2003) [13] stated in their previous works that the plant parts decoctions are applied in handling of wounds, burns, skin and respiratory tract infections affecting people in Northern Nigeria. More so, in the South-western Nigeria, the plant leaf preparation relieves body, abdominal pain, stress and toothache (Benjamin *et al.*, 2008) [10]. Previous work has revealed that the plant bark decoction when spread on cuts of tribal and tattoo mark incisions prevents infections and aids in wound healing (Oladeji *et al*., 2020) [31]. According to Ajibesin, *et al.* (2008) [3], leaf decoctions of C. *alata* are applied in the handling of mycosis and skin diseases. In Cameroon and Egypt, the stem, bark, and leaves have been applied to take care of gastroenteritis, hepatitis, and ringworms (Leung *et al.*, 2011) [25]. *C. alata* plant decoction is also used for treating wounds as it functions as an anti-inflammatory agent (Monkheang *et al*., 2011) [27]. *Cassia alata* has been documented to possess many strong pharmacological properties such as anti-allergic, anti-inflammatory, antioxidant, anti-microbial, anti-cancer, and anti-diabetic, It has been in use in the practice of herbal and complementary medicine in handling skin diseases existing in some cultures. (Alalor *et al*., 2012; Fatmawati and Bakar 2020) [4. 15]. Hence the study investigates the chemical composition and the antioxidant activity of the *C. alata* plant which may give credence to the therapeutic properties and pharmacological activities of the plant.

2. Materials and Methods

2.1 Sample collection

Fresh *Cassia alata* leaves sample was collected from a swampy area within the school botanical garden. It was identified and authenticated by Dr (Mrs) S.E. Obasi of Botany Unit, Department of Science Laboratory Technology of Akanu Ibiam Federal Polytechnic Unwana, Afikpo Ebonyi state, Nigeria. It was deposited in the school herbarium with number BU/ 02315.

2.2 Sample preparation

The sample was carefully washed under running tap water and later rinsed with distilled water. It was allowed to dry within room temperature on a laboratory bench 14 days. with careful turning and monitoring. Later the dried sample was milled to coarse powder with a milling machine. Then 500 g of the powdered sample was macerated in 1 L of methanol and allowed to stay for 72 hours with sparse stirring and later filtered with Whatman filter paper (no 42) 125mm to get the filtrate. A rotar evaporator set at temperature of 45˚C was used to concentrate the filtrate and later placed on a laboratory bench with a covering to get a dark brown oily liquid of 35.7 g as the methanol extract.

2.3 Antioxidant activity potential of the plant methanol extract. 2,2-Diphenyl-1-picryl- hydrazyl (DPPH) radical scavenging and Ferric reducing activity power(FRAP) techniques were done to determine the antioxidant capacity.

2.3.1 DPPH free radical scavenging activity

DPPH free radical inhibition activity of the sample was done by studying the decolorization of the reagent (Mensor *et al*., 2001) [26]. 0.1 mM ethanolic solution of DPPH which was purple in colour was used. One milliliter (1 ml) of sample extract was added to 2 ml ethanolic DPPH. The mixture was shaken in a vortex and allowed to stand for 30 minutes in a dark place. Thereafter, it was re-shaken for some minutes. The absorbance of the solution was read at 517 nm. DPPH in

methanol served as blank. Vitamin C was used as standard. The level of discoloration of the solution effectively shows the scavenging strength of the sample. The percentage inhibition of DPPH activity was calculated using this formula.

$$\frac{D_0 - D_1}{D_0} \times 100\%$$

Where
D_0 = Blank sample Absorbance value
D_1 = test sample absorbance value

2.3.2 Ferric reducing antioxidant power (FRAP)

The principle of the analysis is based on quantification of ferric degradation product, by its condensation with the plant sample.

The reducing activity potential of the plant extract was determined using the method described by (Pulido *et al.*, 2000) [35].

About 0.5 mL of *C. alata* methanol extract was added to 0.25 mL of sodium phosphate buffer pH 3.6 and 0.25 mL of 1% potassium ferrocyanide was added to the solution. The mixture was incubated at 37ºC for 20 minutes. Thereafter, 0.25 ml of 10%Trichloro acetic acid was added and centrifuged at 2000 rpm for 10 minutes. 0.9 mL of the supernatant was mixed with 0.25 mL of distilled water and 0.1 mL of ferric chloride was added. Absorbance was measured at 593 nm. Vitamin C served as standard.

2.4 Gas chromatography-mass spectrometry (GC-MS) analysis

Gas chromatography-mass spectrometry analysis was done with Perkin Elmer GC Claurus 500 system. The gas chromatograph linked to a mass spectrometer (GC-MS) was equipped with an Elite-1 fused silica capillary column (30 x 0.25 mm ID- x 1 µMdf made of 100% Dimethyl polysiloxane). An electron ionization system with an energy of 70 ev was applied for the GC-MS detection. Pure helium inert gas was used at a steady flow rate of 1mL/min. An injection volume of 2 µl was used at a split ratio of 10: 1 and Injector temperature of 250ºC and ion – source at 280 ºC. The oven temperature was programmed from 110ºC (isothermal for 2 min) with a rise of 10ºC/min to 200 ºC, then 5 ºC/min to 280 ºC terminating with 9 minutes at an isothermal of 280 ºC. The mass spectra were recorded at 70 ev, at a scanning interval of 0.5 seconds, and fragments from 45 to 450 Da. Total GC running time was recorded, the relative percentage amount of each component was evaluated by comparing its average peak area to the total areas. The software that was adopted to have the mass spectra and the chromatogram is Turbo mass version 5.2.0 and NIST standard (Hema *et al.*, 2011) [17].

3.0 Results and Discussion

Table 1: Result of DPPH scavenging activity of *Cassia alata* leaf

Concentration µg/ml	DPPH Activity of *Cassia alata* (%)	Vitamin C (standard) (%)
50	4.14±0.00	18.41±0.17
100	12.99±0.55	32.07±0.30
200	19.55±0.06	67.28±0.54
400	25.73±0.17	88.39±.09

Values are means triplicate determinations ± standard deviation

The plant *Cassia alata* has been used for many medicinal purposes. The health benefits associated with this plant come from its possession of some pharmacological properties which antioxidant is among them. The antioxidant activity profile of the *C. alata* plant was evaluated with DPPH free radical scavenging inhibition and Ferric reducing activity power techniques.

The result of radical scavenging and inhibition activity of DPPH and vitamin C by *Cassia alata* plant extract is shown in table 1. The result is expressed in percentage mean ± standard deviation. All indicated significant differences at ($p<$ 0.05) as concentration increases. At a low concentration of 50 ug/ml, the DPPH inhibition activity was 4.14%. At concentration of 100 ug/ml, the DPPH scavenging activity stood at 12.99%. So as concentration increases to 400 ug/ml the percentage inhibition of DPPH free radical species rose to 25.73%. This shows that it is concentration-dose dependent. This is applicable to vitamin C used as the standard. From this study, the standard (vitamin C) showed higher inhibitions at different concentrations compared to the *Cassia alata* extract. This shows that vitamin C has a higher capacity to scavenge free radicals of DPPH than the sample. 2, 2-Diphenyl-1-picrylhydrazyl is a stable free radical due to resonance. This prevents dimerization of the compound and confers on it a deep violet colouration. To achieve scavenging this radical is by protonation or donation electron which may stabilize it and change its color. The antioxidant species may achieve this by the donation of hydrogen atoms to the DPPH species (Shaala and Nahi, 2021) [38].

Table 2: Results ferric reducing activity potential of *Cassia alata* plant leaf

Concentration µg/ml	FRAP Activity of *Cassia alata* (%)	Vitamin C (standard) (%)
50	0.00±0.00	5.09±0.16
100	2.72±0.02	9.18±0.12
200	5.66±0.06	20.31±0.14
400	11.5±0.34	52.99±0.21

Values are triplicate determinations ± standard deviation

The result of ferric reducing activity power (FRAP) of *Cassia alata* extract is displayed in table 2. The result indicates that at different sample concentrations of 50, 100, and 200,400 ug/ml the percentage reduction of ferric ion to ferrous ion increases from 0.00% to 11.54% while that of standard vitamin C increases from 5.09 to 52. 99%. The result shows that the activity is concentration-dose dependent. This shows that the standard displayed higher antioxidant activity than the sample. The values obtained from the ferric activity-reducing work show the transformation of Fe^{3+}ion to Fe^{2+} ion by the *Cassia alata* extract and the vitamin C which served as standard.

Cassia alata has been reported to have many therapeutic potentials, this could be as a result of possessing this antioxidant property which suppresses the oxidation of molecular cells and tissue that generate oxidative stress.

Table 3: Result of GC- MS analysis of *Cassia alata* methanol extract

S/N	Retention Time (s)	Compound name	Molecular formular	Molecular weight	% Relative Abundance
1	11.557	1-Butanol-3-methyl	$C_7H_{14}O_2$	139	28.2
2	26.683	Pentadecane 2, 6, 10, 14-tetramethyl	$C_{19}H_{40}$	268	11.3
3	28.068	β-D-Glucopyranose-4-O-β-β-galactopyranosyl	$C_{12}H_{22}O_{11}$	342	14.1
4	31.420	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270	77.1
5	32.297	n-Hexadecanoic acid	$C_{19}H_{32}O_2$	256	16.4
6	34.816	9, 12, 15-Octadecatrienoic acid methyl ester [ZZZ]	$C_{20}H_{40}$	292	69.6
7	35.120	Phytol	$C_{21}H_{36}$	296	50.9
8	35.691	9, 12, 15-Octadecatrienoic-2-3- dihydroxyl propyl ester	$C_{21}H_{36}O_4$	352	8.6
9	42.620	Ethyl -iso-allocholate	$C_{26}H_{44}O_5$	436	24.5
10	51.731	Vitamin E	$C_{29}H_{50}O_2$	430	14.0

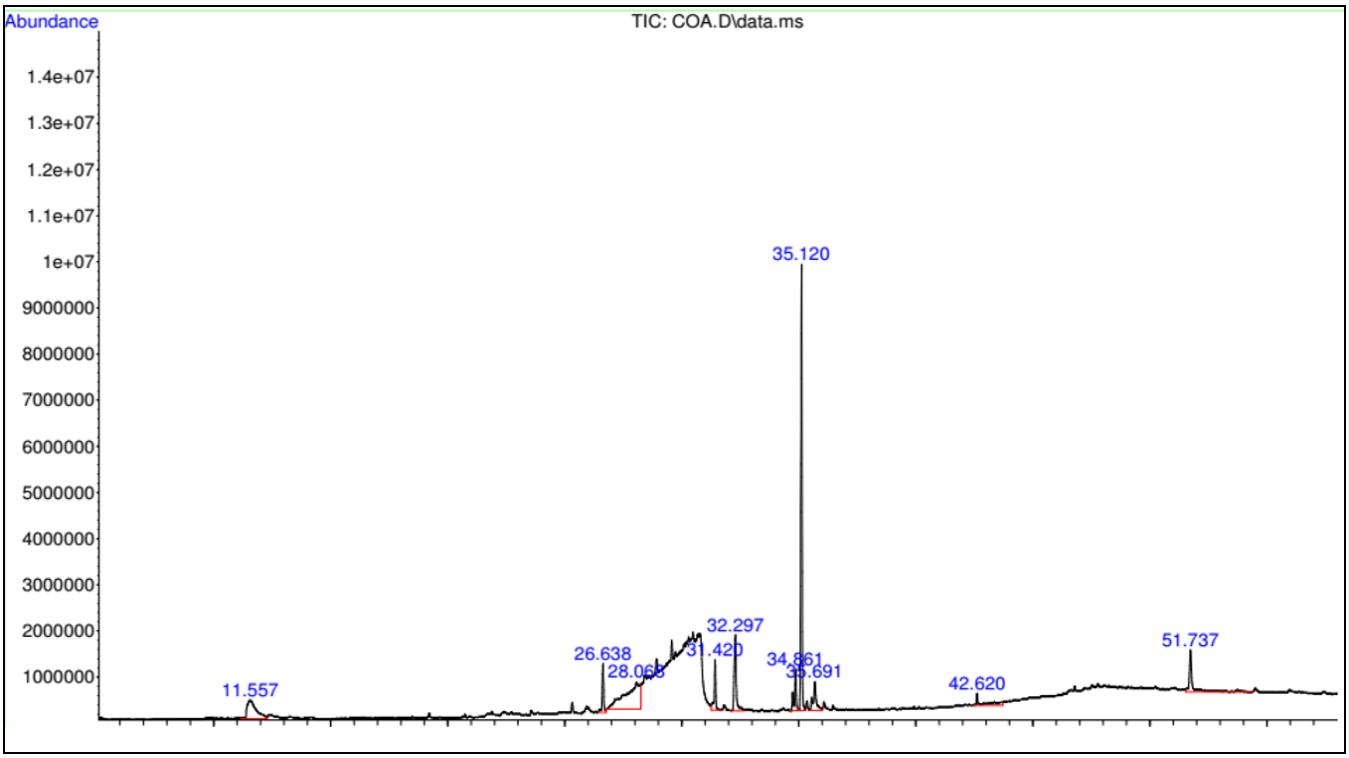

Fig 1: Chromatogram of GC-MS result of *Cassia alata* extract.

GC-MS chemical analysis of the plant leaves as shown in table 3 gave ten compounds. Knowledge of phytochemical constituents of the plant poses great benefit for development of therapeutic agents which can be isolated, synthesized and applied in treatment of some ailments. The GC-MS technique applied in analyses of plant constituents has become an acceptable method as a result of its simplicity, sensitivity and differentiation of mixtures. The identified components may possess some biological and pharmacological properties. For example, 9,12,15-Octadecatrienoic acid methyl ester [ZZZ] (Rt 34.861), a fatty acid ester possesses anti-inflammatory, cancer preventive, insectifuge, hepato-protective, anti-eczemic, hypocholesterolemic and anticoronary properties It also plays role as medicine in handling and managementof hyperlipidemia and atherosclerosis (Lan *et al*.,2017; Yao *et al*., 2013) [24, 42]. n-Hexadecanoic acid – a palmitic acid (Rt 32.297) has shown to be antioxidant, hypocholesterolemic, nematicidal, and flavour enhancing (Lan *et al*., 2017; Aparna *et al*., 2012) [24, 6]. Oluwaseun and Morakinyo (2015) [32] reported that the chromatogram of chloroform/methanol leaf oil extract of *C. alata* also afforded n-Hexadecanoic acid. Igwe and Onwu (2015) [21] work also afforded n-Hexadecanoic and hexadecanoic acid methyl ester in the plant. According to Kavipriya and Chandran (2018) [23], GC-MS Chemical analysis of *Cassia alata* showed 13 compounds that possess various pharmacological properties. The revelations on the plant constituents are in agreement with the work done.

Phytol – A diterpene (Rt 35.120) is found to show antimicrobial, anticancer, anti-inflammatory, antioxidant, andanti-diabetic activities (Silva *et al*., 2014) [39]. It has also been detected to improve immunological response in the early phase of tumor and carcinogenesis (Mukund *et al*., 2014) [28]. It has been detected to be useful in the treatment of Masoni schistosomiasis a serious endemic disease ravaging millions of people world over as it exhibited antischistosomal properties (De Morales *et al*., 2014) [12]. It is also a cholesterol-lowering agent.

Vitamin E was detected in the plant. The vitamin is soluble in fat, digested and conveyed inside plasma by lipoproteins. In the form of α-tocoferol a strong antioxidant, it can effectively inhibit lipid peroxidation of the plasma membrane (Atta *et al*., 2017) [8]. The antioxidant activity of α-tocoferol may be due to possession of phenolic hydrogen which it can donate to free radical species, by this it scavenges the free radical (Atta *et al*., 2017) [8]. Vitamin E and other antioxidant compounds in *Cassia alata* leaf have gone a long way to confer antioxidant properties to the plant.

Statistical analysis: The analyses were done in triplicates. Means are determined and results stated as means ± S.D. Results are done statistically using Microsoft Excel (Roseile USA).

Conflict of interest: No conflict of interest was declared by the authors.

Conclusion

No work on medicinal plants is an exhaustive search as more revelations are made every time. Scientists are interested in natural products of plant origin as a result of their health and pharmacological benefits. This study on *Cassia alata* has shown the antioxidant capacity of the plant. The free radical scavenging activity on 2, 2-Diphenyl-1-picrylhrazyl and the degree of reducing the activity of ferric ion to ferrous ion though less effective than the standard are enormous evidence to demonstrate that *Cassia alata* possesses the ability to inhibit oxidation. The revelations of some of these compounds from *C. alata* which have been reported to have some biological and pharmacological activities show that the plant has medicinal value. The above findings go a long way to give credence to the use of the plant in herbal medicine and pharmaceutical formulations.

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