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Antioxidant Activity of Bioactive and Mineral Constituents of *Jatropha curcas linn. (Euphorbiaceae)* Leaf and Seed Extract

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ABSTRACT

In recent years, there has been a surge in research on natural products, particularly plant-based ones, for pharmaceutical formulations and food supplements. Natural products rich in bioactive components like phenols, flavonoids, alkaloids, and saponins, and elemental minerals like Fe, Mg, Mn, and Cu, serve as antioxidants, anti-inflammatory, and antimicrobial agents. This study investigates the antioxidant capacity of *Jatropha curcas* leaves and seeds and the qualitative and quantitative phytochemical analysis and mineral compositions of the plant's leaves and seeds. The samples were collected and treated before being extracted through cool maceration in methanol. The antioxidant activities of the methanol extracts were evaluated using the DPPH Assay after their concentration was achieved using a rotary evaporator. Both quantitative and qualitative analysis was conducted to investigate the presence of phytochemicals in both the seed and leaves of J. curcas. The presence of elemental minerals such as Fe, Mn, Cu, Zn, and Mg was investigated using AAS. The phytochemical analysis of *J. curcas* revealed the presence of phenols, flavonoids, tannins, saponins, and steroids in both leaves and seeds, while alkaloids and terpenoids were found in the leaves. The mineral composition indicates Mg levels of 1161.65 mg/Kg in leaves and 590.66 mg/kg in seeds, with Cu absent in leaves while Mn highest in leaves. The study found that *Jatropha curcas* leaves have the highest antioxidant activities (82.6%), competing with the ascorbic acid standard (86.2%), with less activity in seeds (52.7 %), suggesting the high potential of the leaves for pharmaceutical formulations and food supplements.

Keywords: Antioxidant, DPPH, *J. curcas*, Free radicals, Oxidative Stress

ABSTRAK

Beberapa tahun terakhir telah terjadi peningkatan penelitian terhadap produk alami, khususnya produk nabati, untuk formulasi farmasi dan suplemen makanan. Produk alami yang kaya akan komponen bioaktif seperti fenol, flavonoid, alkaloid, dan saponin, serta unsur mineral seperti Fe, Mg, Mn, dan Cu, berfungsi sebagai agen antioksidan, antiinflamasi, dan antimikroba. Penelitian ini menyelidiki kapasitas antioksidan daun dan biji jarak pagar serta analisis fitokimia kualitatif dan kuantitatif serta komposisi mineral daun dan biji tanaman. Sampel dikumpulkan dan diolah sebelum diekstraksi melalui maserasi dingin dalam metanol. Aktivitas antioksidan ekstrak metanol dievaluasi menggunakan DPPH Assay setelah konsentrasinya dicapai menggunakan rotary evaporator. Analisis kuantitatif dan kualitatif dilakukan untuk mengetahui keberadaan fitokimia pada biji dan daun J. curcas. Keberadaan unsur mineral seperti Fe, Mn, Cu, Zn, dan Mg diselidiki menggunakan AAS. Analisis fitokimia J. curcas menunjukkan adanya fenol, flavonoid, tanin, saponin, dan steroid baik pada daun maupun biji, sedangkan alkaloid dan terpenoid ditemukan pada daun. Komposisi mineral tersebut menunjukkan kadar Mg sebesar 1161,65 mg/Kg pada daun dan 590,66 mg/kg pada biji, dengan kandungan Cu tidak terdapat pada daun sedangkan Mn tertinggi terdapat pada daun. Studi ini menemukan bahwa daun jarak pagar memiliki aktivitas antioksidan tertinggi (82,6%), bersaing dengan standar asam

askorbat (86,2%), dengan aktivitas lebih sedikit pada biji (52,7%), menunjukkan tingginya potensi daun untuk formulasi farmasi dan makanan suplemen.

Kata Kunci: Antioksida, DPPH, *J. curcas*, Radikal Bebas, Stres Oksidatif.

1. Introduction

Medicinal plants have long been thought to be a significant source of phytochemicals, a broad category of bioconstituents. Traditional medicine has made use of these bio-constituents to treat various illnesses. Today's chemists are concentrating on scouring the natural world for these kinds of bio-constituents derived from plant products, which have the potential to treat severe human ailments, including cancer, diabetes, heart failure [1], uncontrollably aging, and even the newest coronavirus outbreak. Most of these health issues are brought on by oxidation reactions that occur in the human body because of an imbalance in free radicals, which sets off uncontrollable free radical chain reactions. These kinds of reactions are very harmful to the human system and thus are the reasons for the increasing mortality rate observed daily all over the globe.

Oxidation is a normal phenomenon that takes place in the body. Oxidative stress, on the other hand, occurs when there is an imbalance between free radicals' activities and antioxidant activities. When functioning properly, free radicals can help fight pathogens, but infections can set in when there are more free radicals present that can be kept in balance by an antioxidant. The free radicals can start damaging fatty tissue, DNA, and proteins in the body via oxidative stress [2]. Hence, the reasons for the emergence of several diseases. To salvage the cell from oxidative stress, antioxidants such as phenolic compounds, nitrogen compounds, derivatives of cinnamic acids, ascorbic acids, and carotenoids obtained from medicinal plants, and minerals such as Mn, Zn, Mg, and Se can be integrated into pharmaceutical formulations and food supplements in other to complement for the body's natural antioxidants [3]. Even though synthetic antioxidants are in use, they pose significant risks in that some of the techniques for their synthesis, like click reactions [4], can leave residual toxic chemicals that may be harmful, causing severe side effects when used, hence the need for natural products from plants. *J. curcas* have been studied in recent years for medicinal value and have been shown to provide great medicinal advantages amongst other plant products. Research has shown that the medicinal values, like the antioxidant capacity, vary depending on the region of plant cultivation. Hence, this research intends to investigate this property in *J. curcas* grown in Shada Kwali Area Council Abuja, Nigeria, and compare the medicinal values of the leaves and the seeds of the plant of which, from our best knowledge, this plant within this region have not been investigated yet.

Jatropha curcas is a large shrub or a small perennial tree with a height of about 30-10 meters [5]. The plant is an evergreen shrub with high resistance to aridity, which allows it to grow in deserts. The life span of the plant is up to 50 years [6]. It is a deciduous plant with an articulated growth habit with morphological discontinuity [7]. The root system constitutes a main tap root and four shallow lateral roots [8]. The branches are glabrous with smooth greenish-bronze-colored bark and translucent latex. The leaves are smooth, simple, 5-lobed, and heart-shaped, 10-15cm long, dark green, cordate or round, acute at the apex, cordate at the base, alternate, and may fall once a year [9-10]. The flowers are in axillary clusters with a stalk of 3-5cm long, bracts entire, lanceolate, or linear, densely pubescent, yellowish green, with prominent glandular discs in the flowers [11, 12]. The fruits are ovoid capsules 3-4 cm long, slightly trilobite, splitting into three cells. The seeds are three per fruit: large, oblong, 2cm long, and sweet tasting [13]. The antioxidant and antimicrobial activities of the Kernel meal extract of Jatropha curcas L. have been reported by Oskoueian et al. [14].

Plant Morphology

Figure 1. Jatropha curcas plant (left) and seeds (right)

The processing of the seed oil of *Jatropha curcas* for biofuel development microbial and larvicidal properties has attracted a lot of research on the plant with subtle negligence to explore both the seed and the leave for their medicinal antioxidant characteristic. However, plants generally are the major sources of drugs. Notwithstanding, this research aimed to study the antioxidant activity of the leaf and seed of the plant by comparison and to account for the most effective part of the plant as an active antioxidant agent. This will be of great importance for medicinal purposes and beneficial for commercial sectors such as pharmaceutical, food, and chemical industries. Thus, it could serve as a basis for drug development as both the leaf and seed of *J. curcas* are effective pharmacological agents.

2. Material and Methods

2.1. Equipment

The equipment used in this study include: atomic absorption spectroscopy (ICE 3000 SERIES), ultraviolet-visible spectrophotometer (CE 7500, 7000 SERIES), analytical balance (A-200D and AR2140), glassware (beakers, test tubes, conical flask, etc.), rotary evaporator, micro syringe, spatula, and aluminum foil, hot plate, fume cupboard, and ballmer bottle

2.2. Materials

The materials used in this study include: methanol, ethanol, formaldehyde, ethyl acetate, concentrated pentanol, lead acetate, chloroform, acetic acid, acetic anhydride, Dragendoff"s reagent, ferric chloride, DPPH (2,2-diphenyl-1-picrylhydroxyl radical), sulphuric acid, hydrochloric acid, ammonium hydroxide solution, ammonia.

2.3. Biomass Collection

The plant samples were collected as raw material because of their availability and potency in pharmacological studies. The leaves and seeds of *Jatropha curcas* were collected from several points in Sheda kwalli area council, Abuja FCT.

2.4. Biomass Drying

The collected plant leaf was air-dried for 14 days until the moisture content was found to be very low. The nuts bearing the seeds were cracked manually to remove the seeds, which were further air dried for 21 days until the moisture content was at its minimum. The plant parts were pulverized into smaller sizes of about 1mm using a blender to increase the surface area for proper mixing with the solvent to enhance extraction

2.5. Cold Extraction

About 100g each of both the leaf and the seed of *Jatropha curcas* was dissolved in 500 mL of methanol in a reagent bottle and placed on an orbital shaker overnight at 120 rpm. The mixture extracted from the plant parts was then filtered using Whatman No.1 filter paper to obtain a crude extract of the plant part.

2.6. Concentration of Extract

The crude solvent obtained from the extraction was concentrated for each sample (leaf and seed) using a rotary evaporator at a temperature of about 78 ℃ to obtain a concentrated extract of both the leaf and the seed. The concentrated crude extract of both plant parts was poured into a separate specimen bottle before analysis.

2.7. Preliminary Phytochemical Analysis

Alkaloid Test

About 2 mL of the crude extract was dissolved in 5 mL of 1% Hydrochloric acid HCl. It was filtered and treated with three drops of Dragendoff" 's reagent. The development of an orange-brown precipitate was used to indicate the presence of an alkaloid compound [15].

Phenol Test (Ferric Chloride Test)

About 2 mL of the crude extract was added to 2 mL of 5 % Ferric chloride $FeCl_3$ solution. The appearance of blackish-green coloration indicates the presence of phenolic compounds.

Flavonoid Test

One mL of the crude extract was added to 1 mL of 10 % lead acetate Pb(OAc). The appearance of a yellow precipitate signifies the presence of a flavonoid compound. [16].

Tannin Test

Two mL of the crude extract was added to 2 mL of distilled water. This was boiled, followed by the addition of a few drops of 1% Ferric chloride. The appearance of brownish green signifies the presence of the Tannin compound [17].

Saponin Test (Froth Test)

One mL of the crude extract was added to 1 mL of distilled water and heated for 5 minutes. It was allowed to cool, the mixture was shaken, and the appearance of froth, which persisted was used to identify the presence of saponin in the sample.

Steroid Test (Salkwoski Test)

About 2 mL of the crude extract was dissolved in 1 mL of chloroform, followed by the addition of concentrated Sulphuric acid in drops to form a lower layer, which is yellow with green fluorescence. A reddish-brown color on the upper layer signifies a steroid ring [15].

Glycosides Test (Liebermann's Test)

Two mL of the crude extract was dissolved in 2 mL of chloroform, followed by the addition of 2 mL of concentrated acetic acid. A violet-to-blue-to-green coloration indicates the presence of glycosides.

Terpenoid Test

About 2 mL of the crude extract was added to 2 mL of acetic anhydride, followed by 2-3 drops of concentrated sulphuric acid. The appearance of deep red coloration signifies the presence of a Terpenoid compound [17].

2.8. Quantitative Phytochemical Screening

Total Phenols

The quantity of phenol was determined using the spectrophotometric method described by Gupta et.al [18]. 0.5 g of the sample was boiled with 50 mL diethyl ether for 15 minutes, and 5 mL of the boiled sample was then transferred into a 50 mL flask using a pipette. 10 mL of distilled water was added, followed by the addition of 2 mL of ammonium hydroxide solution and 5 mL of concentrated propanol to the mixture. This was made up to the mark and left for 30 minutes to react for color development. Standard solutions of Galic acid concentrations (0.003, 0.009, 0.015, 0.021, and 0.030 mg/mL) were prepared alongside the sample. The absorbance of both the sample and the standard solution was taken at 505 nm.

Total Flavonoid

The determination of the total flavonoid of the leaf and seed of the sample was done by acid hydrolysis using the spectrophotometric method. 0.5 g of the processed sample was mixed separately with 5 mL of dilute Hydrochloric acid and boiled for 30 minutes. The boiled extract was allowed to cool and filtered. 1 mL of filtrate was added to 5 mL of ethyl and 5 mL of 1 % Ammonia. Quercetin standard solutions of concentration (1.00, 0.75, 0.50, 0.25 µg/mL) were prepared alongside the sample. Both the sample and the standard were scanned at 420-520 nm for the absorbance [18].

Total Alkaloid

About 0.2 g of the sample was dissolved in 96 % ethanol-20 % tetraoxosulphate (VI) acid (1:1). It was filtered, and 2 mL of the filtrate was added to 5 mL of 60 % tetraoxosulphate (VI) acid. This was allowed to stand for 5mins followed by the addition of 2 mL 0.5 % Formaldehyde and allowed to stand for 3 hours. Strychnine standard solutions of concentrations (0.005, 0.010, 0.015, 0.020, 0.025, 0.030, and 0.035 µg/mL) were prepared alongside the sample. The absorbance of both the standard and the sample was taken at 565nm [18].

2.9. Elemental Analysis

The method of Odey et.al [19] was employed, where 5 g and 2 g of the seed and leaf of the sample respectively were digested using 20 mL of concentrated Nitric acid at 50 ℃ for 30 minutes until a clear solution of both samples were obtained. The solutions were filtered into separate 100 mL standard flasks and made up to the mark with distilled water. This was transferred into a specimen bottle and stored in a cooled environment. The digest for both samples was analyzed using AAS for the metals Fe, Cu, Mn, Mg, and Zn using different cathode lamps for the element of interest to be analyze.

2.10 Antioxidant Activity

The free-radical scavenging activity was evaluated by accessing its discoloration of 2, 2-diphonyl-1-picrylhydrozyl radical (DPPH) in methanol by a slightly modified method of Mg bahurike et.al [16]. A stock solution of the sample in mg/mL was prepared, and the following concentrations of the extract were tested (0.1, 0.3, 0.5, 0.7, and 1.0 mg/mL). The decrease in absorbance was monitored at 517 nm. Vitamin C was used as the antioxidant standard at concentrations (0.1, 0.3, 0.5, 0.7, and 1.0 mg/mL). A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{A-B}{A} \text{x } 100\%$$

Where,

A = Absorption of the blank sample without extract.

B = the absorption of the extract.

Note: The Negative value shows there is no anti-aging activity at that absorbance range.

3. Results and Discussion

3.1 Qualitative Phytochemical Screening

The preliminary phytochemical screening of the methanolic extract of *Jatropha curcas* seed and leaf revealed several phytochemicals, as shown in Table 1. The main phytochemicals present in both the leaves and the seeds of *J. curcas* are phenols, flavonoids, and steroids which agrees with the report of Rampadarath et al., [20], except for alkaloids only being present in the leaves and absent in the seeds of *J. curcas* obtained from Sheda Kwali area council Abuja, Nigeria, which is in contrast with the report of Rampadarath et al., [20]. Of all these phytochemicals, the phenolic compounds have been reported to be active antioxidant agents [21], and therefore, their presence in both the leaf and seed extract of *J. curcas* presents a significant influence in the antioxidant activities of both plant parts as presented in this research work. Flavonoid is another important phytochemical of antioxidant importance present in both plant parts, whereas alkaloids are present in the leaves and absent in the seeds. Exhaustive work has been done on microbial activities and larvicidal properties of most of the plant parts of *J. curcas*, but the antioxidant properties remain unexplored. This is reported here as a promising property that is explorable in the pharmaceutical industry for medicinal formulation and food supplements.

Table 1. Preliminary phytochemical screening of seed and leaf extract of *Jatropha curcas*

S/N	Bioconstituent	Test	Leaf	Seed
1	Phenol	Ferric chloride test	+	+
2	Flavonoid	Lead acetate test	+	+
3	Tannins	Ferric chloride test	+	+
4	Alkaloid	Dragendoff's test	+	-
5	Saponin	Froth test	+	+
6	Glycosides	Liebermann's test	-	-
7	Terpenoid	Liebermann's test	+	-
8	Steroid	Salkwoski test	+	+

3.2 Quantitative Phytochemical Screening

The phytochemicals necessary for inducing antioxidant activities in the leaf and seed extract of *J. curcas* were quantified and presented in Table 2. The quercetin equivalent of flavonoid and the gallic equivalent of phenol in the leaves were obtained to be higher than that of the seeds. In contrast, the leaf presents a very low Strychnine alkaloid Equivalent, which is absent in the seed of *J. curcas*. The higher concentration of phenols in the leaves also helps elevate the antioxidant activity, hence making the leaves of *J. curcas* compete favorably with standard ascorbic acid, as shown in the curve of Figure 2 (right).

Table 2. Total phytochemicals of the leaf and seed extract of *J. curcas.*

Phytochemical	Leaves	Seed
Flavonoid (QE µg/g)	31.980	14.069
Phenols (GE µg/g)	56.659	31.357
Alkaloids (SE µg/g)	1.197	-

Key: QE= Quercetin Equivalent
GE= Gallic acid Equivalent
SE= Strychnin Equivalent

3.3 Mineral Constituents

Table 3. Quantitative mineral analysis

Minerals	Conc. mg/kg Leaves	Conc. mg/kg Seed
Fe	64.34	47.25
Zn	13.23	27.64
Cu	-	10.00
Mn	39.66	9.21
Mg	1161.65	590.66

Various minerals of medicinal importance have also been characterized in both the leaves and seeds of *J. curcas*. Amongst these, elemental Fe, Zn, Mn, and Mg were identified in the acid-decomposed seeds and leaves of the plant, except for Cu, which is only present in the seeds. From the quantitative analysis, Mg presents the highest concentration of 1161.65 and 590.66 mg/Kg for the leaves and seeds, respectively. The elemental mineral with the lowest concentration obtained was Zn (13.23 mg/Kg) for the leaves and Mn (9.21 mg/Kg) for the seeds. Mn and Mg content in the leaves is almost three times that of the seeds, and the Fe content of the

leaves is higher than that of the seeds. The seeds' Zn content is twice that of the leaves. This result is consistent with the report of Méndez et al. [22], which presents high elemental Fe, Mg, and Mn contents in the leaves of *J. curcas*. Zn, which was quantified in this work, and Cu were not identified by Mendez et al. [22]. The identification of these elemental minerals in *J. curcas* could be of pharmaceutical importance in the formulation of food supplements and other pharmaceuticals.

3.4 Antioxidant Activity

Table 4. Antioxidant Capacity expressed as mean ± standard deviation

Conc. of crude extract (mg/mL)	% Inhibition Vitamin C	% Inhibition Leave	% Inhibition Seed
0.1	86.2±0.22	82.6±0.07	3.5±3.04
0.3	85.5±0.78	82.4±0.14	8.0±2.05
0.5	85.0±0.32	82.1±0.22	18.6±5.80
0.7	84.9±0.36	81.2±0.28	39.1±3.25
1.0	84.5±0.21	80.1±0.28	52.7±3.89

Table 4 presents the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the leaves and seed extracts of *J. curcas* compared to that of vitamin C (ascorbic acid) as standard. The inhibition capacity for ascorbic acid and leave extract showed a linear decrease with increasing concentration of the crude extract, while for the seed extract, the antioxidant capacity increases with increasing concentration of the crude extracts, which is a similar case observed by Oskoueian et al., [14]. From observations, the antioxidant activity of the leaf extract of *J. curcas* competes favorably with that of the vitamin C standard, which could be associated with the presence of phytochemicals and mineral constituents in the leaf extract. The seeds show very little antioxidant activity at a low extract concentration, but when the extract concentration increases, the antioxidant activities shut up to 50 %.

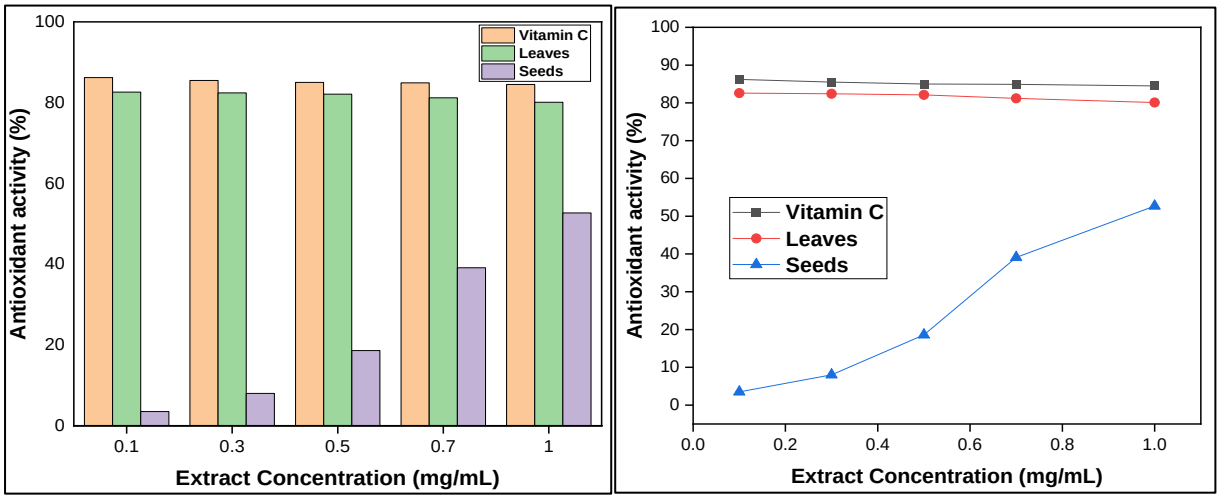

Figure 2. Antioxidant capacity of the leave and seed extract of *J. curcas*

Table 5. Antioxidant IC_{50} for the leaves and seed extract of *J. curcas* and vitamin C standard.

S/N	IC_{50} for Vitamin C (mg/mL)	IC_{50} for Leave (mg/mL)	IC_{50} for Seed (mg/mL)
1	7.44	11.98	0.96

The therapeutic benefits of medicinal plants are attributed to the free radical scavenging capacity of the plant extracts, which is a function of phytochemicals such as phenols, alkaloids, flavonoids, vitamin C, etc. *J. curcas* was evaluated for phytochemicals in the leaves and the seed of the plant, and the result revealed the presence of antioxidant phytochemicals such as phenols, alkaloids, and flavonoids in both the leaves and seed of the plant. The antioxidant activity of the methanol extract of both plant parts (leaves and seeds) was evaluated using DPPH assay. The antioxidant capacity of the leaves increases as the dosage of the crude extract was reduced, with the highest activity of 82.6 % in the leaves and, thus, having an IC_{50} of approximately 11.98 mg/mL, which competes favorably with the antioxidant capacity of standard ascorbic acid (86.2 %) having

IC_{50} of 7.44 mg/mL. The antioxidant capacity of the seed was observed to be directly proportional to the dosage of crude extract, and hence, it shows IC_{50} 0.96 mg/mL, as shown in Table 5. Thus, it is less effective than the leaves of the plant. These values are very high for the leaves compared with the IC_{50} of 1.6 mg/mL for *J. curcas* kernel meal reported by Oskoueian [14], except for the very low IC_{50} of the seed whose antioxidant capacity depends on the increasing concentration of the extract. Also, the results reported by Mgbahurike et al. [16] show that the leaves of *J. curcas* have an IC_{50} of 0.17320 mg/mL, which is lower than the IC_{50} of the leaves of *J. curcas* obtained in this work. Shikhar et al. [23] reported an IC_{50} of 0.046 mg/mL approximately for the ethanolic seed extract of *J. curcas*, which also varies from the IC50 of the seed extract (0.96 mg/mL) obtained from this work. Based on the results of the IC_{50} obtained in this work, both the standard ascorbic acid and the leaves of J. curcas, whose antioxidant capacity varies inversely with concentrations, have high IC_{50}. In contrast, the IC_{50} of the seeds, whose antioxidant capacity varies directly with concentration, is very low. This suggests that the IC_{50} is higher for high-capacity antioxidants.

Generally, it is believed that antioxidant activity is higher at lower concentrations of crude extract; this gives more explanation to why the antioxidant activity of both vitamin C and crude extract of the leaves of *J. curcas* are higher as the concentration of the crude extract is reduced and vice-versa. The activity of the seed does not confirm the assertion above and, hence, requires a higher concentration of the crude extract to maximize its activity, as shown in Figure 2. The unusual antioxidant activity observed with the seed could be attributed to the fact that the seed contains high lipid (oil), which may be activated by the DPPH radical, resulting in lipid peroxidation assay. Hence, it takes more of the antioxidant phytoactive chemicals to neutralize both the DPPH radical and the generated radical through lipid peroxidation assay formed by the action of DPPH radical on lipids of the seed. With the low antioxidant phytoactive chemicals such as phenols, flavonoids, and alkaloids present in the seed, it becomes clearer why the seed shows an unusual trend of activity with the DPPH assay; therefore, the higher the crude extract, the higher the activity as observed with the seed.

The elevated antioxidant capacity of J. curcas crude extract was accounted for by the quantitative evaluation of such phytoactive components in both the leaves and seeds of the plants. The leave was observed to have a higher quantity of these phytoactive chemicals which are given in Table 2. The seeds show a lower concentration of phytochemicals, therefore having lower antioxidant capacity. From the result of antioxidant activity and available phytoactive chemicals in Jatropha curcas, both plant parts can certainly be used for medicinal and pharmaceutical formulations, with much priority given to the leaves for formulating free radical inhibiting agents.

4. Conclusion

Plausible hypotheses have been constructed implicating oxidative stress as the cause of cell and tissue damage. The complex web of the antioxidant defense system plays a key role in protection against oxidative damage, most of which is sourced from plants. From the result obtained, fact states that the leave of Jatropha curcas, having a high phenolic content of 56.659 (GEμg/g), will do better as a precursor for antioxidants than the seed, having a phenolic content of 31.357 (GEμg/g) therefore, the leaves of J. curcas is regarded as a valid antioxidant agent. The presence of Fe, Mg, Zn, and Mn in both plant parts could serve as an indicator for excellent precursors for medicinal and pharmaceutical formulations.

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