



METHANOL LEAF EXTRACT OF *Jatropha tanjorensis* Ellis and Saroja POSSESS PHYTOCONSTITUENTS WITH FREE RADICAL SCAVENGING ACTIVITY

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ABSTRACT

The body during normal metabolic function produces free radicals which are highly reactive species. Free radicals could also be introduced into the body from the environment. The oxidation induced by reactive oxygen species can result in DNA mutation, membrane protein damage and cell membrane disintegration. The present study assayed the preliminary phytochemicals, total phenolics and total flavonoids and free radicals scavenging ability of methanol extract of *Jatropha tanjorensis* leaf. Standard methods for determining phytochemicals, reducing power, nitric oxide scavenging, hydroxyl radical, and lipid peroxidation scavenging activity were employed. The phytochemical screening result revealed the presences of phenols, flavonoids, saponin, alkaloids, tannins, terpenoids and steroids. The total phenolic content of methanol extract of *Jatropha tanjorensis* leaf measured by Folin-Ciocalteu reagent in terms of gallic acid equivalent was $11.35 \pm 0.82 \text{ mgGAE/g}$. The flavonoid content of the plant sample calculated as Quercetin equivalent was $15.91 \pm 1.60 \text{ mgQCE/g}$. GC-MS results revealed relevant pharmacological bioactive compounds. The antioxidative activity of the plant's extract correlated with total phenolic content. The radical scavenging activity showed a dose dependent increase in the reducing power. The minimum NO inhibitory activity was 8.88 ± 0.63 at $200 \mu\text{g/ml}$ and the maximum activity was 32.70 ± 2.71 at $800 \mu\text{g/ml}$. The minimum percentage H₂O₂ radical inhibitory activity was 8.30 ± 0.88 at $200 \mu\text{g/ml}$ and a maximum activity was 22.80 ± 2.28 at $800 \mu\text{g/ml}$. There was also an increase scavenging effect of lipid peroxide radical in concentration dependent manner. The results of this study indicate that the leaf of *Jatropha tanjorensis* possess antioxidant properties and could serve as free radical inhibitor.

Keywords: Antioxidants, Scavenging, Free radicals, Gallic acid, *Jatropha tanjorensis*

INTRODUCTION

Some pathological conditions are often due to production of free radicals from metabolic side reactions and exogenous sources (Atanu et al., 2018). The activities of these reactive oxygen species (ROS) and reactive nitrogen species (RNS) results in distortion of normal metabolic activities thereby presenting health challenging conditions (Pavithra and Vadivukkarassi, 2015). Generally, radicals implicative role in disease pathology are attributed to loss of cell membrane stability, consequently leading to interactions with basic molecules of proteins, carbohydrates, nucleic acids and lipids (Rahman et al., 2015).

Since several pathological conditions are attributed to oxidative stress, in finding remedy to these pathological conditions, scientists are considering perspective of preventing oxidative stress mediated process which can be achieved via free radicals scavenging and radicals quenching with compounds commonly known as antioxidants (Meles et al., 2019).

Plant species serve as a major source of several novel biologically active compounds and some of these bioactive compounds are beneficial and serve as natural antioxidants (Danborno et al., 2019). The adverse effects of the reactive oxygen species produced in living things could be inhibited by natural antioxidants. Humans based on this principle depended on plants and plants products to cure various diseases and maintain healthy living. It is on record that natural products have proved to be of great health impact on humans traditionally and scientifically (Oyewole et al., 2011).

The nature remains the right source for health promotion and for the supplementation of safe drugs. Great attention is

mandatory to explore many unexplored plants with highly effective antioxidant activity. Phenolics and polyphenols are the most widely researched class of bioactive compound (Asif, 2015). A great number of modern medicines have been derived from plants hence plants are considered as important sources of medicinal agents used to treat different diseases (Njoku and Chidi, 2009). Bioactive compounds such as flavonoids, tannins, phenols, and alkaloids, in medicinal plants, play an important role in drug development (Khan, et al., 2019). In recent years, the use of herbs have received considerable attention as an alternative way to compensate for perceived deficiencies in orthodox pharmacotherapy worldwide (Arika et al., 2015). Plant-based natural medicines are popularly acclaimed to be safe, scientists advocate for proper toxicological studies in other to ensure safety in the use of natural medicines (Asif, 2015). The therapeutic effect of these medicinal plants can justifiably be attributed to the phytochemicals in them.

Jatropha tanjorensis is green leafy medicinal plant that has found usage in folk medicine. The plant is a common weed of field crops belonging to the family Euphorbiaceae (Oladele et al., 2020). It is usually grown in rain forest zones of West Africa. *Jatropha* is understood for its use as purgative/laxative and other medicinal purposes. All parts of the plant including seeds, leaves and bark; fresh or as a decoction, are used in traditional and folk medicine as well as veterinary purposes (Chigozie et al., 2018). In southern part of Nigeria like in Edo state the leaf of the plant is locally consumed as vegetable added to daily meal as well as in treating diabetes mellitus due to its anti-hyperglycemic property (Olayiwola et al., 2004). In other part of Nigeria the

plant is consumed as soups and as a tonic with the claim that it increases blood volume. There has been claim that it cures anaemia, diabetes and cardiovascular diseases (Omoregie and Osagie, 2011).

Jatropha tanjorensis has received tons of attention due to its potential health benefits, availability and affordability. This study was carried out to determine the phytochemical content and antioxidant activity of the plant leaf.

MATERIALS AND METHOD

THE PLANT

The leaves of *Jatropha tanjorensis* were obtained from the premises of Federal Polytechnic Nekede, Owerri and subsequently authenticated by a botanist at the Department of Plants Science, Michael Okpara University of Agriculture Umudike.

Phytochemical Screening

The qualitative phytochemical screening was carried out using the methods of Harborne (1973) and Trease and Evans (1989).

GC-MS Analysis

The analysis of bioactive compounds from the extract was carried out at Springboard, Awka, Anambra State, using Agilent Technologies Gas Chromatography systems 7890A coupled with Mass spectrometry 5975C model equipped with HP-5MS column (30 m in length \times 250 μ m in diameter \times 0.25 μ m in thickness of film). Helium gas was used as the carrier gas with flow rate of 1.5 mL/min. The initial temperature was set at 70 for 0.5 min to 280 °C with increasing rate of 12

°C/min and holding time of about 5 min. Furthermore, One microliter was injected into 250 °C inlet with a splitless mode. The detection of compounds involved an electron ionization system which involves high energy electrons (70 eV). The relative quantity of the compounds present in the extract of henna was expressed as percentage based on peak area produced in the chromatogram.

Characterization of Compounds

The database of National Institute of Standard and Technology (NIST) was used in the interpretation of mass spectra of GC-MS. The mass spectra of the unknown compounds was compared with that of the known components stored in the NIST-library.

In Vitro Antioxidant activity

Total phenolic content was determined by Folin Ciocalteu's method as described by Bhalodia et al. (2011) and Patel et al. (2010) with slight modification. The aluminium chloride colorimetric assay as used by Pallab et al. (2013) was used in the determination of total flavonoid content. The hydrogen peroxide (H₂O₂) scavenging activity of the extract was determined by the method of (Srinivasan et al., 2007) with slight modification. In the determination of reducing power, the method described by Kumaran and Karunakaran (2007) was used. The method of Daljit and Priyanka (2010) was employed in the determination of Nitric Oxide (NO) scavenging activity of the extract. The inhibitory Capacity of Extract on Lipid Peroxidation by Malondialdehyde (MDA) assay was determined colorimetrically using thiobarbituric acid (TBA), as described by Okolie et al. (2009) with slight modifications.

RESULTS AND DISCUSSION

Table 1: Phytochemicals present in methanol extract of *Jatropha tanjorensis* leaves

Phytochemicals	Specific Test	Inference
Phenols	Ferric chloride test	+
Flavonoids	Sodium Hydroxide test	++
Saponins	Frothing test	+
Alkaloids	Wagner's test	++
Tannins	Ferric chloride test	+
Terpenoids	Salkowski test	+
Steroids	Liebermann test	+

The phytochemical content of *Jatropha tanjorensis* revealed the bioactive compounds shown in the table above. + = present, ++ highly present.

Table 2. Results of Biomolecules (Total phenols and Total Flavonoids) and IC₅₀ of the Various in vitro antioxidant analyzed from *Jatropha tanjorensis* leaves

Antioxidant	Value
Total Phenol	11.35 \pm 0.82mgGAE/g
Total Flavonoid	15.91 \pm 1.60mgQCE/g
Nitric Oxide (NO) Scavenging	252 \pm 6.74 μ g/ml
Hydrogen Peroxide (H ₂ O ₂) Scavenging	80 \pm 0.08 μ g/ml
Lipid Peroxidation activity	520 \pm 4.80 μ g/ml

Values are Mean \pm SEM of triplicate determination

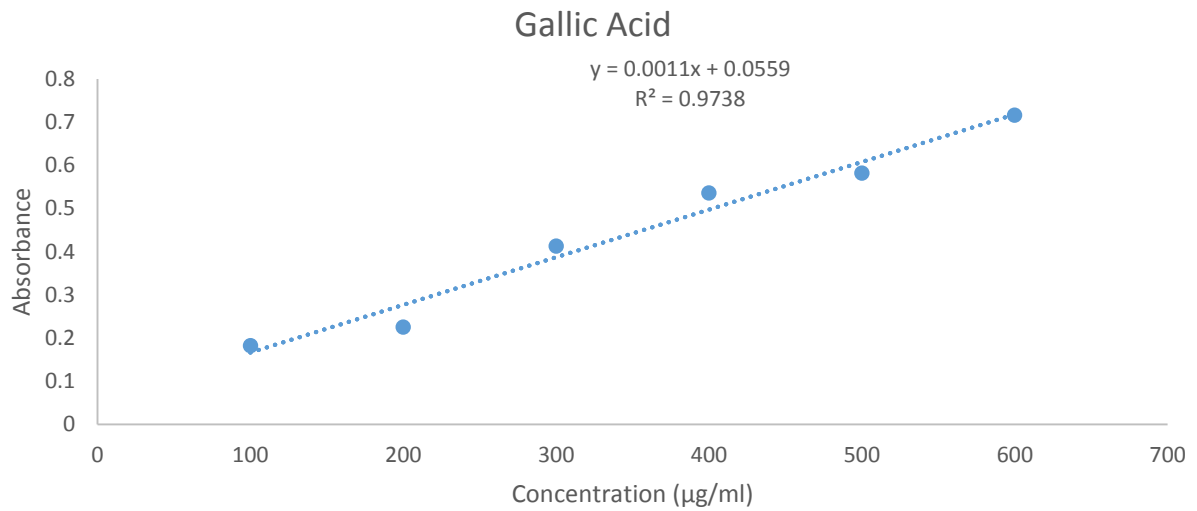


Figure 1: Standard curve of gallic acid

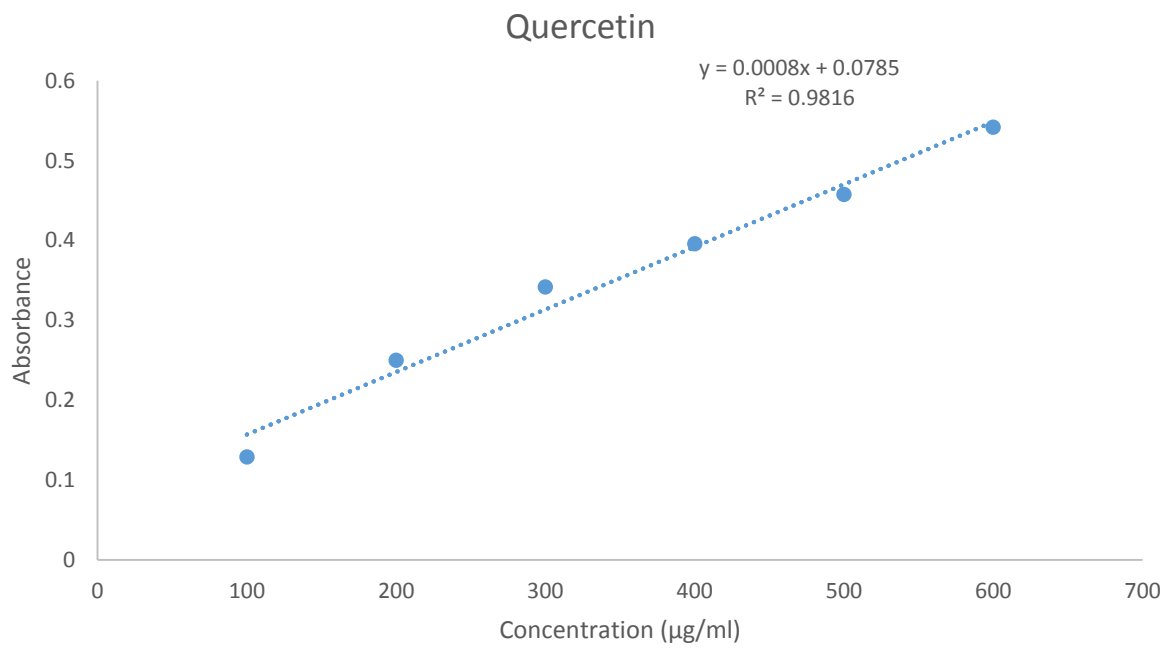


Figure 2: Standard curve of Quercetin

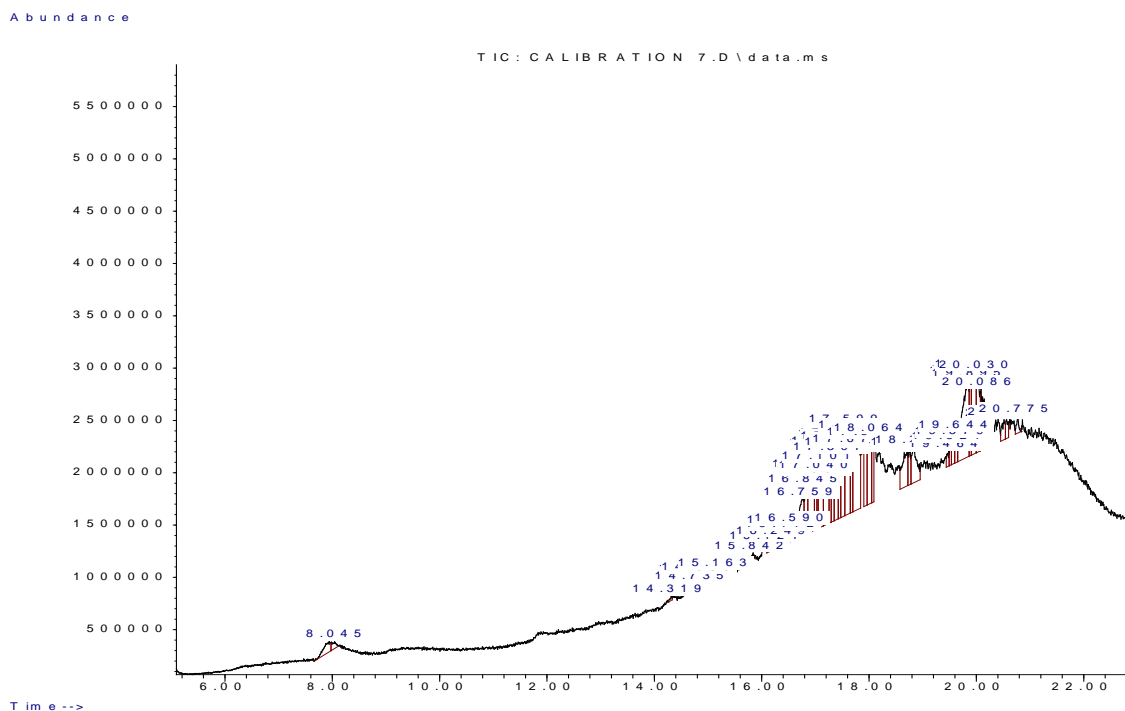


Figure 3: GC Chromatogram of methanol fraction of *J. tanjorensis*

Table 3: Phytochemicals identified in methanol fraction of *J. tanjorensis* by GC –MS

S/N	RT	% AREA	NAME OF COMPOUND	MOLECULAR FORMULAE	MWT g/mol
1.	7.932	1.67	Benzene,1,4-dimethoxy-2-methyl-5-iso propyl-	C12 H18 O2	194
2.	14.316	0.12	Vitamin E.	C29 H50 O2	430
3.	14.857	0.18	di-.alpha.-Tocopherol	C29 H50 O2	430
4.	15.160	0.25	1,22-Docosanediol	C22 H46 O2	342
5.	16.590	2.26	Lup-20(29)-ene-3-ol, acetate, (3-beta.)-	C32 H52 O2	468
6.	16.842	2.15	Sesquirosefuran	C15 H22 O	218
7.	16.916	4.79	Methoxyacetic acid, 2- pentadecyl ester	C18 H36 O3	300
8.	17.101	4.12	Cholest -4 -ene, 3.beta. -(methoxymethoxy)-	C30H54Osi	458
9.	17.242	7.15	1H-Benzimidazole, 2,2 - (2,5-furandiyl) bis-	C18H12 N4O	300
10.	17.271	2.09	D- Homopregn-17a(20)-ene, (5.alpha., 17aE)-	C 22H36	300
11.	17.405	3.93	4a,8-Dimethyl-2(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene	C 15H24	204
12.	17.442	2.37	.1,3-Benzodioxole, 5-(1-(4-ethoxyphenyl)ethyl-6-methoxy-	C18 H20 O4	300
13.	17.597	7.81	D- Friedoolean-14-en-3-one	C30 H48 O	424
14.	17.775	10.96	1,2-Thiagermolane, 2,2- dibutyl-	C11 H24 GeS	261
15.	18.708	12.80	4,4,6a,6b,8a,11,12,14b-Octamethyl-1,4,4a, 5, 6, 6a, 6b,7,8,8a,9,10,11,12,12a,14,14a, 14b- Octadecahydro-2H-picen-3-one	C30 H48 O	424
16.	19.849	15.5	12-oleanen-3-yl acetate, (3.alpha.)-	C32 H52 O2	468
17.	20.775	7.89	Beta-amyron	C30 H48 O	424

Table 4: Bioactivity of identified compounds in J. tanjorensis

S/N	NAME OF COMPOUND	BIOACTIVITY
1	Benzene, 1,4- dimethoxy-2	
2	methyl-s-isopropyl-	Antileishmammal, Antitrypanosomal
2	D – Friedoolean-14-en-3- one (Teraxerone)	Antifungal,Antioxidant,Inhibits nitric oxide generation
3	Lup-20(29)-en-3-ol, acetate, (3.beta) -	Antitumor,Antiarthritis,Anti-inflammatory,
4	Vitamin E.	Antileukemia, Hepatoprotective, Cardioprotective. Antiageing,Analgesic,Antidiabetic, Anti-inflammatory, Antitumor, Anticancer, Antidermatitic, Antileukemic, Hepatoprotective,Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antiplasmodic, Antibronchitis, Anticoronary. Antioxidant,
5	di-.alpha.-Tocopherol	Antioxidant, Anticancer,
6	1,22-Docosanediol	Emulsifier, Emollient, Thickener in cosmetics
7	4,4,6a,6b,8a,11,12,14b-Octamethyl-1,4,4a, 5, 6, 6a, 6b,7,8,8a,9,10,11,12,12a,14,14a, 14b-Octadecahydro-2H-picen-3-one	Antifungal,Antioxidant, Inhibits nitric oxide generation
8	12-oleanen-3-yl acetate, (3.alpha.)-	Antihyperglycemic,Anti-trichonomal, Cytotoxicity,Inhibits platelet aggregation. Anti- inflammatory,
9	Beta-amyrone	Antifungal, Anti-acetylcholinesterase

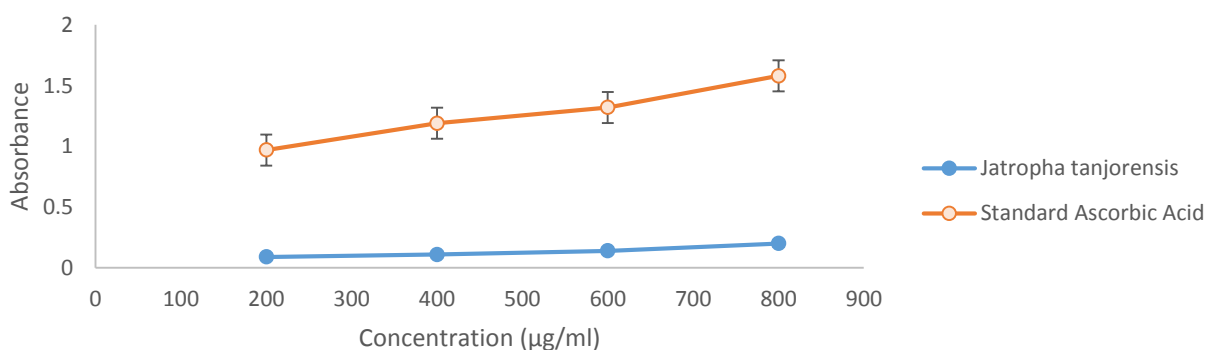


Figure 4: Result on reducing power of Jatropha tanjorensis. The values of the line chart are mean of triplicate determination and SEM was derived and served as the error bar as seen above (figure 4). Standard ascorbic acid served as the positive control. The ability of methanol extract of

Jatropha tanjorensis leaves to donate electron and stop radical chain reaction by converting free radicals to a more stable product was displayed in the reducing power ability of the extract. There was a dose dependent increase in the reducing power, though the ascorbic acid had more and better reducing power than the extract.

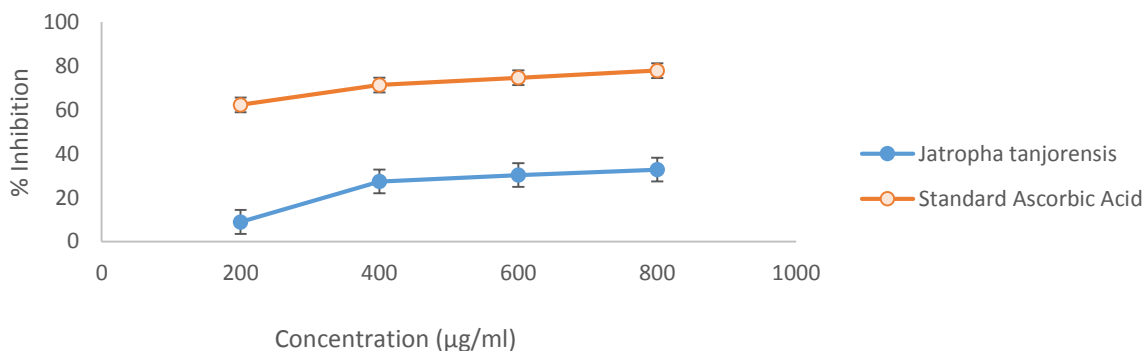


Figure 5: Result on Nitric oxide (NO) scavenging activity of Jatropha tanjorensis

Values represented with line chart are % inhibition of NO scavenging and each value is mean of triplicate determination (figure 5). SEM derived was as the error bar. Standard ascorbic acid served as the positive control. The percentage inhibition of nitric oxide was increased with increase in

concentration of the extract. The minimum inhibitory activity was 8.88 ± 0.63 at $200 \mu\text{g/ml}$ while the maximum activity was 32.70 ± 2.71 at $800 \mu\text{g/ml}$. The ascorbic acid used as standard control showed more inhibitory activity.

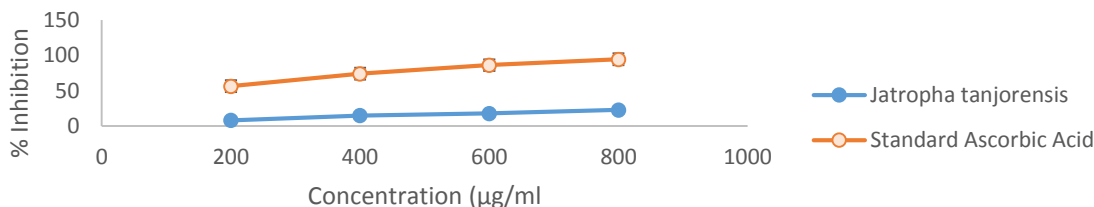


Figure 6: Result on Hydrogen Peroxide (H₂O₂) scavenging activity of *Jatropha tanjorensis*

Values represented with line chart are % inhibition of H₂O₂ and each value is mean of triplicate determination. SEM derived served as the error bar (figure 6). Standard ascorbic acid served as the positive control. The potential of methanol extract of *Jatropha tanjorensis* leaves to inhibit hydroxyl radical mediated cell damage was assayed at a concentration

of 200-800µg/ml. The extract showed a minimum percentage (%) inhibitory activity of 8.30 ± 0.88 at $200 \mu\text{g/ml}$ and a maximum activity of 22.80 ± 2.28 at $800 \mu\text{g/ml}$. This implies that the hydroxyl radical scavenging activity occurred in a dose dependent manner (fig 6).

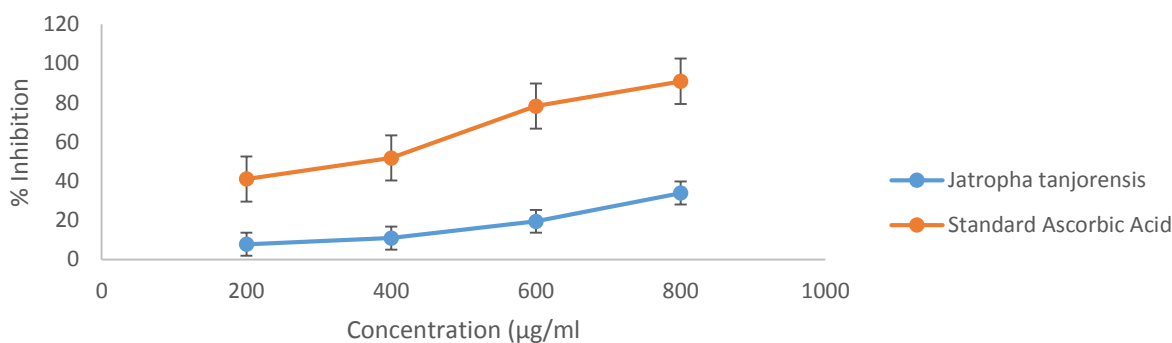


Figure 7: Result on Lipid peroxidation activity of *Jatropha tanjorensis*

Values represented with line chart are % inhibition of Lipid peroxidation and each value is mean of triplicate determination. SEM derived was the error bar (figure 7). Standard ascorbic acid served as the control. The scavenging effect of lipid peroxide radical increased with concentration. The methanol extract of *Jatropha tanjorensis* was effective scavenger of lipid peroxide radical.

DISCUSSION

The phytochemical content of *Jatropha tanjorensis* revealed the bioactive compounds present (Table 1). The result indicates a high presence of flavonoids and alkaloids in *Jatropha tanjorensis* leaves. The total phenolic content of the plant leaf extract was calculated using the standard curve gallic acid with regression values of $Y = 0.0011x + 0.0559$ and $R^2 = 0.9738$. While the total flavonoid content of *Jatropha tanjorensis* leaves was calculated using the standard curve Quercetin with regression values of $Y = 0.0008x + 0.0785$ and $R^2 = 0.9816$.

The high concentration of flavonoids and alkaloids can be attributed to the reason for therapeutic values of *Jatropha tanjorensis* in the treatment of diabetes mellitus due to its antihyperglycemic property as proposed by Olayiwola et al.,

(2004). There has been claim that *Jatropha tanjorensis* leaves cure anaemia, diabetes and cardiovascular diseases (Omeregbe and Osagie, 2011). This claim can be justifiably attributed to the high presence of these flavonoids and alkaloids in *Jatropha tanjorensis* leaves.

Flavonoids are believed to have various therapeutic values such as antihyperglycemic effect (Muriithi, et al., 2015), inhibition of cell proliferation and free radical scavenging activity (Holst, et al., 2008). The improvement of cardiac function, decrease anginas and lowering of cholesterol levels by medicinal plant is attributed to its flavonoids content (Nyamai, et al., 2016).

Alkaloids on its own are known to have antidiabetic and antioxidant activity (Yang, et al., 2001). Alkaloid fractions have shown hypoglycemic potential in mice (Cassidy, et al., 2000). The antihypertensive effects, antimalarial activity and anticancer as well as anti-arrhythmic effects of alkaloids have been reported (Dholi, et al., 2015; Chiu-Yin, 2002). Studies have shown that alkaloids have antimicrobial, cytotoxic and trypanocidal activity (Nyamai, et al., 2016).

The result of the present work also indicated the presence of other phytochemicals in *Jatropha tanjorensis* leaf. These

include phenols, saponins, tannins, terpenoids and steroids, this is similar and in agreement with the work of Oyewole and Akingbala (2011) which revealed the presence of these phytochemicals in in *Jatropha tanjorensis* leaf extract.

Phenols have been reported to have high antioxidant properties a great attribute to its therapeutic effect (Joshi, et al., 2001).

Terpenoids have antioxidant properties and also interact with most regulatory proteins (Wagner and Elmadfa, 2003). Saponins have been reported to have beneficial therapeutic effects they are known to have hypocholesterolaemic, immunostimulant, hypoglycemic effect and anticarcinogenic properties (Ros, 2000).

Clinical studies have shown that phytosterol intake leads to up to 15% reduction of LDL-cholesterol (Katan, et al., 2003; O'Neill, et al., 2005). Vanhanen et al., (1993) had reported that Intake of plant sterols reduces both plant sterol and animal cholesterol concentrations in the serum.

The indicator that shows a compound potential antioxidant activity is regarded as the reducing power. This reducing properties are generally related with the presence of reductones, which exhibit antioxidant activity by breaking the chain reactions by donating hydrogen atoms. Some precursors of peroxide could react with reductones, thus preventing formation of the oxidants (Meir et al., 1995). In this study, the extract exhibited considerable good reducing power activity though the ascorbic acid has more pronounced activity.

Mammalian cells produce nitric oxide which is a free radical involved in the regulation of various physiological processes.

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However, there is a close link between excess production of nitric oxide and several diseases. New research target for treating chronic inflammatory diseases is geared towards the development of substances that could prevent the overproduction of nitric oxide (Shen et al., 2002). The result from this study showed that *Jatropha tanjorensis* leaf could prevent overproduction of Nitric oxide thereby maintaining the normal physiological state of the human system.

Hydroxyl radicals are highly potent oxidants, which can react with biomolecules in living cells and cause severe damage (Gulcin, 2006). In the present study, the hydroxyl radical activity was significantly inhibited by the administration of methanol extract of *Jatropha tanjorensis* leaf to the reaction mixture.

Conclusion

Due to the increase in human diseases especially metabolic diseases such as diabetes, liver disease, myocardial infarction and hypertension, the role played by highly reactive oxygen species such as free radicals has become increasingly relevant and the study on medicinal plants for natural antioxidants is now imperative. This study indicates that *Jatropha tanjorensis* leaf possess antioxidant properties and could serve as free radical scavengers and act as essential antioxidants.

Conflict of Interest.

Authors declared no conflict of interest.

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