ANTIOXIDANT ACTIVITIES AND PHENOLIC CONTENTS OF THE LEAF AND STEM EXTRACTS OF JATROPHA *TANJORENSIS*

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ABSTRACT

The leaves and stem of *Jatropha tanjorensis* were extracted with methanol and ethyl acetate and these extracts were investigated for their antioxidant properties using 2, 2- Diphenyl-1-picryl hydrazyl radical (DPPH)assay and ferric reducing antioxidant potential (FRAP) assay. Total phenolic content was also determined by the Folin-Ciocalteu method. The results showed that percentage inhibition of (leaf and stem) extracts on DPPH radical as well as their reducing capacity was concentration dependent. Antioxidant activity of the extracts for DPPH scavenging assay was between 57.4 –87.37% to IC_{50} (67.00 – 101.09 µg/ml) and the ferric reducing capacity ranged from 0.034 -0.422 and IC_{50} (0.06 -1.408 µg/ml). The total phenol content ranged from 0.045±0.01- 0.067 ± 0.04mg/g for leaf extract while the content varied from 0.050± 0.01 to 0.057± 0.04mg/g for stem extracts. From the results obtained, all the extracts showed appreciable overall antioxidant activity in all of the methods used which may be linked to phenolic contents of the plant.

INTRODUCTION

Antioxidants are substances that combat free radicals such as superoxide anion (O^{2-}), hydroxyl (HO) radicals and non-free radical species such as H_2O_2 and singlet oxygen (O_2). These free radicals are extremely reactive species and can cause the oxidation of various biomolecules (lipids, proteins and nucleic acids) present in human beings thereby causing diverse illnesses such as cancer, neurodegenerative disorders, cardiovascular diseases, diabetes and other chronic diseases (Onoja *et al.,* 2014;).Oxidative stress occurs when the production of reactive oxygen metabolites exceed the capacity of the antioxidant system of the cell, tissue or body. Therefore, under the conditions of prolonged oxidative stress an exogenous supply of antioxidants is warranted in order to maintain the redox homeostasis and keep the debilitating diseases in check.

Researches over the years have produced convincing evidence towards application of natural antioxidants in place of the synthetic molecules as the later have associated toxicities (Tseng, 2006). Plants have long been a source of exogenous (dietary) antioxidants which contain many phytochemicals that are useful sources of natural antioxidants such as phenolic diterpenes, flavonoids, tannins and phenolic acids (Krishnaiah *et al.,* 2011). Polyphenols, especially flavonoids are generally known as the antioxidant agent in plant extracts and well known as radical scavengers, metal chelators, reducing agents, hydrogen donors and singlet oxygen quenchers (Bernardi *et al.*, 2008). Moreover, polyphenols are known to interact with other physiological antioxidants such as ascorbate or tocopherol and to synergistically amplify their biological effects (Croft, 1998).

Nigeria is blessed with enormous biodiversity resources, which some of them maybe unexpected food or remedy for the natives. *Jatropha tanjorensis* is a member of the Euphorbiaceae family, commonly called “hospital too far” or “Catholic vegetable” in southern Nigeria (Omoregie and Osagie, 2012). *Jatropha tanjorensis* is a native of Central America and has become naturalized in many tropical and subtropical countries, including Africa, India and North America (Prabakaran and Sudjatha, 1999). Other species are *Jatropha curcas*, *Jatropha glandulifera,*

Jatropha gossypifolia, Jatropha multifida, Jatropha podagrica and Jatropha intergerrima.Jatropha tanjorensis is predominantly grown in southern Nigeria (Oboh and Masodje, 2009). The leaves of the plant are a source of edible leafy vegetable and taken as a tonic in herbal medicine, with the claim that it increases blood volume (Omoregie and Sisodia, 2011).

Jatropha tanjorensis has been vastly studied due to its potential health benefits, availability and affordability. Traditionally, decoction of the leaves is used to treat anemia (as a haematinic agent), diabetes, skin diseases, malaria, and cardiovascular diseases (Oduola*et al*., 2005). The antibacterial, antiarthritic hypolipidemic, hepatroprotective and antioxidant activities of *Jatropha tanjorensis* have been reported (Ezeonu *et al.,* 2017). Reports have shown that *J. tanjorensis* is rich in antioxidant nutrients like phosphorus, selenium, zinc and vitamins C and E (Omobuwajo *et al*., 2011). It has equally been reported that *Jatropha* leaves are rich in beta blockers and anti-cancer components (; Ahaotu *et al.,* 2013). According to Egbon *et al.,* (2013), proximate analysis of *Jatropha tanjonrensis* revealed high carbohydrate content, soluble sugar and crude fibre as well as the total ash. In the mineral composition, high values were also recorded in Calcium, sodium, phosphorus and iron. The present study is aimed at investigating the *antioxidant* potentials of the different extracts of *Jatropha tanjorensis.*

MATERIALS AND METHODS.

Collection and Identification of Plant Materials

Different parts (leaves and stems) of *Jatropha tanjorensis* were collected from Mbiabam village in Ibiono Ibom Local government Area of Akwa Ibom State, Nigeria. The plant sample were identified by traditional users and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Nigeria. A voucher specimen of the plant species used for this research has been deposited in the University of Uyo Herbarium.

Preparation of Extracts

The plant parts were thoroughly washed with distilled water and air dried to constant weight at room temperature for 10 days. The air-dried leaves and stems were ground separately to fine powder and then stored in an air-tight container until further use. 1000g of each of the plant parts (leaves and stem) was extracted separately by cold maceration method with methanol and ethyl acetate and left for 72 hours with intermittent shaking. The plant extracts were filtered and then concentrated using rotary evaporator at 40 °C, and each extract was transferred into well labeled sterile glass vials and stored at 4 ℃ before use.

Determination of Polyphenols

The total phenolic content in the extracts were determined by the modified Folin-Ciocalteu method by Singleton *et al.* (1999) and Ayoola *et al.* (2008). Hydro alcoholic extracts of the sample in different concentrations ranging from 10µg/ml - 50µg/ml were prepared by dissolving the sample extract in methanol. An aliquot of each plant extract was mixed with 5 ml of Folin-Ciocalteu reagent which was previously diluted with distilled water (1:10 v/v) and 4 ml (75 g/l) of sodium carbonate (Na_2CO_3). The tubes containing the mixtures were allowed to stand for 30 min at 40 °C todevelop color. Absorbance was then read at 765 nm using the spectrophotometer. Results were expressed as Gallic acid equivalent in (mg/g) of extracts. All samples were analyzed in triplicate.

2, 2- Diphenyl-1-Picryl Hydrazyl (DPPH) Radical Scavenging Assay

The radical scavenging activity of the plant extracts against 2, 2- Diphenyl-1-picryl hydrazyl radical was determined by measuring UV absorbance at 515 nm. Radical scavenging activity was measured by a slightly modified method of Brand-Williams *et al.* (1995). Sample solutions at different concentrations ranging from, 10 - 50µg/ml was prepared. Vitamin C was used as standard and the same concentrations of it were prepared as the test solutions. All the solutions were prepared with methanol. One ml of each prepared concentrations was placed into test tubes and 0.5 ml of 1M DPPH solution in methanol was added. The experiments were carried out in

triplicates. The test tubes were incubated for 15 min and the absorbance was read at 517 nm. A blank solution was prepared and measured containing the same amount of methanol and DPPH.

The radical scavenging activity was calculated using the following formula:
% inhibition = {(AB – AA)/AB} x 100.
Where AB is the absorption of blank sample and AA is the absorption of tested extract solution. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and represented as IC_{50} value for each of the test solutions.

Ferric Reducing Antioxidant Power Assay
The reducing capacity of the different extracts under study was determined using a standard procedure as described by Oyaizu (1986). Briefly, Methanol and ethyl acetate extracts from leaves and stem at different concentrations (10, 20, 30, 40 and 50 µg/ml) were mixed with phosphate buffer (0.2 M, 2.5 ml, pH 6) and potassium ferric cyanide (2.5ml, 1%). The mixture was incubated at 50ºC for 20 min, trichloroacetic acid (TCA) (2.5 ml, 10%) was added and the mixture was centrifuged at 100ºC for 10 min. Supernatant (upper layer) (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as positive reference. The experiment was done in triplicate.

RESULTS AND DISCUSSION

Phenolic compounds are the most important groups of secondary metabolites in medicinal herbs and dietary plants that characterized by at least one aromatic ring (C_6) bearing one or more hydroxyl groups. Examples include benzoic and cinnamic acid, coumarins, tannins, lignins, lignans, flavonoids etc. These groups of compounds possess a diverse range of beneficial biological functions, including antioxidant activity. Plant phenols constitute the major group of compounds that act as primary antioxidant due to their redox properties that allow them to act as reducing agents, hydrogen donors and metal chelators. Besides their role as antioxidants, these compounds present a wide spectrum of medicinal properties, such as antiallergic, anti-inflammatory, anti-bacterial and anti-thrombotic, plus present cardio protective and vasodilator effects showing a broad field of application for the phenolics in these plants. (Balasundram *et al.*, 2006; Khoddami *et al.*, 2013; Bhatt *et al.*, 2012The total phenolic contents of *Jatropha tanjorensis* extracts determined by Folin-ciocalteu method are reported as mg of Gallic acid equivalents per gram of extract and are presented in Tables 1 and 2. The total phenolic content of the leaf extracts ranged from 0.045±0.01 - 0.053±0.02 mg/g for methanol extract while 0.059±0.02 - 0.067±0.04mg/g was the range for the ethyl acetate leaf extract. For the stem extracts, 0.050±0.01- 0.058±0.02mg/g was the range for methanol extract while the total phenolic content for ethyl acetate stem extract varied from 0.051±0.02 to 0.057±0.04mg/g. From the results obtained, the leaf extract specifically the ethyl acetate recorded the highest phenolic content; however, there was no significant difference in the phenolic content in the two extracts.

Table 1: Total phenolic content of the leaf extract of *Jatropha tanjorensis*

Volume (µg/ml)	Methanol extract mg/g	Ethyl acetate extract mg/g
10	0.045±0.01	0.059±0.02
20	0.047±0.05	0.062±0.05
30	0.048±0.04	0.063±0.04
40	0.049±0.05	0.065±0.01
50	0.053±0.02	0.067±0.04

Table 2: Total phenolic content of the stem extract of *Jatropha tanjorensis*

Volume (µg/ml)	Methanol extract mg/g	Ethyl acetate extract mg/g
1	0.050±0.01	0.051±0.02
20	0.053±0.05	0.052±0.05
30	0.055±0.04	0.054±0.04
40	0.057±0.05	0.056±0.01
50	0.058±0.02	0.057±0.04

There are various methods to estimate the antioxidant activity of compounds in plant extracts. However, one method alone is unable to recognize all possible mechanisms characterizing an antioxidant (Erkan *et al.*, 2008). In this study, DPPH and FRAP methods were used in determining antioxidant in samples since analyzing data obtained from these two methods could provide a more precise description of antioxidant activity of these samples. DPPH is a very stable free radical and it is widely used to evaluate antioxidant activities in a relatively short time. The assay is based on the reduction of DPPH radicals in methanol which causes an absorbance to drop at 515 nm

The DPPH free radical scavenging activity of the leaf and stem extracts of *Jatropha tanjorensis* are presented in Figures 1 and 2. From the results obtained, the relationship is dose dependent and the activity increased with increase in concentrations of both extracts. For the leaf extract, ethyl acetate extract exhibited the highest scavenging activity (87 .37 ± 60% at 50µg/ml) while for the stem extracts, methanol extract had the highest scavenging activity (72.00±1.63% at 50µg/ml). The variation may be attributed to the chemical nature of the endogenous extractable compounds which can be polar or non-polar. The research findings have shown that the plant leaves contained high amount of antioxidant as revealed in the pronounced free radical scavenging activity. This was more prominent in the ethyl acetate extract. It was evident that the plant extracts showed proton-donating potential which could serve as free radical inhibitors.
The IC_{50} (50% of inhibition) value was calculated following a linear regression analysis of the observed inhibition percentage versus concentration, where a lower IC_{50} value shows higher antioxidant activity ()..

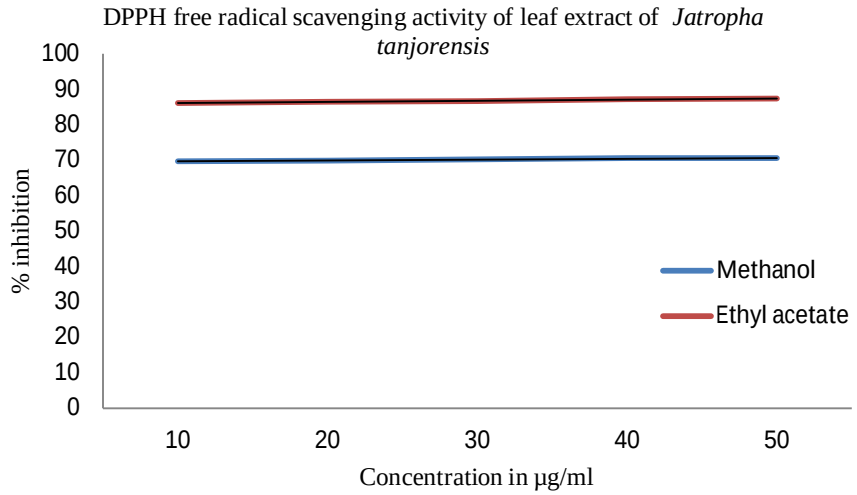

Figure 1: DPPH free radical scavenging activity of leaf extract of *Jatropha tanjorensis*
M= Methanol leaf extract; E = ethyl acetate leaf extract

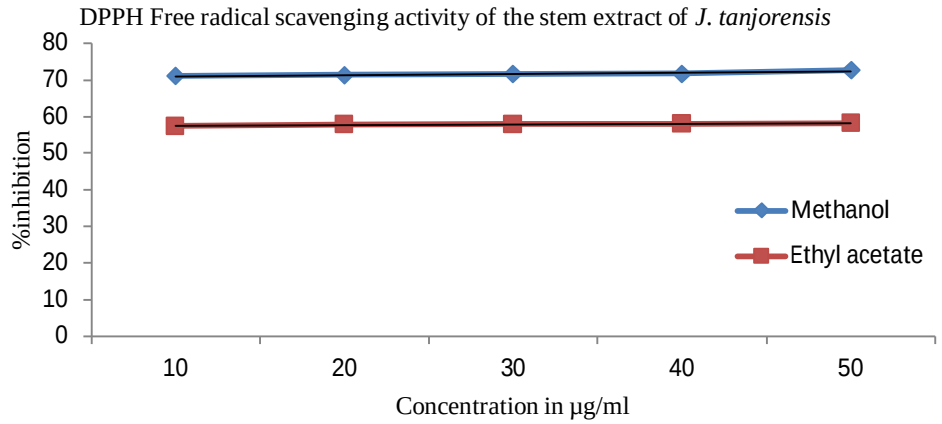

Figure 2: DPPH free radical scavenging activity of stem extract of *Jatropha tanjorensis*

Total antioxidant activity of plants has been monitored using Ferric Reducing Antioxidant Power (FRAP) assay as it is the only assay that directly measures antioxidants or reductants in a sample. The FRAP assay, in contrast, uses antioxidants as reductants in a redox-linked colorimetric reaction (Sharma *et al.*, 2009). Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant. In the reducing power assay, the presence of antioxidants in the samples would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron (Antia *et al.*, 2015). The ferric reducing activity of the leaf and stem extracts of *Jatropha tanjorensis* are presented in Figures 3 and 4. The reducing capacity of the extract, another significant indicator of antioxidant activity was also found to be appreciable comparable with ascorbic acid. Like the radical scavenging activity, all the extracts showed concentration-dependent reducing power. However, the methanol leaf extract exhibited the highest reducing power whereas for stem extracts, ethyl acetate extract exhibited the highest reducing activity. The reducing power of these extracts might be due to their hydrogen-donating ability.

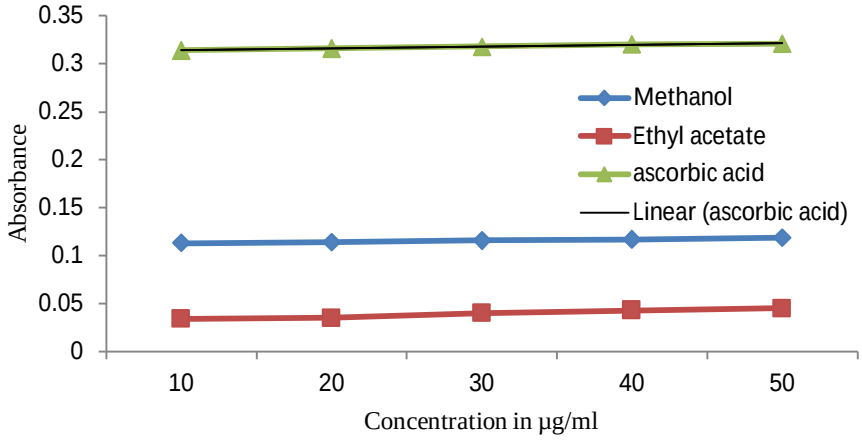

Figure 3: Ferric reducing power activity of leaf extract of *Jatropha tanjorensis*

Table 3: IC_{50} in µg/ml for antioxidant activity of *Jatropha tanjorensis* extracts

Activity	Methanol leaf extract (µg/ml)	Ethyl acetate leaf extract (µg/ml)	Methanol stem extract (µg/ml)	Ethyl acetate stem extract (µg/ml)
DPPH	81.79	101.09	88.15	67.00
Ferric reducing (FRAP)	0.06	0.18	1.152	1.408

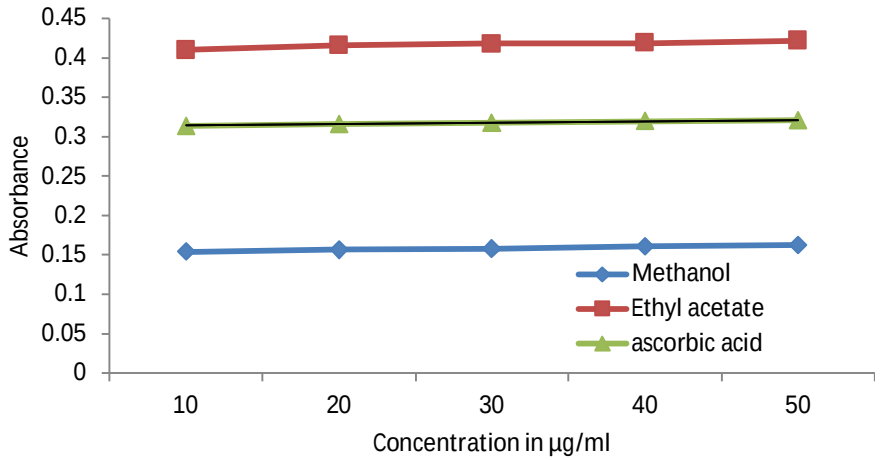

Figure 4: Ferric reducing power activity of stem extract of *Jatropha tanjorensis*

On comparing the IC_{50} values obtained for FRAP assay with that of DPPH, the result indicated that the IC_{50} for reducing power were better since it was significantly lower. The results also showed that the leaf extract had a lower FRAP value, where a lower IC_{50} value shows higher antioxidant activity). FRAP antioxidant capacity were higher than that of DPPH free radical scavenging activities. The reason may probably due to the difference in compounds that are reactive towards the two different methods. Despite the various mechanisms of the methods used, the relative antioxidant activity of different parts of *Jatropha tanjorensis* combined results of these in vitro tests gave an idea of the relative or otherwise medicinal activity of the plant thereby validating its use in rural areas.

CONCLUSION AND RECOMMENDATION

The methanol and ethyl acetate leaf and stem extracts of *Jatropha tanjorensis* contained appreciable level of total phenols with ethyl acetate having the highest concentration in the leaf extract, while methanol extract contained the highest phenolic content in the stem extract. The extracts exhibited significant antioxidant activity – DPPH radical scavenging and ferric reducing capability when compared with standard compounds. The present investigation suggests that *Jatropha tanjorensis* possess good antioxidant potential that can be exploited for pharmaceutical uses.

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