

Evaluation of the antioxidative properties of different Fractions of Ethanol extract of *Jatropha tangorensis*

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Abstract

The antioxidant potential of crude ethanolic as well as ethyl acetate and n-hexane soluble fractions of *Jatropha tanjorensis* which is widely used in indigenous system of medicinal for different purposes were studied. The antioxidant potential of extract different Fractions were evaluated using different in vitro antioxidant models which includes Ascorbic acid, Beta carotene, flavoniods, total phenols, ABTS, H₂O₂, FRAP and reducing power. The estimation of the crude extract showed ascorbic acid (93.51mg/kg), beta carotene (10.564mg/kg), flavoniods (7.88mg/kg), total phenols (64.03mg/kg), ABTS (64.21%), H₂O₂ (6.39%), FRAP (0.221μmol/ml) and Reducing power (2.913μmol/ml). That of ethyl acetate fraction and n-hexane fractions for Ascorbic acid, beta carotene, flavoniods, Total phenols, ABTS, H₂O₂, FRAP and reducing power are as; 227.8mg/kg, 9.21mg/kg, 0.59mg/kg, 33.94mg/kg, 96.47%, 2.5%, 1.1911 μmol/ml, 4.500μmol/ml and 228.7mg/kg, 9.21mg/kg, 0.59mg/kg, 33.94mg/kg, 98.93%, 2.32%, 1.578μmol/ml, 4.456μmol/ml respectively. However, the result of this Study reveals the plant contains some appreciable amount of the antioxidant models analyzed which is vital to illnesses associated with oxidative stress thereby revealing that the plant is a good source of natural antioxidant.

Keywords: Antioxidant, Oxidative stress, Free radicals, *Jatropha tangorensis*.

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INTRODUCTION

Free radicals like reactive oxygen species (ROS) and reactive nitrogen species (RNS) are known to cause damages to the cellular biomolecules resulting in degenerative diseases (Atere *et al.*, 2018). However, antioxidants serves as agents used in mitigating these oxidative process which is harmful to the human health. These free radicals tends to cause an imbalance in the body leading to oxidative stress that is being suggested as the root cause of agings and various human diseases such as stroke, diabetes, cancer, atherosclerosis and neurodegenerative diseases (Olaniyi *et al.*, 2013).

Nature has provided remedies in form of herbs as medicinal plants are abundantly rich in antioxidant compounds as a result of various phyto- constituents in them. So disease linked with free radicals can be prevented by antioxidant therapy which is obtained from natural herbs, has gained an immense importance.

Current research is now directed towards finding naturally occurring antioxidants particularly of plant origin (Sannigrahi *et al.*, 2010).

Jatropha tanjorensis belongs to the family Euphorbiaceae and is common weed of field crops in the higher rain forest zones of West Africa. It is called "Hospita too far" or "Catholic vegetable" in Nigeria (Madubuike *et al.*, 2015). Furthermore, methanolic extract of *Jatropha tanjorensis* exhibited antioxidative effect as revealed in a study (Madubuike *et al.*, 2015). Another study showed that *Jatropha tanjorensis* has a potent but does dependent free antioxidant activity due to it's high Phenolic content (Atansuyi *et al.*, 2012).

Currently available synthetic antioxidants have been suspected to cause negative health effects and some of these drugs are expensive to afford. There is still demand to derive new leads from natural herbs. Therefore, there is dearth of information on the

contribution of the Phenolic component of the plant to its widely reported pharmacopotency in order to understand the mechanism behind vast therapeutic relevance of *J tanjorensis*. Therefore, the objectives of this present study were to determine and evaluate the antioxidative properties of different Fractions of Ethanol of *Jatropha tanjorensis*.

MATERIALS AND METHODS

Collection of plant material and preparation of plant extract

Fresh leaves of *J. tanjorensis* used were harvested from Umuokomoche community in Nekede, Owerri West L. G. A. Imo State, Nigeria and taxonomically authenticated by a Botanist at Federal University of Technology Hebarium, Imo State. The leaves were washed, rinsed and air dried for 1 week after which they were pulverized into fine powder. 100g of powdered material was extracted with 1L of absolute Ethanol at room temperature using maceration method for 3 days. The combined extract was filtered using muslin cloth and the filtrate was further concentrated using rotary evaporator apparatus at 78°C. The crude extract was dried in the dessicator before finally stored in refrigerator prior to use.

Fractionation

The ethanolic crude extract was prepared for separation by adding methanol silica gel after which the mixture was smoothened and homogenized. The chromatographic column was set up, rinsed with distilled water. Silica gel was Introduced half the column. The crude extract was dissolved and partitioned with n-hexane and ethyl acetate to give n-hexane, ethyl acetate and water soluble fractions.

Sample Quantitative Analysis

Some quantification tests were carried out on the crude extract and fractions for Ascorbic acid presence, beta carotene, total phenol, total flavoniod, ABTS scavenging effect, Hydrogen peroxide, ferric reducing properties and TAC using UV-Spectrophotometer.

Estimation of Ascorbic acid

Ascorbic acid was analyzed by the Spectrophotometric method described by Reo (1943). 0.5ml of the sample was collected and was transferred into a test tube, 2ml of TCA was added and then incubated for 3hours. After incubation, 2ml of sulfuric acid mixed with 2ml of 25% ammonia solution was added. The mixture was filtered and filtrate was analyzed using Uv-spectrophotometer at 540nm. Amount of Ascorbic acid = [(Absorbance of sample X Conc. Of standard) / Absorbance of standard]

Estimation of Beta carotene

0.5ml of the extract and fractions were collected and transferred to a test tube 2.5ml of KOH (alcohol) was added, 10mlnod petroleum ether was

added. A change in colour was observed with two layers, the upper layer which is light in green color and lower layer which is dark in green color. The upper layer was collected and analyzed using uv-spectrophotomer at 450nm. The amount if total beta carotenoids was calculated;

Amount of Beta carotene= [(Absorbance x volume of the sample x 100 x 4) / Weight of sample].

Estimation of Total phenols

The samples of about 0.5ml was collected and was put in a test tube. 5ml of n-butanol was added and shaken well then allowed to settle. The supernatants were evaporated to dryness. The residue was then dissolved with 1ml of distiller water. 1ml or ammonia solution was added, 5mlnof isopropyl alcohol was added to give a green colour change. The mixture was analyzed using a Uv Spectrophotometer at 650nm against a blank reagent (Mallick and Singh, 1980).

Concentration of phenols is expressed as mg/g.

Amount of phenol = [(Absorbance of sample x Conc. of standard) / Absorbance of standard].

Estimation of flavoniods

0.5ml of the samples were measured into a test tube , then 1ml of methanol, 1ml of adrenaline were added and the sample was filtered then it was subjected to spectrophotometric analysis (Cameroun *et al.*, 1943). Amount of flavoniods = [(Absorbance of sample x Conc. Of standard) / Absorbance standard].

ABTS Scavenging effects

0.5ml of the samples were collected and transferred into a test tube. 0.5ml of ABTS and 1ml of methanol was added then the mixture was filtered. The filtrate was subjected to UV spectrophotometer for analysis at 745nm.

% Inhibition = [(Conc. Of Standard - Sample Absorbance)/ Conc. Of Standard] x100. (Stares *et al.*, 1980).

Estimation of Hydrogen peroxide

The method or Ruch *et al.*, (1989) was employed. 0.5ml of sample was collected and transferred into a test tube. 1ml of phosphate buffer was added and incubate for 5 minutes. 2ml of 3% Hydrogen peroxide was added. The mixture was filtered and filtrate was examined. Analysis was done using uv spectrophotometer at 430nm. A blank solution containing phosphate buffer without Hydrogen peroxide was prepared. The extent of Hydrogen peroxide Scavenging of the samples were calculated as; % Scavenging of hydrogen peroxide = [(A₀ -A₁)/A₀] x 100

A₀= Absorbance of control

A₁= Absorbance in the presence of sample.

Ferric Reducing property

The reducing property of the extracts was evaluated employing the method of pulido *et al.*, (2000). 0.5ml of the sample was transferred into a dry test tube. 1ml of 1% potassium buffer was added into it and then heated for 5mins. 0.2ml of potassium ferricyanide was added, 2ml of trichloroacetic acid was added then 1ml of water was added. 0.5ml of 10% ferric chloride was added to give a deep-blue colour. The mixture was filtered and the filtrates were analyzed at an absorbance of 700nm.

FRAP = [(Absorbance of sample x vol of sample x conc. Of standard) / Absorbance standard]

Reducing Properties

The principle depends on the ability of the extract to chelate Iron (II) in which the method of pulido *et al.*, (2000) was employed. 0.5ml of the samples were Transferred into a dry test tube. 1ml of 0.05M NH_4FeSO_4 , 1ml of 0.1M of Tris-HCL, 0.5ml of 0.5M NaCl were added and also 0.5ml of phenanthroline was added also. The mixture was filtered and analyzed using uv-spectrophotometer at 510nm.

Reducing Power = [(Absorbance of Sample x vol. Of sample) / (Absorbance standard x Conc. Of standard)]/1

RESULTS AND DISCUSSION

Parameters	Values of Analysis		
	Crude extract	Ethyl acetate fraction	n-Hexane
Ascorbic acid	93.51mg/kg	228.7mg/kg	228.7mg/kg
Beta carotene	10.56mg/kg	9.21mg/kg	9.21mg/kg
Flavoniods	7.88mg/kg	0.59mg/kg	0.59mg/kg
Total phenols	64.03mg/kg	33.94mg/kg	33.94mg/kg
ABTs	64.21%	96.47%	98.93%
H_2O_2	6.39%	2.5%	2.32%
FRAP	0.221 $\mu\text{mol/ml}$	1.911 $\mu\text{mol/ml}$	1.578 $\mu\text{mol/ml}$
Reducing power	2.913 $\mu\text{mol/ml}$	4.500 $\mu\text{mol/ml}$	4.456 $\mu\text{mol/ml}$

DISCUSSION

With a view to develop an antioxidant agent of plant origin a common plant available in and around Southeast region, *Jatropha tanjorensis* belonging to euphorbiaceae is selected. Plants have diverse groups of Phenolic compounds and all these Phenolic classes have gained extensive attention because of their physiological functions (Adebiyi *et al.*, 2017). According to pietta (2000), the antioxidant activity of phenolics is largely due to the redox properties which makes them functions as reducing agents, hydrogen donors, singlet oxygen quenchers and as well as potential metal chelators. In this study, high level of phenolics was observed majorly in the crude extract than in fractions of ethyl acetate and n-hexane. This way give claim to the folkore use of the plant.

The Ascorbic acid estimated in the plant was found to be higher in both the ethyl acetate fraction and n-hexane fraction having a value of 228.7mg/kg and 228.7mg/kg respectively. However, Ascorbic acid has a substantial potential of not only Scavenging ROS, but enhances oxidative defense potentials. The Beta-carotenoids result revealed a higher level of carotenoids in the crude extract on comparison to the fractions. Szalay (2016) in his article expounded that beta-carotenoids help lower the risk of metabolic syndrome in the middle age and elderly men. Furthermore, the crude extract of *Jatropha tanjorensis* contains higher level of flavoniods (7.88mg/kg) and phenols (64.03mg/kg) on comparison to the ethyl acetate and n-hexane fractions. Previous studies has revealed antioxidant activities which have been associated to these phenol and flavoniod compounds.

The ABTs assay has been employed as an index that reveals antioxidant activity. The scavenging of the ABTs radical by the n-hexane fraction in this study was found to be high (98.93%). This shows that *Jatropha tanjorensis* n-hexane fraction presents a higher ability to scavenge the ABTS radical than ethyl acetate fraction and therefore the ABTS assay of crude extract showed a lower activity.

The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine complex and produce a colored ferrous tripyridyltriazine (Adebiyi *et al.*, 2017). However, the increasing concentration of n-hexane and ethyl acetate fraction than the crude extract shows that *Jatropha tanjorensis* should be able to donate electrons to free radicals stable in the actual biological and food systems.

The reducing power of the samples were investigated for transformation of $\text{Fe}^{3+} \rightarrow \text{Fe}^{2+}$. The increased concentrations of the n-hexane and ethylacetate than the crude extract showed higher reducing power as reducing power increases significantly with increase in concentration.

The ethanolic crude extract showed a good scavenging ability of 6.39% compared to ethylacetate and n-hexane fraction of 2.5% and 2.32% respectively being that hydrogen peroxide is a weak oxidizing agent that inactivates enzymes directly, usually by oxidation of essential thiol (-SH) groups.

CONCLUSION

Based upon the results obtained in the present study, it is concluded that ethanolic crude extract and fractions of *Jatropha tanjorensis* contains considerable amount of phenol, Ascorbic acid, flavonoids and carotenoids exhibits high antioxidant and free radicals Scavenging functions vital to illnesses associated with oxidative stress. It also chelates and has reducing power. These reveals that plant is a significant source of natural antioxidant which might be helpful in preventing the advancement of various oxidative stress. However, further isolation of bioactive compounds would be helpful in ascertaininh it's potency and safety as a lead candidate of antioxidant for further pharmacological uses.

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