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A COMPARATIVE STUDY OF THE ANTIOXIDANT AND PROTEOLYTIC ACTIVITIES OF METHANOL EXTRACTS OF JATROPHA GOSSYPIFOLIA AND JATROPHA CURCAS LEAVES

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ABSTRACT

Jatropha gossypifolia and Jatropha curcas are plants used in Nigeria and other parts of the world as folk medicine in the treatment §and cure of various diseases. The aim of this study is to evaluate and compare the antioxidant and proteolytic activities of these plants. Phytochemical screening revealed the presence of phenolic compounds, saponins, flavonoids and cardenolides (*J gossypifolia*) and carotenoids, alkaloids, cardenolides, saponins, phenolic compounds and flavonoids (*J curcas*). The most distinguished radical scavenging potential was observed for methanol extract of *J curcas*. The IC_{50} of *J curcas* was 173.2± 0.01µg/mL while *J gossypifolia* revealed an IC_{50} of 337.5±0.02 µg/mL. A higher milk-clotting property was observed for *J curcas*. The milk clotting activity was shown to be concentration dependent. The overall results suggested that the extracts *J gossypifolia and J curcas* could be a potential source of natural antioxidants in addition to possessing potent proteolytic properties.

KEYWORDS: Antioxidants, proteolytic effects, methanol extract, clotting time.

INTRODUCTION

For many decades, man has utilized spices, fruits, vegetables, and herbs which are known to contain potent secondary metabolites against diseases.[1] Vegetables, spices, and herbs contain important natural substances such as antioxidants and proteolytic compounds.

Antioxidants play important key roles in health care to prevent and scavenge free radicals; alleviate debilitating chronic diseases and degenerative ailments like cancer, autoimmune diseases, hypertension, atherosclerosis etc.[2-4] Free radicals are produced in all living cells as part of normal cellular and physiological function. However, production of excess free radicals plays a key role in the development of many diseases. Examples of such diseases include immune-suppression, many chronic and degenerative diseases, including cancer, atherosclerosis, diabetes mellitus, and neurodegenerative diseases as well as ageing.[5] They are said to be associated with free radical mediated oxidative stress.[6] Naturally, cells employ different cellular antioxidant systems and mechanisms to protect themselves from free radical-mediated oxidative stress such as low molecular mass antioxidants (glutathione, tocopherols, ascorbic acid); enzymes interacting with reactive oxygen species (ROS) such as superoxide dismutase, peroxidases, catalases; and other enzymes generating reduced forms of antioxidants.[7] Scientific studies have demonstrated a positive correlation between the consumption of fruits and vegetables and prevention of diseases associated with oxidative stress.[5,8] Plant tissues contain wide array of antioxidants like flavonoids, tannins, and lignin precursors, which act as ROS-scavenging compounds.[7]

Proteases have been shown to play diverse roles in health and disease.[9] These roles are important for normal physiological functions in the body, including digestion, maintenance of normal blood vessel, angiogenesis, formation and dissolution of blood clot, bone remodeling and ovulation.[10,11] Nevertheless, their breakdown can lead to a variety of pathological processes, including cancer, pancreatitis and thrombosis.[12] A very promising strategy to control proteases could involve the development of selective low-molecular weight inhibitors from natural sources with minimal toxicity. Thus, proteolytic activities by these inhibitors pose a promising strategy in combating these pathologies.

Jatropha gossypifolia (Euphorbiaceae) is a traditional medicinal shrub plant traditionally used in Nigeria and other parts of the world for the management of skin diseases, diabetes, and cancers.[13] Leaves of Jatropha gossypifolia (pignut or fignut) plant are used locally in Nigeria and other parts of the world to treat a wide array of ailments. Extracts from the leaves are used for bathing or dressing wounds[14] treating sores, sprains, rashes in Nigeria, Latin America and the Caribbean.[15,16] Scientific reports have highlighted the use of the poultices of the plant treating sores and pain in Trinidad.[17] Oduola and co-workers established the anticoagulant properties of the leaf extract but were unable to further validate its coagulant

properties.[16] Studies have established that different parts of *J. gossypifolia* contain phenolics, flavonoids, and alkaloid compounds.[18,19] Reports by Aboaba et al., highlighted the presence of phytol, germacrene, and linalool as some of the leaf volatile oil constituents of J. gossypifolia.[20]

Jatropha curcas is a large shrub or a small perennial tree with a height of about 3-10 meters.[21] This plant is mostly found in tropical and subtropical regions of Southeast Africa, Central and Latin America, Asia and India. For many years, extracts of different parts of the plant (such as seed, leaf, stem bark, fruit, and latex) have been used in traditional medicine and for veterinary purposes.[22] Anti-hyperglycemic effects of the methanolic extract of leaves of *Jatropha curcas*. L have been reported *in vivo;*[23] antiulcer activity of alcoholic extract of the leaves have been demonstrated *in vivo*;[24] in addition, methanolic/aqueous extracts of leaves of *Jatropha curcas* L. have also been shown to inhibit drug-resistant HIV strains and hemagglutinin protein of influenza virus.[25,26] The aim of this study was to evaluate and compare the antioxidant and proteolytic activities of methanol leaf extracts of *Jatropha* gossypifolia and Jatropha curcas.

MATERIALS AND METHODS

Standards and Reagents

Ascorbic acid (Emzor, Nigeria), Calcium chloride, methanol were obtained from Fisher Scientific, Toronto, ontario), 2,2-diphennyl-1-picrylhydrazyl (DPPH), chloroform, ethyl acetate, formic acid, tetraoxosulphate (IV) acid, layers reagent, dragendortff's reagent, glacial acetic acid, sodium hydroxide, ferric chloride, ammonia solution and then were obtained from Sigma aldrich, Marvel dried skimmed milk was obtained from Chivers Ireland ltd, Cooklock dublin) chymotrypsin was obtained from pymepharco ltd. All chemicals used were of analytical grade.

Plant Materials

Fresh plant materials were collected from Iju (Azure, Ondo state, Nigeria) in September 2011. The leaves were identified in the department of Plant science and biotechnology, University of Port Harcourt and a voucher deposited in the herbarium.

Sample processing and extraction

After identification, the samples were washed with clean tap water to remove dirt on the leaves. The leaves were kept in a mesh and allow water to drain off, it was then cut in small

pieces with stainless steel knife and dried under shade. Thedried leaves were then ground to obtain a fine powder using electric grinder.

Extraction

One hundred grams (100g) of the ground *J. gossypifolia* leaf powder was weighed and macerated in 1 litre of methanol in a stoppered container and allowed to stand for 96 hours with constant agitation. The mixture was filtered with a suction pump and the filtrate was allowed to evaporate using water bath set at 45^{o}C.

Phytochemical screening

Basic phytochemical screening was carried out to detect the presence of tannins, saponins, anthraquinones, alkaloids, and other phenolic compounds in accordance with the standards method of Trease and Evans,1996; Soforowa, 2006.[27,28]

Test for alkaloids

About 20g of the powdered drug sample was stirred with 20 mL 1% H_2SO_4 on a water bath. The mixture was centrifuges and supernatant collected. 1ml of the supernatant was treated with a few drops of Mayer`s reagent (Mercuric iodide solution) and another 1ml portion was treated similarly with Drangendorffs reagent (iodine in KI solution). Cream and orange colour observed indicated the presence of alkaloids.[27,28]

Test for flavonoids

Dilute sodium hydroxide (NaOH) was added to 5 mL of the extract, a yellow colour on addition of HCL confirms the presence of flavonoids.[27]

Test for tannins

5mls of plant extract was stirred with 2mls of distilled water, filtered and 5 drops of $FeCl_3$ reagent added to the filtrate. A blue-black, green, blue-green precipitate is evidence of the presence of tannins.[27]

Test for saponins

The frothing test method of Wall et al.was used for the experiment.[29] 10 mg of the extract was shaken with 1.5 mL of normal saline in a test tube. Frothing which persists on warming was taken as preliminary evidence for the presence of saponins.

Test for glycosides

Test Salkowski test described by Sofowora, 2006 was used.[28] 5 mL of chloroform was added to 5ml of extract. H_2SO_4 was carefully added to form a lower layer. A reddish brown colour at the interface indicates the presence of a steroidal ring which is a glycone portion of a cardenolide glycoside.

Test for phenols

10 mL of 70% alcohol was added to 500 mg of the powdered extract. This was warmed on a water bath for 2 minutes. Two drops $FeCl_3$ were added and a bluish colour indicates the presence of phenolic compound.

Test for carotenoids

500 mg of the powdered sample was weighed out and 30 mL of chloroform was added. The resultant solution was filtered and then evaporated to dryness. This was reconstituted with 0.5 mL of chloroform and then 3ml of concentrated $H_2SO_4$was added to form a layer. A reddish-brown precipitate colouration at the interface formed indicated the presence of carotenoids.

Antioxidant assay

Rapid radical scavenging screening

With the aid of a capillary tube, stock solutions from the *J. gossypifolia* were spotted on silica gel thin layer chromatographic (TLC) plates and developed with the solvent systems of chloroform, ethyl lactate and formic acid were mixed in the ratio of 5:3:1. After development, the chromatograms were dried and sprayed with a 0.4 mM solution of the stable radical, DPPH. Yellow spots formed against purple background were taken as positive results. The time (duration) for the development of the yellow colour indicated whether the antioxidant activity was strong or not.[30,31]

DPPH photometric assay

The free radical scavenging activities of *J. gossypifolia* extract and ascorbic acid (positive control) were analysed by the DPPH assay.[32] A 1.0 ml of the test extract, at gradient final concentrations of 5 - 200 µg/ml, was mixed with 3 mL of 0.3 mM DPPH solution in MeOH in a cuvette. The absorbance was taken at 517 nm after 20 minutes of incubation in the dark at room temperature. The experiment was done in triplicates. The percentage antioxidant activity was calculated as follows:

%Antioxidant Activity [AA] = 100 - [{(Abs $_{sample}$ − Abs $_{blank)}$ X 100}/Abs $_{cantrol}$]. Where Abs $_{sample}$ was the absorbance of sample solution (2.0 ml) + DPPH solution (1.0 ml, 0.3 mM), Abs $_{blank}$ was the absorbance of Methanol (1.0 ml) + sample solution (2.0 ml), Abs $_{control}$ was the absorbance of DPPH solution (1.0 ml, 0.3 mM) + methanol (2.0 ml).

Milk clotting assay

The substrate (10% skim milk, Marvel®, Chivers ltdDublin, Ireland) was prepared in distilled water or in 0.05 mM $CaCl_2$ in water, and pH was adjusted at 5.8. The milk (2.0 ml) was incubated with *J. gossypifolia* extract at 50 °Cand curd formation was observed. The end point was recorded when the full separation between whey and curd was observed. Chymotrypsin was used as positive control.

Statistical analysis

The data was analyzed using one-way analysis of variance (ANOVA) followed by students t-test using Microsoft excel.

RESULTS

Phytochemical screening

The phytochemical screening of leaves of *J gossypifolia* and *J curcas* showed the presence of different types of secondary metabolites, namely saponins, cardenolides, flavonoids, phenolic compounds, terpenes and flavonoids (Table 1). These phyto-compounds were present in both the extracts. However, our tests were showing the absence of glycosides and anthraquinone in both leaves. *J curcas* showed the presence of carotenoids and alkaloids which were absent in *J gossypifolia.*

Table 1: Phytochemical analysis of ethanol extract of *J gossypifolia* and *J curcas* leaves extract.

Tests	*J. gossypifolia*	*J. curcas*
Carotenoids	-	+
Alkaloids	-	+
Cardenolides	+	+
Saponins	+	+
Phenolic compounds	+	+
Flavonoids	+	+
Anthraquinone	-	-
Glycosides	-	-

Key: += present; - = absent

Thin layer chromatography

Extracts of *J gossypifolia* and *J curcas* were analyses using thin layer chromatography to ascertain the presence of antioxidant constituents. Table 2 showed the retardation factors (Rfs) of six eluted constituents of the plant extracts. The six spots were labeled beginning from the spot closest to the line where the extract was spotted.Spots 1-4 showed yellow coloration after spraying with 1% DPHH solution indicating the presence of antioxidant constituents in both extracts.

	J gossypifolia		*J curcas*	
	Retardation Factor (Rf)	**Colouration after spraying**	**Retardation Factor (Rf)**	**Colouration after spraying**
Spot 1	0.15 ±0.01	Yellow	0.29 ± 0.01	Yellow
Spot 2	0.35 ±0.02	Yellow	0.33±0.01	Yellow
Spot 3	0.40 ±0.02	Yellow	0.42 ± 0.01	Yellow
Spot 4	0.56 ± 0.02	Yellow	0.67 ±0.03	Yellow
Spot 5	0.67 ± 0.03	Light Green	0.84 ± 0.03	Light Green
Spot 6	0.93 ±0.03	Green	-	-

Key: Rf value= Distance moved by extract/Distance moved by the solvent

Milk clotting Activity

Results obtained revealed a concentration-dependent increase in milk clotting times. *J gossypifolia* showed a lesser milk clotting times as compared to J curcas (Figure 1).

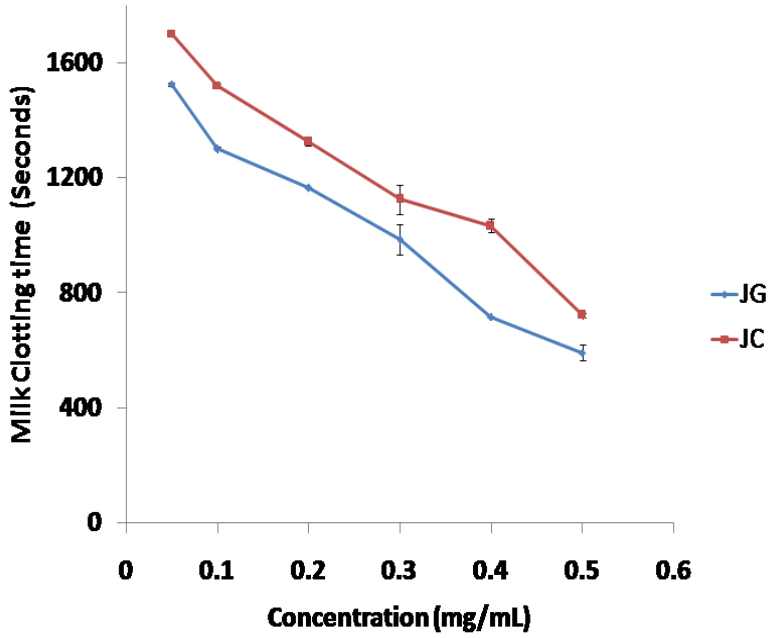

Figure 1: Milk clotting times of *J gossypifolia* and *J curcas.*

DPPH free radical scavenging assay

The DPPH radical scavenging activity of leaves J gossypifolia and J curcas were shown in Figure 2 and Table 4. Figure 2 showed the scavenging effects (%) of J gossypifoliaand J curcas leave extract on DPPH radical. J curcas revealed a higher scavenging activity as compared to J gossypifolia indicative of a stronger antioxidant property. The values for IC_{50} for J gossypifolia was 337± 0.02 µg/ml while that of J curcas was 173.2 ± 0.01 µg/ml ($P<0.05$). Ascorbic acid was used as standard with an IC_{50} of 51.56µg/ml. The results obtained revealed J curcas as having an IC_{50} close to that obtained for the standard (Ascorbic acid) indicative of a more potent antioxidant property.

Table 3: IC_{50}values (µg/mL) of crude methanol extracts of *J gossypifolia* and *J curcas* in DPPH free radical scavenging evaluation assay.

Sample/Extract	IC_{50} (µg/mL)
J gossypifolia	337.50 ± 0.02
J curcas	173.20 ± 0.01
Ascorbic Acid	51.56 ± 0.01

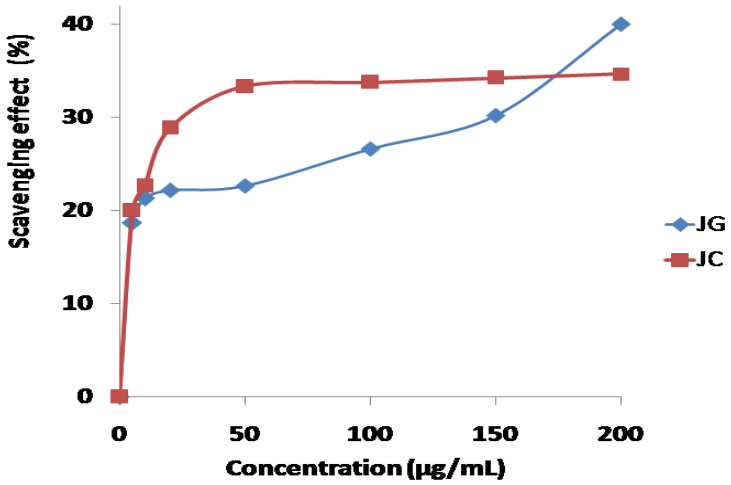

Figure 2: Scavenging effects (%) of *J gossypifolia* and *J curcas.*

DISCUSSION

Enzymatic antioxidant defense systems consisting of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (G-Px), and other endogenous antioxidant molecules, like glutathione (GSH), have been shown to scavenge oxygen derived free radicals produced in physiological and pathological processes. However, the inhibition of such reactive oxygen derived species such as superoxide (O_2), nitric oxide ($NO^{\cdot}$), hydroxyl ($HO\cdot$), and lipid peroxyl

(LP·) produced from body metabolic activities in addition to environmentally induced radicals overwhelms the body's natural defense antioxidants.[33-36] Plant enzymes that catalyse the breakdown of peptide bonds, participate in several biological processes, including utilisation of storage proteins, breakdown of light-damaged chloroplast proteins, protection against phytopathogen attack, tissue differentiation, and floral senescence.[37] Numerous industrial processes make use of proteases such as papain, bromelain, and ficin, and new enzymes with promising physicochemical properties have been studied for such purpose.[38]

Results from Thin Layer Chromatography (TLC) revealed the presence of antioxidant constituents in both extracts of *J gossypifolia and J curcas*. This was confirmed by the yellow colouration following spray with 1% DPPH solution (Table 3).

Previous studies have indicated that a lower IC_{50}value indicates greater antioxidant activity.[1] In the present study, the lowest IC_{50}value (173.20 µg/ml) required to quench at least 50% DPPH radicals was that of *J curcas* extract as compared to *J gossypifolia* (337.5µg/ml) [Table 3]. The result obtained for *J gossypifolia* is consistent with the value obtained by Okoh et al., 2016 (IC_{50} of 320 µg/ml).[1] These results indicate that aforementioned plant extracts are the powerful radical scavengers at very low concentrations. DPPH radical scavenging model is an important tool used in evaluating antioxidant activity of natural compounds and plant extracts. The amount of discoloration obtained indicates the scavenging capabilities of the antioxidant extract, which is due to the hydrogen donating properties.[39] The data obtained from our study revealed that crude methanol extracts of leaves of *J gossypifolia and J curcas* have the effects of scavenging free radicals. Presence of flavonoids-quercetin, apigenin, naphthoquiones, tannins and steroids in *J gossypifolia and J curcas*might contribute towards the DPPH radical scavenging activity since these classes of compounds are known as free radical scavenger).[40] Increased production of free radicals have been implicated in the development of cardiovascular diseases and cancer. Thus, the consumption of *J gossypifolia and J curcas*leaves can be beneficial in preventing oxidative stress related numerous chronic diseases. *In vitro* antioxidant activities of J gossypifolia and J curcas were determined by DPPH[Table 3]. *J curcas* (5–200 µg/ml) showed the highest percentage of DPPH radical inhibition ranging from 20-34.67% respectively [Table 3].

Extracts of J. gossypifolia and J curcas revealed strong milk-clotting activity using milksupplements with Chymotrypsin as positive control, indicative of the presence of

protease inhibitors. The importance ofprotease inhibitors from herbs is that they might provide an alternative approach to synthetic inhibitors, which are associated with numerous side effects and poor bioavailability.[41] Hence, herbs and medicinal plants containingbioactive molecules can alter coagulation, fibrinolytic, complement, and digestive processes via protease inhibition, thereby preventing disease progression.

CONCLUSION

Jatropha curcas(leaf) extract showed the higher antioxidant activity than *J gossypifolia*, and it can be attributed to its high content in phenolic and flavonoid compounds. Thus, the consumption of *J* gossypifolia and J curcasleaves can be beneficial in preventing oxidative stress related numerous chronic diseases. It can be concluded that *J gossypifolia and J curcas* can be used as an effective natural source of antioxidant, as ethnomedicine and as a commercial basis for the development of nutraceuticals. In addition, our results demonstrate that *J gossypifolia and J curcas* leaves maybe potential source for developing protease inhibitors. Nevertheless, further investigations and identification of the anti-protease active components are needed.

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