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PHYTOCHEMICAL SCREENING, PROXIMATE ANALYSIS AND FREE RADICAL SCAVENGING ACTIVITY OF THE ROOTS EXTRACT OF *JATROPHA MULTIFIDA*

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ABSTRACT

Oxidative stress and impaired oxidative system has been implicated in the pathophysiology of many major diseases. In this study, the phytochemical screening proximate analysis and free radical scavenging activity of the ethyl acetate, petroleum ether, chloroform as well as the crude extract of the roots of *Jatropha multifida* were evaluated. The phytochemical screening revealed the presence of carbohydrate, reducing sugars, phenolics, flavonoids, glycosides, saponnins and steroids. The moisture content of the crude sample was found to be 9.48 ± 1.28 while the water extractive index was 1.40 ± 0.0238. The Chloroform extract had the highest antioxidant property compared to other fractions.

KEY WORDS: oxidative stress, Anti-oxidant activity, Phytochemical screening, *Jatropha multifida.*

INTRODUCTION

There is renewed interest in the last decade to search for phytochemicals from naturalized plant for pharmaceutical and nutritional purposes. This arises from the fact that plant derived products hold great potentials as sources of pharmaceutical. Without plants, most medicines that we take would not exist. Over 40% medicine now prescribed contains chemicals derived from plants.[1]

Free radicals are chemical compounds which contain an unpaired election spinning on the peripheral layer around the nucleus. They are chemically aggressive molecules which react with different type of macro-molecules in the body to cause damage to vital cell constituents such as DNA, proteins and lipids.[2] Free radicals react with other molecules by extracting electrons from them in order to obtain stability. Free radicals contribute more than one hundred disorders in humans including atherosclerosis arthritis. Ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS.[3,4] The family of free radical generated from oxygen is called reactive oxygen (ROS) and those generated from nitrogen are called reactive nitrogen specie (RNS). Cells are often equipped with natural mechanism to fight against ROS and to maintain redox homeostasis of cell. For example, antioxidant enzyme such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) play important roles in scavenging the free radicals and preventing cell injury.[5] The harmful effect of free radicals causing potential biological damage is termed oxidative stress. Oxidative stresses are increasingly recognized for their contribution to a number of diseases disorder such as cancer, neuron degenerative disorder, atherosclerosis and aging among others.[6] The process of 'redox regulation' protects living organisms from various oxidative stresses and maintains "redox homeostasis" by controlling the redox status *in vivo*.[7]

Antioxidants are a group of substances that when present in low concentrations compared to those of the oxidase substrate significantly delays or prevent oxidation of that substance while often being oxidized themselves.[8] Oxidation reaction can produce free radicals which in turn can start chain reactions which can cause damage or cell death. Antioxidants have the capabilities to terminate these chain reactions by removing free radical intermediates and inhabit other oxidation reaction.[9]

Jatropha multifida Linn belong to the family Euphorbiaceae. It is a shrub or treelet commonly known as coral plant or physic nut. It is usually cultivated as an ornametal plants and is widely cultivated throughout the tropics.[10] All parts of the plant, but particularly the seeds are reported to have strong purgative activities. The foliage smells like insect repellant and have never been seen to be attacked by insects. The leaves and latex of *J. multifida* are used medicinally. The leaves are used in scabies while the latex is applied over wound and ulcer. The oil is used both internally and externally as abortificient.[11] The stem is employed as chewing sticks used for dental care in Ekiti State, Nigeria.[12]

MATERIALS AND METHOD

Collection of plant materials: Fresh *Jatropha multifida* roots were collected from Owan in Edo State in July 2012. The plant material was identified and authenticated in the Forest Research Institute of Nigeria, Ibadan. A herbarium specimen was deposited with voucher number FH109573.

The roots were carefully washed with water to remove earthy materials after which the bark was carefully removed, air dried and powdered using a mechanical grinder. The crude powdered sample was stored in an air-tight container until ready for use.

Extraction: Exactly 4.6 kg of the powdered sample was macerated with methanol (14 L) for 5 days. This was then filtered and evaporated *in vacuo*. The extract was partitioned using petroleum ether, ethyl acetate and chloroform and the different fractions stored at 4°C until ready for use.

Proximate Analysis: The following quantitative parameters of *Jatropha multifida* root sample were determined using standard methods [13,14]; Moisture content (water loss on drying), total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive value, and water soluble extractive value.

Phytochemical Screening: Chemical tests to detect the presence of alkaloids, tannins, saponins, carbohydrate, protein, flavonoids and other phenolic compounds in *Jatropha multifida* roots were carried out using standard methods.[15-17]

Free Radical Scavenging Activity: The free-radical scavenging effect of crude extracts and fractions of *J. multifida* was estimated using DPPH scavenging method.[18] Exactly 1.0 mL of 0.1 mM DPPH was mixed with 3.0 mL of extract/fraction in methanol of concentrations 0.01-0.2 mg/mL.

The reaction was vortexed thoroughly and left in the dark at room temperature for 30 minutes.

The absorbance of the mixture was measured spectrophotometrically at 517 nm with ascorbic acid as a reference. The ability to scavenge DPPH radical was calculated by the following.

DPPH radical scavenging activity (%) = $\left[\frac{(A_0 - A_1)}{A_0}\right] x\ 100$

Where A_0 = Absorbance of DPPH (0.1 mM in methanol)

A_1 = Absorbance of DPPH + extract/fractions

Determination of Total Phenol

The total phenolic contents in the extract and fractions were determined by the folin-ciocalteu's method. The extract solution (0.5 mL) of 1 mg/mL was dissolved in distilled water and 0.5 mL of Folin ciocalteu's reagent (previously diluted with water, 1:10) was then added to the solution. The mixture was maintained at room temperature for 5 mins followed by the addition of 5 mL of 7% sodium carbonate and 2 mL of deionized distilled water. After mixing, the sample were incubated for 90 mins at room temperature.

The absorbance was measured spectrophotometrically at 750 nm. The standard curve was prepared using gallic acid in six different concentration (12.5, 25.0, 50.0, 75.0, 100.0 and 150.0 mg/mL).

The total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per gram of extract (mg/GAE/g extract).[19]

Determination of Total Flavonoid

Total flavonoid content were estimated using the method described by Ebrahizadeh *et al.* [20] Exactly 0.5 mL of extract sample (1 mg/mL) was mixed with 1.5 mL of methanol and then 0.1 mL of 10% aluminum chloride was added. This was followed by the addition of 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 mins. The absorbance was then measured at 415 nm.

The standard curve was prepared using quercetin in six different concentrations (12.5, 25.0, 50.0, 75.0, 100.0 and 150.0 mg/L). The results were expressed as milligram quercetin equivalent (QE) per gram of extract (mgQE/g extract).

RESULTS

Table 1: Phytochemical composition of *Jatropha multifida root.*

Phytochemical	Inference
Carbohydrates	+
Reducing Sugar	+
Phenolics	+
Flavonoids	+
Alkaloids	–

Glycosides	+
Saponins	+
Steroids	+

+ indicates presence of compound

– indicates absence of compound

Table 2: Percentage (%) values of proximate analysis of *Jatropha multifida* root sample

Parameter	Value ± SEM (%)
Moisture Content	9.48 ± 1.28
Total Ash	10.64 ± 0.0331
Acid Insoluble Ash	8.083 ± 0.0204
Alcohol extractive index	0.64 ± 0.0239
Water extractive index	1.404 ± 0.0238

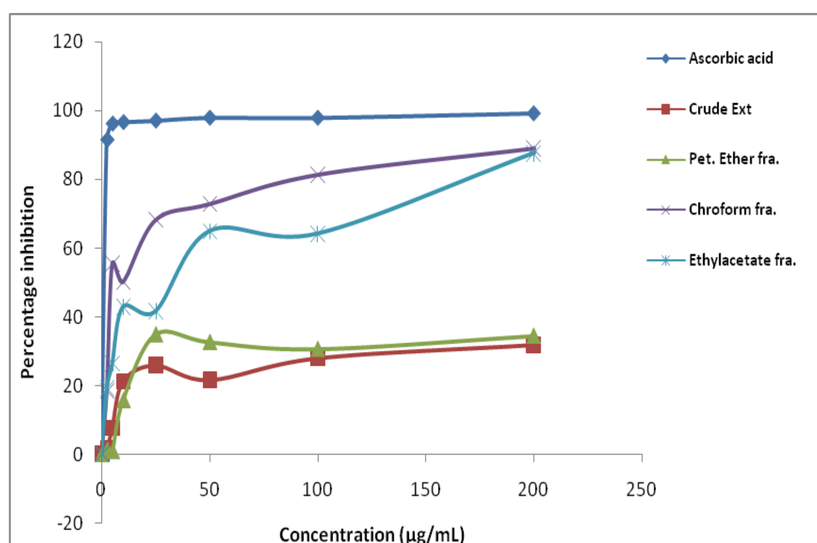

Figure 1: Percentage inhibition of different fractions of *Jatropha multifida*

Table 3: The IC-50 values of the different fractions of the root extract of *J. multifida*

Sample	IC_{50} value (µg/mL)
Ascorbic acid	2.55
Methanol extract	210.59
Pet – Ether fraction	184.95
Chloroform fraction	32.64
Ethyl acetate fraction	76.37

DISCUSSION

The phytochemical screening of the powdered root of *Jatropha multifida* revealed the presence of carbohydrate, phynolic compounds, flavonoids, glycosides, saponins and steriods (table 1).

In this study, proximate analysis was carried out for the purpose of authentication of the crude powdered plant material. The maximum permissible range of moisture content for a crude drug is between 6 – 8 % (African Pharmacopoeia, 1986). The total ash is a measure of the non-volatile inorganic constituents remaining after ashing. A moisture content of 9.48 ± 1.28% (table 2) obtained from this study suggest that the crude plant material is not susceptible to microbial degradation or hydrolytic break down of the chemical constituents.

The DDPH Assay is based on the measurement of the reducing ability of antioxidant towards DPPH. This ability can be evaluated by electron spin resonance (ESR) spectrometry or by measuring the decrease of the absorbance.[21] The DPPH assay is considered to be mainly based on electron transfer (ET) reaction and hydrogen atom abstraction is a marginal reaction pathway.[22]

The result of the DPPH showed that *J. Multifida* extracts have appreciable DPPH radical scavenging activity with the chloroform fraction having the lowest scavenging. The 50% inhibitory concentration (IC_{50}) of the Chloroforn extract was significantly lower than values obtained from the other fractions and the crude extract (table 3). The scavening activity also increased as the concentration of the extract increased.

The antioxidant activity could be attributed to presence of phenolic compound present.

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CONCLUSION

From the study, the root of *Jatropha multifida* possess significant antioxidant property and could be a potential source of antioxidant drug.

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