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Research Article

Evaluation of the *In Vitro* and *In Vivo* Antioxidant Potentials of *Jatropha tanjorensis* Methanolic Leaf Extract

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ABSTRACT
The antioxidant activity of *Jatropha tanjorensis* leaf methanolic extraxt was investigated following different antioxidant models. The antioxidant methods used include: *in vitro* 2,2-diphenylpicrylhydrazine (DPPH) spectrophotometric assay and *in vivo* assay of serum malondialdehyde, superoxide dismutase and catalase activity. The extract (50 – 400 µg/ml concentration) produced in a concentration-dependent manner, significant ($p < 0.05$) antioxidant effect in the DPPH spectrophotometric assay. In the *in vivo* antioxidant tests, 400 mg/kg of the extract evoked significant ($p < 0.05$) increase in serum superoxide dismutase and catalase activity and also significantly decreased the level of malondialdehyde when compared with the control group. These findings suggest that *J. tanjorensis* possess potent antioxidant property, which may be responsible for some of the reported pharmacological actions of the plant.

Keywords: antioxidant, Jatropha tabhirebsusm leaf extract

INTRODUCTION

An antioxidant may be defined as any substance which, when present in low concentrations compared to that of an oxidisable substrate, significantly delays or inhibits the oxidation of that substrate[1]. The normal metabolic processes within the body result in the production of certain dangerous substances called reactive oxygen species (ROS), which comprise free radicals, such as superoxide anion (O^{2-}) and hydroxyl (HO) radicals, and non-free radical species, such as H_2O_2 and singlet oxygen ($1O_2$)[2,3]. Reactive oxygen species are also produced by radiation, viral and bacterial toxins, tobacco smoke, alcohol, metabolism of certain solvents, drugs and pesticides as well as emotional or psychological stress[4]. Excessive production of ROS leads to oxidation of biomolecules and this has been implicated in the etiology of several diseases such as diabetes, atherosclerosis, Alzheimer's disease, ischemic reperfusion injury, cancer, arthritis, neurodegenerative diseases, ageing and liver-related diseases[1,5]. Antioxidants are known to prevent oxidative damage by ROS by reacting with free radicals, chelating catalytic metals and also by acting as oxygen scavengers[6]. Hence they are capable of stabilizing or deactivating free radicals before they damage cells [7]. The antioxidants in biological system can be enzymatic or non-enzymatic. The enzymatic antioxidants are catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, etc, while the non-enzymatic antioxidants include: ascorbic acid (vitamin C), selenium, tocopherols (vitamin E), carotenoids and polyphenols[2,4]. In recent years, there has been upsurge of interest in natural phytochemicals believed to possess antioxidant potential [8]. It has been reported that consumption of natural antioxidants is associated with reduced risks of diabetes, cardiovascular disease, cancer and other diseases associated with ageing[9]. Plants, herbs and spice rich in phenolic compounds like flavonoids, have been demonstrated to have anti-inflammatory, antiallergic, antiviral, antiageing, and anticarcinogenic activities which can be traced to their antioxidant properties[10]. The best strategy to counter oxidative stress appears to be supplementation of food rich in herbal bioactives[11]. *Jatropha tanjorensis* belongs to the family *Euphorbiaceae* and is a common weed of field crops in the higher rain forest zones of West Africa. It is called "hospital too far" or "catholic vegetable" in Nigeria. The plant has been investigated for hematopoietic activity, hypolipidemic activity, hypoglycaemic and antidiabetic activity, as well as antimicrobial effect. A preliminary phytochemical analysis of methanolic extract of *J. tanjorensis* revealed the presence of saponins, glycosides, flavonoids, alkaloids, anthroquinones and tannins [12]. The present study is aimed at investigating the *in vivo* antioxidant potentials of the methanolic leaf extract of *Jatropha tanjorensis* (JTE).

MATERIALS AND METHODS

Plant collection and preparation of extract

Fresh leaves of the plant were collected from its natural habitat and authenticated at the Department of Botany, Michael Okpara University of Agriculture, Umudike. The leaves were dried under mild sunlight, then reduced to coarse powder, using electric blender. Two hundred grams

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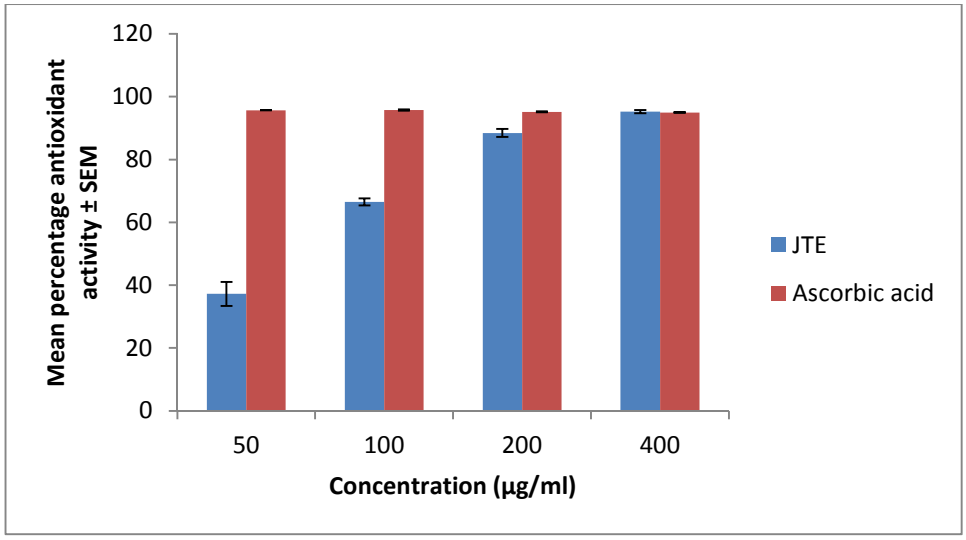

Figure 1: The *in vitro* antioxidant effect of JTE using DPPH photometric assay

of the powder were exhaustively extracted with 80% methanol, using the method of cold maceration with intermittent shaking for 72 hours. Following filtration, the solvent was evaporated and the extract dried in hot air oven (40 ^{0}C) and stored in the refrigerator (4 ^{O}C) before use. Percentage yield was determined using the formula:

$$\% \text{ yield} = \frac{\text{weight of extract}}{\text{weight of plant material}} \times 100$$

Animals

Albino rats (120 – 160g) were procured from the laboratory animal house of the University of Nigeria, Nsukka. The animals were allowed two weeks for acclimatization and fed *ad libitum* with standard rat chow and clean drinking water. All animal experiments were carried out in accordance with the recommendations of the Guide for the care and use of laboratory animals.

Acute toxicity test

Twenty five albino rats of both sexes were randomly divided into 5 groups (*n = 5*). Group A received distilled water (10ml/kg) orally while groups B, C, D and E were treated with 500, 1000, 2000 and 4000mg/kg of methanolic extraxt of *Jatropha tanjorensis* (JTE) *per os*. The animals were observed for 48 hours for signs of acute toxicity and death.

Determination of the in vitro antioxidant activities of J. tanjorensis using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) photometric assay

The free radical scavenging activity of the extract was analyzed by the DPPH Assay [13] using spectrophotometer. The test extract (2 ml) at different concentrations (50, 100, 200 and 400 µg/ml) each were mixed with 0.5 mM DPPH (in 1 ml of methanol) in a cuvette. The absorbance at 517 nm was taken after 30 minutes of incubation in the dark at room temperature. The concentrations were prepared in triplicates and the percentage antioxidant activity calculated as follows:

% antioxidant activity (AA) = 100-[{(absorbance of sample — absorbance of blank) ×100}/ absorbance of control].

One mililiter of methanol plus 2.0 ml of the extract was used as the blank while 1.0 ml of the 0.5 mM DPPH solution plus 2.0 ml of methanol was used as the negative control. Ascorbic acid (vitamin C) was used as reference standard[14].

Determination of the in vivo antioxidant activities of JTE

Twenty four adult albino wistar rats were randomly divided into four groups of six animals each. Group 1 served as the control and received distilled water (10 ml/kg). groups 2 and 3 received 200 and 400 mg/kg respectively. Group 4 received no treatment. The animals were medicated orally, for 14 days. On day 15 blood samples were collected from each rat through the retro orbital plexus of the median canthus of the eyes, using capillary tubes.

Analytical methods

Serum preparation

The serum was prepared using standard method as described by Yesufu *et al*[15]. Blood was allowed to clot for 30 minutes, then centrifuged at 2500 rpm for 15 minutes and serum was harvested.

Determination of the lipid peroxidation (LPO) in serum

The level of thiobarbituric acid reactive substance (TBARS) and malondialdehyde (MDA) production was measured in serum by the modified method as described by Draper and Hadley[16]. The serum (50µL) was deproteinized by adding 1 mL of 14% trichloroacetic acid and 1 mL of 0.6% thiobarbituric acid. The mixture was heated in a water bath at 100ºC for 30 min to complete the reaction and then cooled on ice for 5 min. After centrifugation at 2000 rpm for 10 min, the absorbance of the colored product (TBARS) was measured at 535 nm with a UV spectrophotometer. The concentration of TBARS was calculated using the molar extinction coefficient of malondialdehyde (1.56×10^5 mol/L/cm) using the formula, $A = \Sigma CL$, where A = absorbance, Σ = molar coefficient, C = concentration, and L = path length. The results were expressed in nmol/mg of protein.

Estimation of catalase (CAT) activity

Table 1: *In vivo* antioxidant effect of *J. tanjorensis* in rats.

treatment	MDA (nanomole/mg protein)	SOD (U/mg protein)	CAT (micromole/mg protein)
No treatment	0.10 ± 0.05*	15.30 ± 1.28*	0.08 ± 0.01*
water 10ml/kg	0.24 ± 0.05	6.35 ± 0.142	0.04 ± 0.02
JTE 200 mg/kg	0.10 ± 0.05*	7.70 ± 3.17	0.07 ± 0.16*
JTE 400 mg/kg	0.06 ± 0.02*	18.82 ± 4.42*	0.20 ± 0.01*

The catalase activity in serum was determined using the modified method as described by Atawodi [17]. Briefly, the method is as follows: serum (10µL) was added to test tube containing 2.80 mL of 50 mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1 mL of fresh 30 mM hydrogen peroxide and the decomposition rate of hydrogen peroxide was measured at 240 nm for 5 min on a spectrophotometer. A molar extinction coefficient of 0.041 $mM^{-1}cm^{-1}$ was used to calculate catalase activity. The results were expressed in µmol/mg of protein.

Estimation of superoxide dismutase (SOD)

Superoxide dismutase activity was assayed according to the method of Sun *et al*[18]. In this method, xanthine-xanthine oxidase system was used to generate a superoxide flux, and nitroblue tetrazolium (NBT) was used as an indicator of superoxide production. SOD activity was hence measured by the degree of inhibition of the reaction unit of enzyme providing 50% inhibition of NTB reduction. Results are expressed as U/mL.

Statistical analysis

Data obtained were analyzed following one –way analysis of variance (ANOVA) and the variant means were separated using least significant difference (LSD) of the different groups. Significance was accepted at the level of $p < 0.05$.

RESULT

Extraction and acute toxicity study

The extract gave a yield of 8.45% w/w dry matter. It was oily and dark green in color. The acute toxicity study recorded neither death nor sign of acute toxicity even at the highest dose tested (i.e. 4000mg/kg).

The in vitro antioxidant effect of JTE using DPPH photometric assay

The extract evoked a concentration – dependent increase in percentage antioxidant activity in DPPH spectrophotometric assay. The highest concentration of the extract (400 µg/ml) gave an antioxidant activity of 95.22% which is comparable to the effect (94.97%) of the same concentration of ascorbic acid, the reference drug. (Figure 1).

In vivo antioxidant effect of J. tanjorensis in rats

The result of the in vivo antioxidant activity of JTE is presented in table 1. The extract produced in a dose – dependent manner significant ($p < 0.05$) decrease in the serum level of MDA in the test animals when compared to control. The extract also evoked a dose – related significant ($p < 0.05$) increase in the level of CAT in the serum of treated rats when compared to control. However only the higher dose (400 mg/kg) of the extract caused a significant ($p < 0.05$) increase in the serum level of SOD in the treated animals in comparism to control.

DISCUSSSION

The antioxidant activity of *J. tanjorensis* was evaluated using various antioxidant models. The DPPH spectrophotometric assay is extensively employed to determine the antioxidant potential of different samples *in vitro*[19]. DPPH (2,2-Diphenyl-1-picrylhydrazyl) is a dark colored crystalline powder composed of stable free radical molecules[20]. The antioxidant compounds neutralize the free radical activity of DPPH by transferring either electrons or hydrogen atoms to DPPH[21], there by changing the color from purple to the yellow colored stable diamagnetic molecule, diphenylpicrylhydrazine. The degree of discoloration indicates the scavenging power of the extract or antioxidant in terms of hydrogen donating ability[22]. From the result of DPPH spectrophotometric assay, JTE caused a significant ($p < 0.05$) antioxidant activity comparable to that of ascorbic acid. Superoxide dismutase is an important defense enzyme and catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen, thereby reducing the tendency of superoxide anion reacting with nitric oxide to form reactive peroxynitrite[23]. Catalase is a hemoprotein which catalyzes the reduction of H_2O_2 and known to be involved in detoxification of hydrogen peroxide concentrations[24]. In the present study the methanolic leaf extract of *J. tanjorensis* significantly ($p < 0.05$) increased the activity of serum SOD and CAT, which indicates that the extract possesses *in vivo* antioxidant property and is capable of ameliorating the effects of ROS in biological system[25]. Furthermore, the *in vivo* antioxidant assay showed that JTE significantly ($p < 0.05$) reduced the serum level of MDA in the treated rats when compared to control. Malondialdehyde is an endogenous genotoxic product of enzymatic and reactive oxygen species-induced lipid peroxidation. MDA level is widely used as a marker of lipid peroxidation in states of elevated oxidative stress[26]. Hence the results suggest that JTE is effective in diminishing lipid peroxidation, a common causative agent in cell damage[27]. The antioxidant activity of JTE as demonstrated in this study could be attributed to some of its phytochemical constituents. Polyphenols and flavonoids which are gaining importance for their antioxidant activities are effective hydrogen donors and present in most plants[28]. Several studies have demonstrated that flavonoids possess potent antioxidant properties capable of scavenging lipid peroxy radicals, superoxde anions and hydroxyl radicals[29]. Many pharmacological properties (anti-inflammatory, antibacterial, hepatoprotective, anti-ulcer and antiallergic

actions) of flavonoids have been ascribed to their potent antioxidant potential[30].

CONCLUSION

The results obtained from this study demonstrate that *Jatropha tanjorensis* possesses potent antioxidnant property, which could be responsible for some of its pharmrcological actions.

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