Determination of Some Micronutrient and Antioxidant Components of *Ipomoea digitata*

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Abstract

Ipomoea digitata tubers were harvested, dried and pulverized. Known quantity of the powdered tuber was exhaustively extracted with methanol. Chemical classes of constituents present in the dried tuber and the extract were detected and the LD_{50} of the powder and the extract were determined in mice orally and intraperitoneally, respectively. Colorimetric methods were used to estimate the quantities of vitamins: B_1, B_2, A, C and E; as well as zinc, calcium, selenium and iron in the powder. The antioxidant properties of the powdered tuber and the extract were evaluated in vivo in rats. The extraction gave 14.02 % (w/w) yield while the LD_{50} of the powder and the extract in mice were 4500 and 2500 mg/kg respectively. The micronutrient levels in one gram of the powder were vitamins (mg): B_1 (0.599), B_2 (0.351), C (0.025), E (0.156) and A (0.083); and metals (ug): Ca (9600), Fe (937.5), selenium (2.25) and zinc (2.75). The powder and methanol extract showed antioxidant activity which were comparable to that of vitamin C.

Keywords: *Ipomoea digitata*, Antioxidants, Colorimetry, Vitamins, Mineral elements

Introduction

Plants are factories of chemical compounds and sourcing for drugs in them can be described as inexhaustible. Man has over the years acquired massive knowledge concerning the use of plants around him to maintain health and cure sickness. Scientific research into plants has led to the development of many valuable drugs. Reserpine, quinidine, coedeine and vinblastine are obtained from *Rauwolfia* plants, cinchona tree, opium plant and *Catharanthus roseus*, respectively. The knowledge of the use of plants in traditional medicine rely mostly on past experience and observation handed down from generation to generation either verbal or in writing (Trease and Evans, 2002).

Oxidative stress occurs when the quantity of free radicals the body has to cope with exceeds the available antioxidants (Halliwell, 1994a). This concept is relatively new and is closely linked to the growing awareness that our environment is a potent source of diseases due to pollutants in it. Air pollution has been linked to heart attacks asthma, and other respiratory disease. (Halliwell, 1994b; Jenner 1994). Heart disease, lung cancer and host of other diseases are induced by cigarette smoking. Hip fractures, cancer and Alzheimer's diseases have been linked to chemicals in our drinking water (Grisham, 1994; Stadtman, 1992). Nitrites and other food additives have been linked to cancer (Hans, 1995). Excessive amounts of radiation can cause cancer and allergens. The environment is made up of toxic chemicals from agricultural, industrial and transportation waste that can induce oxidative stress in human body. Oxidative stress is seen as the main causes of cancer, cardiovascular disease, arthritis, inflammatory disease, Parkinson's disease, Alzheimer's disease, cataract formation and Crohn's disease (Hatch, 1995; Danielson, 1992; Ganlt, 1992).

Superoxide dimutase is the body's main defense and is especially effective in deactivating superoxide radicals. Another natural antioxidant which effectively scavenges free radicals is the selenium–containing antioxidants, glutathione peroxides. With much increase in factors that induce oxidative stress, the natural antioxidants are no longer able to cope with oxidative stress the body is exposed to (Diplock, 1991). There is need for increased in-take of antioxidants. The dietary in-take is far too low to provide significant help to ward off degenerative diseases. There is need to boost in-take through supplementation. The common antioxidants are vitamins: C, E and B; equally selenium and zinc are implicated.

Ipomoea digitata is a climber with large ovoid and tuberous roots (Kokwaro, 1976). The leaves are 10-15 cm long, palmately 5-7 lobed, obvate, lanceolate, acute, glabrous and with prominent herves beneath. The flowers are widely campanulate and few to many in the auxiliary corymbose cymes. The corolla is purple and campanulate – infundibuliform.

A decoction of the roots of *Ipomoea digitata* is used as antidote against opium or arsenic poisoning or polluted water. A decoction of the leaves is a remedy for cough, leprosy, tuberculosis and hair problems (Sofowaro, 1982). The powder is given for liver and spleen disease. In India and Philippines, the root of the plant is considered tonic, aphrodisiac, and demulcent; and is used for treatment of fever and burns (Adesany and Idowu, 1969). *Ipomoea digitata* contains sesquiterpene lactone which include alantolactone, isoalantolactone, dihydroalantolactone, dihydro-isolantolactone, betasitosterol, inunolide and laxaxerol (Pankey, 1965). B-carotene, vitamin A and magnesium had been confirmed in the plant. It contains 16 amino acids both hydrolysate and free forms and was shown to have stimulant as well as depressant actions (Elujoba, 1972).

This work investigated the methanol extract of *Ipomoea digitata* tuber and the pulverized tuber for antioxidant effect and evaluated their acute toxicities in mice. The chemical classes of constituents present in the extract were confirmed. Vitamins and mineral elements, most especially the

antioxidant related ones, were determined in the pulverized tuber.

Materials and Methods

Plant: *Ipomoea digitata* tubers were harvested from Akaeze, Ebonyi state, Nigeria in February, 2005. It was authenticated by Mr. C. C. Ozioko of Botany Department, University of Nigeria, Nsukka. The tubers were washed, cut into small pieces, air-dried, pulverized and stored in an air tight container.

Animals: Thirty adult mice of both sexes weighing between 18-26 g and 50 Wistar abino rat of both sexes weighing between 150-250 g were purchased from the Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were housed in white metal cages and given standard feed and water *ad libitum.*

Reagents and instrument: Methanol, hydrochloric acid, hydroxylamine solution, ammonium acetate buffer, sodium acetate, hydrogen peroxide, phenol, ammonium hydroxide products of BDH, England were used for the experiment. Potassium permanganate, sodium hydroxide, ferric chloride, EDTA, diaminobenzidine solution, chloroform, acetone, dimethylsulphoxide (DMSO) products of Merck, England were equally used. Other reagents used were laboratory standard. These reagents were obtained commercially and used as supplied. Pye Unicam UV-visible spectrophotometer, England was used for measurement of absorbances.

Extraction and determination of classes of phytocompounds: Five hundred grams of the powder was exhaustively extracted with methanol using soxhlet extractor. The solvent was distilled off and the quantity of the dried residue measured. The chemical classes of compounds present in the extract and the powdered tuber were detected following the procedures outlined by Harborne (1973). The classes tested for include carbohydrates, alkaloids, flavonoids, resins, protein, oils, glycosides and saponins.

LD_{50} determination of the tuber powder and methanol extract: Different doses corresponding to 10, 100, 1000 mg/kg of homogenized suspensions of the methanol extract in 1% aqueous sodium carboxymethylcellulose were respectively given to three groups of mice intraperitoneally. In another set of experiment, doses of 10, 100 and 1000g/kg of homogenized suspension of the powdered tuber (boiled) were given, orally to three groups of mice, respectively. Each group had three mice. The animals were observed for 24 h and the numbers of deaths were recorded. Following the results, other doses of 1500, 2000, 2500 and 300 mg/kg of the extract and 2000, 3000, 4000 and 5000 mg/kg of the powder were respectively given to eight mice. After 24 h, the numbers of deaths were recorded. The LD_{50} was calculated following the method of Lorke (1985).

Colorimetric determination of vitamins and mineral elements: Vitamins B and E were estimated in the pulverized tuber according to the method of Jakutoictz et *al.* (1977) while vitamins A and C were determined following the methods of Beisy *et al.* (1946).

Four grams of the pulverized tuber was digested by warming it with 60 ml of a mixture of nitric acid and perchloric acid (5:1) in Kjeidahl flask. The content of the flask was heated until the mixture was nearly dried. It was extracted with enough water and the extract was made up to 200 ml with water. Standard solutions of zinc, iron, selenium and calcium were prepared by dissolving appropriate quantity of the salt of the metal in required volume of water. Other concentrations were obtained by dilution. Colorimetric methods using dithizone, phenanthroline, diaminobenzidine and strontium chloride solutions were used in the determination of zinc, iron, selenium and calcium, respectively following the methods of Henry, *et al* (1974).

Antioxidant screening: Forty five rats were divided into groups of five rats each. Each rat in seven groups were administered with 1.5 ml/kg body weight of carbon tetrachloride in corn oil (1:3). The rats were allowed to survive for 48 h. After the period, three groups were given intraperitoneally 0.5, 5 and 50 mg/kg of the extract respectively while another 3 groups were given 5, 50 and 500 mg/ kg of the pulverized suspension of the tuber in water. One group was given 1% sodium carboxymethyl cellulose, which was the solvent for the extract suspension. The rats were allowed to stay for 3 days. The glutathione-s-transferase activity in the serum of the rats was determined according to the method of Henry, *et al* (1974). While lipid peroxidation was assayed using the method of Wallin (1993). Superoxide dimutase activity was determined according to the method of Fridovich (1975).

Statistical analysis: The results were analysis statistically and were reported as mean ± standard deviation.

Result and Discussion

The methanol extraction gave 14% (w/w) yield. The extract and the tuber powder showed presence of glycosides, reducing sugars, tannins, steroids, terpenes; flavonoids and resins on phytochemical screening.

The oral LD_{50} of the powdered tuber in mice was 4500 mg/kg while that of the methanol extract was 2500 mg/kg, intraperitoneally. These indices indicate that the tuber and methanol extract of *Ipomoea digitata* are relatively save for consumption. From the spectrophotometric analysis, powdered tuber of *Ipomoea digitata* was found to contain 0.599 ± 0.007, 0.351 ± 0.002, 0.025 ± 0.004, 0.156 ± 0.003 and 0.083 ± 0.002 mg/g of vitamins B_1, B_2, C, E and A respectively.

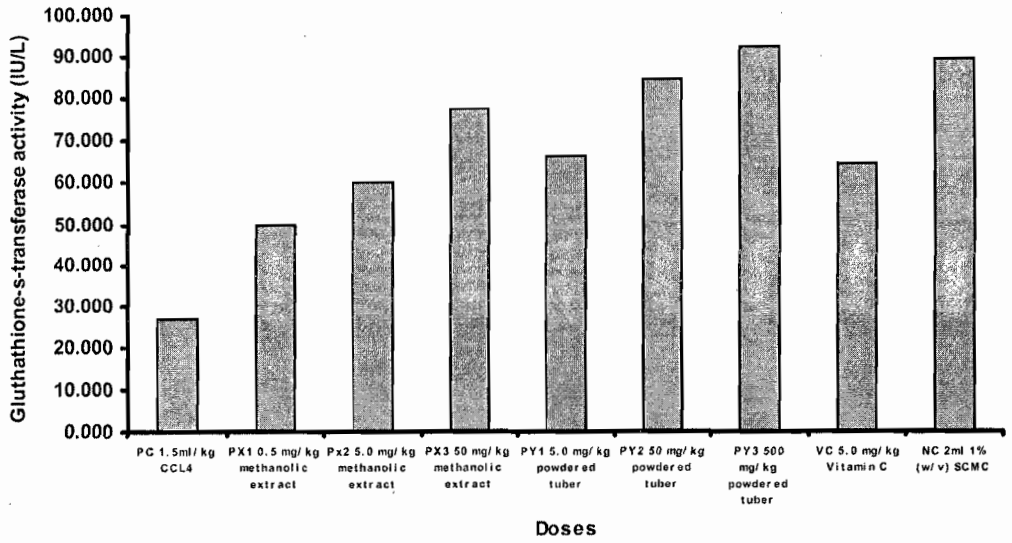

Fig. 1 : Effects of different doses of the powder and methanolic extract of the tuber of Ipomoea digitata on the gluthathione-s-transferase activity

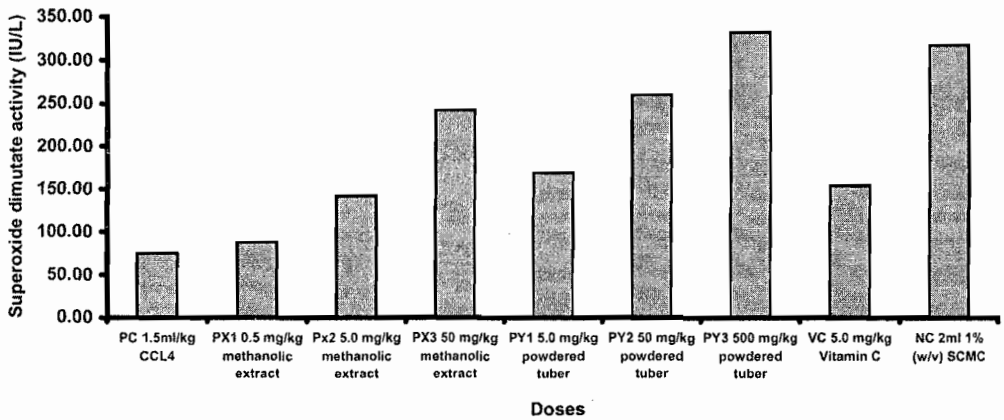

Fig. 2: Effects of the powder and the methanolic extract of I. digitata tubers on superoxide dimutate activities

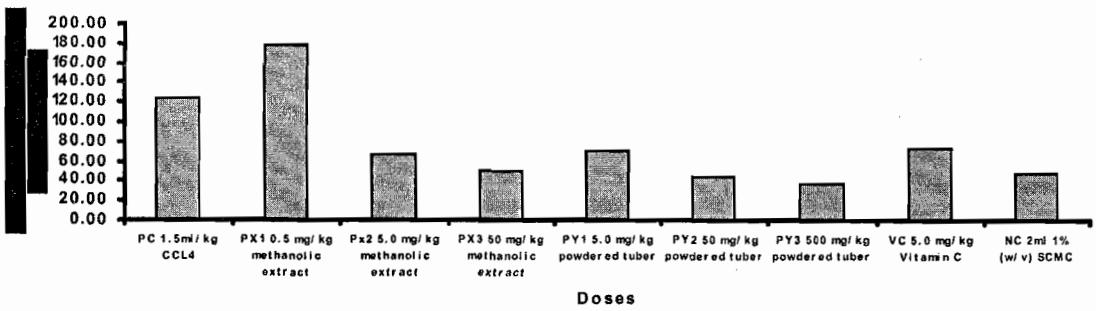

Fig. 3: Malondialdehyde level in the rats treated with CCl4 and different doses of the powder and the extract

The tuber contains reasonable quantities of these vitamins which are known for their antioxidant and antiaging activities. These vitamins have been implicated in the management of many debilitating diseases including cancer, HIV/AIDS, cataract, inflammation and cardiovascular diseases (Halliwell, 1994a).

Calcium, iron, selenium and zinc concentration in one gram of the powdered tuber were estimated colorimetrically to be 9600.0 ± 80.5, 937.5 ± 50.7, 2.25 ± 0.08 and 2.75 ± 0.15 µg respectively. Selenium though at a very low concentration, which is within the recommended supplementary level is a good agent for the formation of human natural antioxidant and an effective antiaging agent. Iron concentration in the tuber is very high, so the tuber can serve as a good agent for management of anemia. Zinc has been implicated in the formation of essential enzymes necessary for synthesis of proteins for cell multiplication, so the tuber is a good antiaging agent.

Glutathione-s-transferase activities were significantly higher in rats given carbon tetrachloride and treated with different doses of the methanolic extract and the powdered tuber when compared with those of rats given only carbon tetrachloride. The effect increased with concentration. The detail of the result is shown as figure 1. Superoxide dimutase (SOD) activities increased in rats given CCl_4 and treated with different doses of the pulverized tuber and methanol extract of the tuber, the summary of the result is shown as figure 2. The increase in the SOD activity was dosage dependent. Ascorbic acid was used as control. A significantly elevated level of malondialdehyde level was observed in tissues of rats treated with CCl_4 while in tissues of rats given CCl_4 and different doses of the powder and the extract showed lower levels, which were concentration dependent (Fig. 3).

The antioxidant effects were more pronounced in the results obtained with different doses of the pulverized powder when compared with those obtained with different doses of the methanol extract. The plant has antioxidant and antiageing activities and equally can be used in the management of anemia.

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