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 LD₅₀ of the powder and the extract were determined in mice orally and intraperitonally, respectively. Colorimetric methods were used to estimate the quantities of vitamins: B₁, B₂, 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 LD₅₀ 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): B₁ (0.599), B₂ (0.351), C (0.025), E (0.156) and A (0.083); and metals (µg): 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 sources 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, quinine, codeine 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 (Treat 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 allergies. 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 cause of cancer, cardiovascular disease, arthritis, inflammatory disease, Parkinson’s disease, Alzheimer’s disease, cataract formation and Crohn’s disease (Hatch, 1995; Danielson, 1992; Galati, 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 peroxidases. 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 intake of antioxidants. The dietary intake is far too low to provide significant help to ward off degenerative diseases. There is need to boost intake through supplementation. The common antioxidants are vitamins: C, E and B₁ equally selenium and zinc are implicated.

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

A decoction of the roots of Ipomoea digitata is used as antitoxin against opium or arsenic poisoning or polluted water. A decoction of the leaves is a remedy for cough, leprosy, tuberculosis and hair problems (Sofowara, 1982). The powder is given for liver and spleen disease. In India and Philippines, the root of the plant is considered toxic, aphrodisiac, and demulcent; and is used for treatment of fever and burns (Adesanya and Idowu, 1989). Ipomoea digitata contains sesquiterpene lactone which include alantolactone, isolantolactone, dihydroalantolactone, dihydromisolantolactone, betasisosterol, inunoxide and laxerol (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 (Eluboja, 1972).

This work investigated the methand 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 Akape. Eboney state, Nigeria in February, 2005. It was authenticated by Mr. C. C. Ozoiko at 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 16-26 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, acetonitrile, 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 phytoconstituents: Five hundred grams of the powder was exhaustively extracted with methanol using soxhlet extractor. The solvent was distilled off and the quantity of the dry 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.

Colorimetric determination of vitamins and mineral elements: Vitamins B and E were estimated in the pulverized tuber according to the method of Jakutick et al. (1977) while vitamins A and C were determined following the methods of Beisy et al. (1948).

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 Keidahl 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 metal in required volume of water. Other concentrations were obtained by dilution. Colorimetric methods using dithizone, phosphonil, diamlinobenzidine and sormium 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 mg/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 LD50 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 safe for consumption. From the spectrophotometric analysis, powdered tuber of Ipomoea digitata was found to contain 0.589 ± 0.007, 0.351 ± 0.002, 0.025 ± 0.004, 0.156 ± 0.003 and 0.083 ± 0.002 mg/g of vitamins B1, B2, 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 glutathione-s-transferase activity

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

Fig. 3: Nontodialdehyde 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 enzyme complexes 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 result of the effect is shown as figure 1. Superoxide dimutase (SOD) activities increased in rats given CCl₄ 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 CCl₄ while in tissues of rats given CCl₄ 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 antiaging activities and equally can be used in the management of anemia.

References


