Antioxidant Properties of *Polyalthia longifolia*

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ABSTRACT: *Polyalthia longifolia* is a plant found in the tropical areas of the world. The plant is used as an ornamental tree. The seeds of the plant were investigated for its toxicity, phytochemicals and antioxidant activities. The acute toxicity studies of the plant revealed LD_{50} of 400mg/kg which indicates that the seeds of the plant are non-toxic and save for human consumption. The plant was found to contain alkaloids, flavonoids, saponins, carbohydrates, fats and oils, tannins, steroids and terpenoids. The antioxidant activities of the ethanol extract of the seeds were assayed using rat liver homogenate. Nitric oxide, ferrous sulphate and carbon tetrachloride-induced lipid scavenging activities carried out showed that there were inhibition in the formation of free radicals; indicating a possible antioxidant property. The use of the plant on humans can alleviate the incidence of oxidative stress.
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INTRODUCTION

Reactive oxygen species (ROS) are the most plant biological free radicals and are generated during normal metabolic reactions. They include the superoxide and hydroxyl radicals, as well as derivatives of oxygen that do not contain unpaired electrons such as hydrogen peroxide, singlet oxygen and hypochlorus acid (Robin, 2002). Free radicals are highly reactive due to the presence of unpaired electron and must be converted to non-toxic form to avoid causing damage to the cell. Antioxidants, on the hand, are substances that counteract the harmful effects of these free radicals. Recently, a good number of phytochemicals have been found from a variety of sources and are used in neutraceuticals.

Polyalthia longifolia, ashoka, is a native of India and has been found to be rich in flavonoids and alkaloids (Zha *et al*., 1991). It is a flowering plant with weeping and grows up to 25-foot tall with a glossy green, long, narrow leaves have attractive wavy edge. The star-shaped light green flowers in the spring are followed by clusters of inch-round black fruits. *Polyalthia longifolia* is a genus of about 120 species in the family Annonaceae and it is mainly distributed in the South and Southeast Asia, as well as tropical Africa (Mitra, 1993).

Free radicals are molecular species which contains one or more unpaired electron. A free radical is easily formed when a covalent bond between entities is broken and one electron remains with each newly formed atom (Karlson, 1997), because, they have one or more unpaired electrons, free radicals are highly unstable. They scavenge the body to grab or donate electrons, thereby damaging cells, protein and DNA (genetic materials). They arise from sources that are both endogenous and exogenous in the bodies (Robin, 2002). Oxidant that develop from processes within our bodies form as a result of normal aerobic respiration, metabolism and inflammation. At high concentrations, free radicals and radical derived, non-radical reactive species are hazardous to living organisms and may damage all the cellular constituents. At moderate concentrations, however, nitric oxide (NO), superoxide anion and reactive oxygen species (ROS) play important roles as regulatory mediators in signaling processes (Droge, 2002).

Free radicals are species capable of independent existence that contains one or more unpaired electrons that make them highly reactive (Dinis *et al*., 1993). They have been implicated in many human diseases due to their increased formation, which accompany tissue injury (Bridges *et al*., 1993). Free radicals are known to react with proteins, lipids, polysaccharides and nucleic ; thus induce damage to cell membranes, organelles and tissue (Fakoya *et al*., 1998).

Antioxidants are effective because they are willing to give up their own electrons ton free radicals. When a free radical gains the electron from an antioxidants it no longer need to attack the cell and the chain reaction of oxidation is broken (Dekkers *et al*., 1996). After donating an electron, an antioxidant becomes a free radical by definition. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive. The human body has a elaborate antioxidant defense system. The first line found in the fat-soluble cellular membrane consists of vitamin E, β-carotene and co-enzyme Q_{10} (Kackma, 1999).

In our attempt to complement plant materials that have antioxidant effects, a study was carried out using ethanol extract of *P. longifolia* to ascertain if the plant which has been found rich in flavonoids and alkaloids have any *in vitro* antioxidant effect.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals used in this study were of analytical grade and products of Sigma GMb'H, England, Randox Laboratories Ltd, Crumlin, United Kingdom and Merck, Darmstadt, Germany.

Plant Materials

Fresh leaves of the various plants were collected from Nsukka, Enugu State, Nigeria and authenticated by Mr. P. O. Ugwuozo of the Department of Botany, University of Nigeria Nsukka. Voucher specimens were deposited in the herbarium unit of the Department.

Animals

Adult male Wistar albino rats of 8 to 12 weeks old with average body weight of 150±12 were used for the lipid peroxidation scavenging tests. Twenty one (21) adult male albino mice were used for the acute toxicity study. The animals were obtained from the Animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka. The animals were kept at average temperature of 30^{o}C and a 12 h light/dark cycle. They were fed with standard pellet diet (product of Bendel Feed Nigeria Ltd) obtained commercially. All animals had free access to food and water ad libitum and were acclimatized to laboratory condition for a week before commencement of the experiment.

Extraction Procedure of *Polyalthia longifolia*

Fresh leaves of *Polyalthia longifolia* were washed, cleaned and air-dried at ambient temperature for two weeks. The dried leaves were pulverized to coarse powder using a mechanical grinder. A weighed quantity, 200 g, of the coarse powder material was extracted by cold maceration in absolute ethanol for 48 h. The extraction mixture was filtered with Whatman No. 1 filter paper. The filtrate was concentrated *in vacuo* using a rotary evaporator (at an optimum temperature between 40 and 45 ºC to avoid denaturation of the active ingredients) to obtain a dark-green semi-solid mass. The extraction yield (%) was estimated gravimetrically. The extract was stored at +4^{o}C until used.

Determination of Extract Yield

The percentage yield of the leaf extract of *Polyalthia longifolia* was determined by weighing the pulverized dry leaves before extraction and the concentrated extracts obtained after extraction and then calculated using the formula;

Percentage yield =

$$\frac{Weight\,(g)\,of\ Extract}{Weight\,(g)\,of\ Pulverized\ Leaves}\times 100$$

Acute toxicity and lethality (LD_{50}) Test

Actute toxicity (LD_{50}) of the ethanol leaf extract of the *Polyalthia longifolia* was determined by Lorke method (1983).

Qualitative Phytochemical Analysis

The fresh leaves of *Polyalthia longifolia* were subjected to phytochemical analysis according to the method outlined by Harbourne (1998) and Trease and Evans (1989). The phytochemical analysis was done to detect the presence of secondary metabolites, such as alkaloids, tannins, saponins, resins, flavonoids, steroids, glycosides and terpenoids.

Preparation of Extract of Celery for Nitric Oxide/Lipid Peroxidation-Induced Scavenging Test

A quantity, 1g of the ethanol extract of *Polyalthia longifolia* was suspended in 100ml of 20% tween 80 in standard phosphate buffer (0.025M, P^{H} 7.4) to give the stock solution containing 10,000µg/ml of the extract. The stock solution was then used for the entire nitric oxide/lipid peroxidation-induced scavenging tests.

Nitric Oxide Scavenging Activity

Nitric oxide scavenging assay was performed as describe by Sreejayan (1997)

Carbon Tetrachloride-Induced Lipid Peroxidation Scavenging Test

Carbon tetrachloride induced lipid peroxidation scavenging test was performed using the method of Comporti (1989).

Ferrous Sulphate-Induced Lipid Peroxidation Scavenging Test

The method of Okhawa *et al.* (1979) was used with minor modification by Tripathi and Sharma (1998).

Statistical Analysis

The data in the results were expressed as mean ± SD and test of statistical significance was carried out using one way ANOVA. The statistical package used was statistical package for social sciences (SPSS), version 18.

RESULTS

Percentage Yield of Extract

Table 1a: Percentage yield of the ethanol extract of the leaves stems of *Polyalthia longifolia*

EXTRACT (g)	Percentage (%)
50.0	18.50

The result of the percentage yield from the table 1 is found to be 18.50

ACUTE TOXICITY STUDIES OF *Polyalthia longifolia*

Table 1b: Acute toxicity studies (LD_{50}) of the ethanol extract of *Polyalthia longifolia.*

Dose (mg/kg)	No of Animals Before Administration	No of Deaths After Administration
250	3	–
500	3	–
750	3	–
1000	3	–
2000	3	–
3000	3	2
Control	3	–

Phytochemical Analysis

From the result of the phytochemical studies as shown in Table 2 above, alkaloids, Flavonoids, Saponons, Proteins, carbohydrate, fat and oils, tannins and steroids were present in the leaves and stems of both fresh and dried *Polyalthia longifolia.*

LIPID PEROXIDATION-INDUCED SCAVENGING TESTS

Effects of Nitric oxide Scavenging Activity of the Extract of *Polyalthia longifolia*

The extract of *Polyalthia longifolia* showed significant free radical scavenging activity on nitric oxide (NO)-induced release of free radicals. Different concentrations exhibited different percentage of inhibition. Ascorbic acid was used as reference standard.

Table 2: Phytochemical analysis of both the fresh and dried leaves of *Polyalthia longifolia.*

Bioactive Constituents	Fresh Sample of *Polyalthia longifolia*	Fresh Sample of *Polyalthia longifolia*
Alkaloids	++	+++
Flavonoids	+	++
Glycosides	–	–
Reducing sugars	–	–
Saponins	+	+
Proteins	+	+
Carbohydrates	++	++
Fats and Oils	+	–
Tannnis	+	+
Steroids	+	++
Terpenoids	+	+
Resins	–	–

Key:-
+++ = Relative abundance of compound
++ = Moderate abundance of compound
+ = Relative low presence of compound
ND = Not detected

Table 3: Percentage inhibitions of different concentrations of the extract of *Polyalthia longifolia* in the nitric oxide scavenging activity test.

	Concentration (µg/ml)	Absorbance 546nm (Mean ± S.E)	Inhibition (%)
Ethanol extract of *Polyalthia longifolia*	100	0.507 ± 0.003	2.30
	200	0.495 ± 0.016	4.99
	400	0.485 ± 0.006	6.91
	800	0.432 ± 0.007	17.08
Ascorbic acid	100	0.426 ± 0.003	18.23
	200	0.421 ± 0.001	19.19
	400	0.403 ± 0.006	22.65
	800	0.333 ± 0.005	36.66
Control		0.521 ± 0.035	

Effect of the extract on carbon tetrachloride-induced lipid peroxidation in rat liver homogenate

Lipid peroxidation induced by carbon tetrachloride was inhibited by the extract of *Polyalthia longifolia* at all tested doses (100µg/ml, 200µg/ml, 400µg/ml and 800µg/ml). The percentage inhibition of peroxide formation increased in a dose-dependent manner. Also, the standard, vitamin C (ascorbic acid), showed significant reduction in lipid peroxidation formation.

Table 4: Inhibition of the extract on carbon tetrachloride induced lipid peroxidation test.

	Concentration (µg/ml)	Absorbance 543nm (Mean ± S.E)	Inhibition (%)
Ethanol extract of *Polyalthia longifolia*	100	1.334 ± 0.017	21.34
	200	1.099 ± 0.003	35.20
	400	0.690 ± 0.036	59.32
	800	0.550 ± 0.051	67.57
Ascorbic acid	100	0.402 ± 0.0055	76.30
	200	0.348 ± 0.0033	79.48
	400	0.267 ± 0.0069	84.26
	800	0.187 ± 0.042	88.95
Control		1.696 ± 0.0126	

Effect of the extract on ferrous sulphate-induced lipid peroxidation in rat liver homogenate

The extract showed significant reduction in the lipid peroxidation formation against ferrous sulphate-induced lipid peroxidation in a dose-dependent manner. Ascorbic acid, the standard antioxidant used also showed significant reduction in the lipid peroxidation.

TABLE 5: Inhibition of the ferrous sulphate on liver homogenate by extract of *Polyalthia longifolia*.

	Concentration (µg/ml)	Absorbance 535nm (Mean ± S.E)	Inhibition (%)
Ethanol extract of *Apium graveolens*	100	0.259 ± 0.03	46.04
	200	0.253 ± 0.006	47.29
	400	0.251 ± 0.047	47.71
	800	0.204 ± 0.007	57.50
Ascorbic acid	100	0.324 ± 0.027	32.50
	200	0.236 ± 0.021	50.83
	400	0.200 ± 0.041	58.33
	800	0.147 ± 0.005	69.38
Control		0.480 ± 0.069	

DISCUSSION

In our search for plant materials that have antioxidant activities, seeds of eucalyptus plant (*Polyalthia longifolia)* was used to determine both the toxicity levels as well as the secondary plant metabolites present in the ethanol extract of the plant. From the studies the acute toxicity of the extract of *Polyalthia longifolia* was found to be 4000mg/kg body weight indicating that the plant is possibly not toxic at this level.

Further Investigation on the phytochemical content revealed the presence alkaloids, flavonoids, carbohydrates, tannins, steroids, proteins, fats and oils and saponins as shown in Table 2. The phytochemicals present in the dried sample of the plant were found to be higher than those found out in the fresh plants. The alkaloids and flavonoids earlier reported by Zhao *et al.* (1991) were equally found in the study, suggesting the plant to be a rich source of these antioxidant phytochemicals (Livingstone *et al.*, 1999).

The presence of a broad spectrum of phytochemicals present in this plant could confer on the plant a lot of pharmacological properties as shown by earlier studies (Dresbach and Ross, 1999). The antioxidant activity of *Polyalthia longifolia* could be attributed in part to the presence of the flavonoids which is present in large amount. Flavonoids and other phenolic compounds (polyphenols) of plant origin have been reported to be good scavengers of free radicals (Formica and Regelson, 1995). Flavonoids have also been reported to have antioxidant activity *in vitro* but no direct antioxidant effect *in vivo* (Formica and Regelson, 1995).

Nitric oxide induced lipid peroxidation study showed that the extract is a potent inhibitor of nitric oxide. From this study, the absorbance of the chromophore generated in the nitric oxide scavenging test reduced as the extract of *Polyalthia longifolia* was added. As the concentration of the extract increased, there was a decrease in the absorbance of the chromophore; showing a corresponding increase in the inhibition of the formation of chromophores.

The extract of *Polyalthia longifolia* also showed similar protection against lipid peroxidation induced by

carbon tetrachloride and ferrous sulphate induced lipid peroxidation scavenging. Activity. Tables 5 and 6 showed that the extract increased as the concentration of the extract increased in both cases. The results of the nitric oxide and lipid peroxidation induced scavenging tests were significant when compared to the standard antioxidant, ascorbic acid.

From this study, it is evident that *Polyalthia longifolia* has some antioxidant activities *in vitro* and this could be an added pharmacological use of the plant which is currently enjoying a lot of publicity, especially as a possible insecticide, antifungal and antiviral drug (Ghani, 2003; Natcha *et al*., 2010).

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