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# Potential Effect of *Alpinia purpurata* rhizome against Free radicals and Lipidperoxidation

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## ABSTRACT

The aim of the study was to determine the free radical scavenging activities as well as the inhibition of *in vitro* lipid peroxidation evoked by *Alpinia purpurata* rhizome. The ethanolic extract of *Alpinia purpurata* was studied for its free radical scavenging property on different *in vitro* models like, Hydroxyl radical scavenging, Nitric Oxide Radical Scavenging, Super oxide radical scavenging activity and *in vitro* antilipidperoxidative assay using goat liver homogenate and RBC Ghost model. The results of ethanolic extract of *Alpinia purpurata* rhizome showed that a dose dependent increase in Hydroxyl radical scavenging, Nitric Oxide Radical Scavenging and Super oxide radical scavenging activity. *In vitro* anti-lipid peroxidative assay using goat liver homogenate and RBC Ghost model. The *in vitro* Lipid peroxidation (LPO) was inhibited to a good extent by the *Alpinia purpurata* rhizome ethanolic extract and the extent of inhibition being higher in the RBC membrane model than the liver homogenate model. The ethanolic extract showed good dose dependent free radical scavenging property in both the models. This study showed that inhibition of LPO and Hydroxyl radical, Nitric Oxide Radical and Super oxide radical scavenging abilities of *Alpinia purpurata* rhizome could be related to the presence of phenolic and flavonoid compounds. Therefore, the ethanolic extract of the rhizome may be a good source of natural antioxidative agent.

***Key words*:** Lipid peroxidation, Freeradical scavenging, *Alpinia purpurata*.

## INTRODUCTION

Lipid peroxidation is an accumulated effect of reactive oxygen species (ROS), which leads to deterioration of biological systems. It may be initiated by reactive free radicals, which subtract an allylic hydrogen atom from a methylene group of polyunsaturated fatty acid side chains. This is accompanied by bond rearrangement that results in stabilization by diene conjugate formation. The lipid radical then takes up oxygen to form peroxy species [1]. Oxygen radicals and other reactive species are generated in biological systems either as by-products of oxygen reduction or by xenobiotics catabolism [2]. These ROS such as superoxide anion ($O_2^{\cdot-}$), hydroxyl radicals ($OH^{\cdot}$), nitric oxide (NO) and peroxy radical ($ROO^{\cdot}$) are unstable and can attack key biomolecules such as lipids, proteins and nucleic acids [3]. The consequences of oxidation of these biomolecules have been linked to a variety of different human disorders, including atherosclerosis, cancer and disease of the nervous system [4]. Cells have a comprehensive array of antioxidant defense mechanisms to reduce free radical formation or limit their damaging effects [5]. These mechanisms are not sufficient when the balance shifts to the side of free radicals generation [6], thus body requires antioxidant supplements to reduce oxidative damage and retard lipid peroxidation. Nowadays, the use of synthetic antioxidants is limited because of inherent toxicity associated with them at optimum concentration. The use of natural antioxidants of plant origin is receiving great attention. Phytochemical constituents of plants have been reported as scavengers of free radicals and inhibitors of lipid peroxidation [7]. *Alpinia purpurata* is very recent and incipient and results show the presence of flavonoids rutin and kaempferol-3-O-glucuronide [8]. Flavonoids are present in several species of *Alpinia* and they are referred as promising therapeutic agents in the treatment of cardiovascular diseases. Many *Alpinia* species are well-known medicinal herbs that have been shown by several previous studies to have various effects, namely, anti-inflammatory, anti-oxidant, anti-microbial, anti-dermatophytic, anti-nociceptive, hepatoprotective, immunostimulatory, and anticancer activities [9].

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## MATERIALS AND METHODS

### *Plant material*

### *Collection*

Fresh plant material was collected from Kovaipudhur, Coimbatore District, and Tamil Nadu State, India. Efforts were made to collect the

plant in rhizomes and flowering conditions for the correct botanical identification. The plant material was brought to the laboratory and identified with the help of Agriculture university of Coimbatore, Tamil Nadu State.

***Preparation of Extract***

About 300g of the coarse dried powder of the rhizome of *Alpinia purpurata* was taken in soxhlet apparatus and extracted using 95% ethanol. The extraction was carried out for about 72 hours. The extract was collected by the filtrate, which was pooled and the solvents were evaporated in a rotator evaporator at temperature below 50°C and the extracts were freeze-dried. The residue was used to analyse the various *in vitro* assays.

***Chemicals***

Chemicals used in the study were analytical grade and procured it. All biochemical assay kits were purchased from Sigma, SD fine-chemicals limited and Himedia, India.

***Preparation of goat liver homogenate***

Fresh goat liver was obtained from local slaughter house, washed free of blood and removed fat deposits, if any, a 5% homogenate was prepared in ice cold TBS (Tris Buffered Saline) and used for assay. The assay procedure [10] has been followed.

***Preparation of Erythrocyte Ghosts***

About 50 ml of fresh venous whole blood of goat was collected into a cleaned sterile bottle and defibrinated immediately using acid-washed stones. The defibrinated blood was then transferred into sterile centrifuge tubes and spun at 3000 rpm for 10min to pellet out the cells and the supernatant was discarded. The pellet of RBCs was washed in isotonic TBS, thrice successively. The washed pellet was then treated with hypotonic TBS and incubated at 37°C for 1 hour for lysis to occur. The lysate was centrifuged at 5000 rpm for 15-20 min at 4°C. The pale pellet containing the erythrocyte ghost membranes' was then suspended in 1.5ml of TBS. The assay procedure [11] has been followed to study the anti-lipid peroxidative effect of the *Alpinia purpurata* rhizome.

***Hydroxyl Radical Scavenging Activity***

Hydroxyl radical scavenging activity of extract was measured according to the method [12], by studying the competition between deoxyribose and test compounds for hydroxyl radicals generated for the $Fe^{3+}$/ascorbate/EDTA/$H_2O_2$ System. The hydroxyl radical attacks deoxyribose, which eventually results in thiobarbituric acid (TBA) reacting substance formation. The reaction mixture contains deoxyribose (28mM), $FeCl_3$ (0.1mM), $H_2O_2$ (1mM), ascorbic acid (0.1mM) and $KH_2PO_4$-KOH buffer (20mM,pH 7.4) and various concentration of the extract in final volume of 1ml.the reaction mixture was incubated for one hour at 37°c. Deoxy ribose degradation was measured with TBA reacting substance and percentage of inhibition was calculated. Percentage of inhibition = (C-T/C) x 100

***Nitric Oxide Radical Scavenging Activity***

Nitric oxide radical scavenging activity of extract was measured according to method [13]. Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction. Sodium nitroprusside (5mM) in standard phosphate buffer saline solution (0.025M, pH: 7.4) was incubated with different concentrations (50-250µg/mL) of the ethanol extract dissolved in phosphate buffer saline (0.025 M. pH: 7.4) and the tubes were incubated at 25° C for 5 hours. Control experiment without the test compound but with equivalent amounts of buffer was conducted in an identical manner. After 5 hours 0.5mL of incubation solution was removed and diluted with 0.5mL of Griess reagent (1% sulphanilamide, 2% O- phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophere formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthyl ethylene diamine was read at 546 nm. The results were expressed in Percentage of inhibition = (C-T/C) x 100

***Superoxide Radical Scavenging Activity***

Superoxide radical scavenging activity of extract was measured according to method [14]. Superoxide radical was generated from the photo reduction of riboflavin and was detected by NBT reduction method. The reaction mixture contained EDTA (6µm), with 3µg NaCN, riboflavin (2µM), NBT (2µM), $KH_2PO_4$ - $Na_2HPO_4$ buffer (67mM, pH 7.8) and various concentrations of the extracts in a final volume of 3.0ml. The tubes were illuminated under incandescent lamp for 15min. The optical density at 530nm was measured before and after illumination the inhibition of superoxide radical was determined by comparing the absorbance values of the control with those of treatments. The results were expressed in Percentage of inhibition=(C-T/C) x 100

**RESULTS AND DISCUSSION**

***Determination of Hydroxyl Radical Scavenging Activity***

The result indicates the antioxidant activity of *Alpinia purpurata* extract at different concentrations 50, 100, 200 and 400 µg/ml, *Alpinia purpurata* extract have showed a dose dependent increase in their antioxidant properties as indicated in (Table 1). The scavenging activity of the rhizome extract of *Alpinia purpurata* and standard Ascorbic acid against hydroxyl radical were found to be 88.48 ± 0.78 % and 92.04 ± 1.23 at 400 µg/ml concentration. The inhibition value of standard Ascorbic acid was high when compared to *Alpinia purpurata*. $IC_{50}$ value of the extract was found to be 32 for the ethanolic extract of *Alpinia purpurata* against standard ascorbic acid where $IC_{50}$ value was found to be 86 µg /ml. Our results are in accordance with that of *Alpinia purpurata* leaves showed high scavenging activity

on hydroxyl radical [15]. [16] reported that *Alpinia vittata* and *Alpinia smithiae* were found to be more effective on scavenging the hydroxyl radical.

**Table 1: Hydroxyl radical scavenging activity of *Alpinia purpurata* extract**

| Sample concentration (µg) | Ethanolic Extract % Inhibition | Ethanolic Extract $IC_{50}$ µg /ml | Standard (Ascorbic acid) % Inhibition | Standard (Ascorbic acid) $IC_{50}$ µg /ml |
|---|---|---|---|---|
| 50 | 80.20 ± 0.69 | | 36.74 ± 0.23 | |
| 100 | 84.74 ± 0.46 | 32 | 58.48 ± 0.45 | 86 |
| 200 | 86.08 ± 0.47 | | 64.35 ± 0.34 | |
| 400 | 88.48 ± 0.78 | | 92.04 ± 1.23 | |

*Values are mean ± SD of three samples in each column*

***Determination of Nitric Oxide Radical Scavenging Activity***

The scavenging of nitric oxide by rhizome extract increased in a dose dependent manner as illustrated in (Table 2). The results were expressed as % Inhibition. *Alpinia purpurata* exhibited inhibition of 83.74 ± 0.56 % at a concentration of 400 µg/ml against standard Butyrate hydroxytoluene (BHT) which showed an inhibition of 86.02 ± 0.76 % at the concentration of 400 µg/ml. The $IC_{50}$ value of BHT was found to be high when compared to that of *Alpinia purpurata* rhizome. It is well known that nitric oxide has an important role in various inflammatory processes. Sustained levels of production of this radical are directly toxic to tissues and contribute to the vascular collapse associated with septic shock, whereas chronic expression of nitric oxide radical is associated with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis [17]. Our result were in accordance with that of the nitric oxide radical scavenging capability of *Alpinia vittata* and *Alpinia smithiae* which showed a dose dependent increase in nitric oxide scavenging activity [16]. [15] observed that *Alpinia purpurata* leaves showed scavenging activity on Nitricoxide radical.

**Table 2: Nitric Oxide Radical Scavenging Activity of *Alpinia purpurata* Extract**

| Sample concentration (µg) | Ethanolic Extract % Inhibition | Ethanolic Extract $IC_{50}$ µg /ml | Standard (BHT) % Inhibition | Standard (BHT) $IC_{50}$ µg /ml |
|---|---|---|---|---|
| 50 | 52.67 ± 0.67 | | 30.20 ± 0.23 | |
| 100 | 75.39 ± 0.42 | 48 | 46.04 ± 0.17 | 192 |
| 200 | 80.49 ± 0.35 | | 52.48 ± 0.23 | |
| 400 | 83.74 ± 0.56 | | 86.02 ± 0.76 | |

*Values are mean ± SD of three samples in each column*

***Determination of Super Oxide Radical Scavenging Activity***

The superoxide radical scavenging activities of *Alpinia purpurata* rhizome and ascorbic acid are represented in (Table 3). The scavenging effects of the *Alpinia purpurata* rhizome extract on the superoxide radical is 97.47 ± 0.56 % for *Alpinia purpurata* and standard ascorbic acid showed the inhibition value of 88.32 ± 0.87 % at 400 µg /ml concentration. The scavenging effect also increased with increase in sample concentration dependent manner. The study was correlated with that of *Alpinia galangal* extract which showed the superoxide scavenging activity was increased with an increasing concentration amount of the extracts [18].

**Table 3: Super oxide radical scavenging activity of *Alpinia purpurata* extract**

| Sample concentration (µg) | Ethanolic Extract % Inhibition | Ethanolic Extract $IC_{50}$ µg /ml | Standard (Ascorbic acid) % Inhibition | Standard (Ascorbic acid) $IC_{50}$ µg /ml |
|---|---|---|---|---|
| 50 | 11.24 ± 0.44 | | 30.12 ± 1.09 | |
| 100 | 32.90 ± 0.23 | 120 | 44.57 ± 0.40 | 170 |
| 200 | 84.18 ± 0.76 | | 59.82 ± 1.63 | |
| 400 | 97.47 ± 0.56 | | 88.32 ± 0.87 | |

*Values are mean ± SD of three samples in each column*

***Determination of in vitro LOP***

The percent inhibition of LPO by the ethanolic extracts of rhizome of *Alpinia purpurata* against goat liver homogenate and RBC ghosts are given in (Table 4). The extracts tested were effective in reducing the production of TBARS in a dose dependent manner, the extent of inhibition by rhizome being higher in RBC membrane model than that of liver homogenate. This difference could be due to the nature of the RBC ghosts, which contain plasma membranes alone, and liver homogenate that may contain other lipid constituents like intracellular membrane having different lipid composition. [19] have reported that the mammalian cells have evolved myriad interrelated antioxidant defense mechanisms, which minimize the injurious events that result from toxic chemicals and normal oxidative products of cellular metabolism. This result was in agreement with that of [16], who reported that, the extract of *Alpinia smithiae* and *Alpinia vittata* which showed the great activity in reducing lipid peroxidation. *Alpinia katsumadai* showed slightly higher lipid peroxidation inhibitory effect [20].

**Table 4: Inhibition of *in Vitro Lipid Peroxidative Effect on Alpinia purpurata* Rhizome**

| Sample concentration (µg) | RBC ghost % Inhibition | RBC ghost $IC_{50}$ µg /ml | Liver Homogenate % Inhibition | Liver Homogenate $IC_{50}$ µg /ml |
|---|---|---|---|---|
| 50 | 29.63 ± 0.24 | | 18.34 ± 0.22 | |
| 100 | 47.10 ± 0.28 | 160 | 37.84 ± 0.38 | 329 |
| 200 | 62.56 ± 0.53 | | 42.88 ± 0.86 | |
| 400 | 83.33 ± 0.32 | | 60.88 ± 0.30 | |

*Values are mean ± SD of three samples in each column*

## CONCLUSION

The present findings demonstrate that the ethanolic extract of rhizome of *Alpinia purpurata* showed better activity of *in vitro* lipid peroxidation and free radicals scavenging studies. *Alpinia purpurata* is a large and economically important among the flowering plants which are commonly known as Zingiberaceae family. Extensive further analysis on the anticancer properties of isolated compound compared with those of an anticancer drug compound as the positive control is currently underway. Efficacy and mechanisms of action in various normal and cancer cell models *in vitro*, coupled with bio-assay guided purification in order to elucidate active anticancer compounds from the crude extract will be reported in due course.

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