Evaluation of in vitro antioxidant and anticancer activity of *Alpinia purpurata*

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[ABSTRACT] AIM: Ethylacetate extract of *Alpinia purpurata* was evaluated for its potential in vitro antioxidant and anticancer activity. METHODS: The antioxidant activity was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method, hydroxyl radical activity, superoxide radical scavenging activity, nitric oxide radical scavenging activity, hydrogen peroxide radical scavenging activity and reducing power activity. The viability of OAW42 cells was evaluated by MTT assay. RESULTS: *A. purpurata* exhibited potential antioxidant activity with a concentration-dependent manner. The extract showed potent anticancer activity at the 48th hour with IC50 of 130.20 μg·mL⁻¹ and exhibited a dose-dependent decrease in cell count for all the concentrations tested. CONCLUSION: The results suggested that long term consumption of *A. purpurata* exhibited antioxidant and anticancer activity and could be further exploited for their anticancer properties.

[KEY WORDS] *Alpinia purpurata*; Ethylacetate; Antioxidants; Anticancer; OAW42


1 Introduction

Medicinal plants have been used to cure disease since antiquity. Plants still constitute one of the major source of drugs in modern as well as traditional medicine throughout the world[1]. The investigation of anti-tumor agents from natural resources has been greatly emphasized in order to find useful drugs for medical treatment of human malignancies, including solid tumors[2]. It has been proposed that sequential use of cytotoxic agents could improve the anti-cancer therapy by inhibiting tumor cell proliferation and phenotypic diversification[3]. Plants belonging to Zingiberaceae (Ginger family) are known for a number of medicinal properties[4-6]. A spectrum of essential oils are present in the members of Zingiberaceae[7]. Rhizome extract of some members of the medicinal Zingiberaceae are widely used in dietary intake as well as in traditional systems of medicine[8]. *Alpinia* is the largest genus in ginger family in which *A.purpurata* (Vieill.) K. Schum. is a very popular garden plant in India[9]. Rhizome has sharp odour, improves appetite, taste and voice. It is also used for headache, rheumatism, sore throat and renal disease[10]. Phytochemical studies on *A. purpurata* revealed that it possess flavonoids, rutin, kaempferol-3-rutinoside and kaempferol-3-oliucronide[11]. The phytochemical constituents of *A. purpurata* promote antimicrobial activity against certain microorganisms[12]. In addition to the purported anti-inflammatory activity, its phytomedicinal potential to treat tuberculosis is also described[13]. *A. purpurata* may serve as potential dietary sources of natural antioxidants.

Ovarian cancer is the fourth leading cause of cancer death and the most frequent cause of death from gynecologic malignancy[14]. The annual worldwide incidence of ovarian cancer exceeds 140 000. Ovarian cancer rates vary enormously between countries and appear to relate to their respective reproductive patterns[15].

Many chemotherapeutic drugs eliminate cancer cells by inducing, a genetically programmed form of cell death[16]. It is, therefore, important to establish the chemopreventive efficacy of the plant by evaluating anticancer and apoptosis induction in cancer cell lines before whole animal studies or clinical trials begin. We, therefore, decided to screen the leaf extract for its antioxidant activity and anti-cancer activity against the human ovarian cancer cell line (OAW42). The
main objectives of this study were to assess the antioxidant and anticancer activity of this extract.

2 Materials and Methods

2.1 Plant material and extraction

*A. purpurata* was collected from Kanyakumari, Tamilnadu, India. The plant specimen was authenticated by Dr. G.V.S. Murthy, Botanical Survey of India, Coimbatore, India. A voucher specimen has been deposited in the laboratory for future reference (BSI/SC/5/23/10-11/Tech). The voucher specimen was deposited at the herbarium of Karlapagam University, Coimbatore. The leaf of *Alpinia purpurata* were washed thoroughly in tap water, shade dried and powdered. The powder (100 g) was exhaustively extracted with ethylacetate in the ratio of 1:5 (W/V) for 24 h using Soxhlet apparatus. The extract was completely evaporated to dryness using rotary flash evaporator (Buchi type).

2.2 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

Sample was mixed with 160 µmol·L⁻¹ DPPH in MeOH. After 20 min incubation at room temperature in the dark, the absorbance was read at 517 nm[19]. The inhibitory percentage of DPPH (% scavenging activity) was calculated as:

\[
\text{[(absorbance of the control – absorbance of the sample) / absorbance of the control]} \times 100
\]

2.3 Hydroxyl radical activity

The reaction mixture contained 2.8 mmol·L⁻¹ deoxyribose, 20 mmol·L⁻¹ potassium phosphate buffer (pH 7.4), 100 µmol·L⁻¹ FeCl₃, 780 µmol·L⁻¹ EDTA, 1 mmol·L⁻¹ H₂O₂ and 100 µmol·L⁻¹ ascorbic acid. After incubating at 37 °C for 1 h, equal volumes of 1% 2-thiobarbituric acid (TBA) and 10% trichloroacetic acid (TCA) were added to the reactants. The mixture was boiled for 5 min, cooled, centrifuged at 13 000 r·min⁻¹ for 5 min and the absorbance was determined spectrophotometrically at 532 nm[18].

2.4 Superoxide radical scavenging activity

Superoxide radicals were generated by the NADH-phenazine methosulfate (PMS) system according to a described procedure[19]. The reaction mixture contained 400 µL of sample and 400 µL of 630 mmol·L⁻¹ nitroblue tetrazolium (NBT). After incubation at room temperature for 10 min, the reaction was started by adding 400 µL of 156 mmol·L⁻¹ NADH. The reaction mixture was incubated at ambient temperature for 5 min. The absorbance (560 nm) of each sample was compared against blank samples.

2.5 Nitric oxide radical scavenging assay

Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, interacts with oxygen to produce nitrite ions which were measured by Griess reaction[20-21]. The reaction mixture (3 mL) containing sodium nitroprusside (10 mmol·L⁻¹) in phosphate buffer saline (PBS) and the extract from 100 to 500 µg·mL⁻¹ was incubated at 25 °C for 150 min. After incubation, 0.5 mL of the reaction mixture was removed and 0.5 ml of Griess reagent (1% (W/V) sulfanilamide, 2% (W/V) H₃PO₄ and 0.1% (W/V) naphthylethylene diamine hydrochloride) was added. The absorbance of the chromophore formed was measured at 546 nm.

2.6 Hydrogen peroxide scavenging assay

Hydrogen peroxide assayed as described[22] proposed an assay for the determination of antioxidant activity of compounds by their ability to scavenge the oxidant hydrogen peroxide. The reaction mixture contained Phosphate buffer (pH 7.4) hydrogen peroxide in phosphate buffer (40 mmol·L⁻¹). A solution of hydrogen peroxide (40 mmol·L⁻¹) was prepared in phosphate buffer. Plant extracts at the concentration of 10 mg/10 µL was added to a hydrogen peroxide solution (0.6 mL, 40 mmol·L⁻¹). The total volume was made up to 3 mL. The absorbance of the reaction mixture was recorded at 230 nm. The blank solution contained phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenged by the plant extract was calculated as follows:

\[
\text{Percentage of scavenged H}_2\text{O}_2 = \frac{(A_0 - A_1)}{A_0} \times 100
\]

A₀: Absorbance of control
A₁: Absorbance in the presence of plant extract

2.7 Reducing power activity

Reducing power activity was determined by the K₃Fe(CN)₆–FeCl₃ method[23]. Each test sample was mixed with an equal volume of 200 mmol·L⁻¹ phosphate buffer (pH 6.6) and 1% potassium ferricyanide (K₃Fe(CN)₆). The mixture was incubated at 37 °C for 20 min. After an equal volume of 10% trichloroacetic acid was added to the mixture, the mixture was centrifuged at 3 000 r·min⁻¹ for 10 min. The supernatant was mixed with deionized water and 0.1% ferric chloride (FeCl₃) at a ratio of 1:1:2. The resulting absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated reducing power.

2.8 Maintenance of cell line

Human Ovarian Cancer cell line (OAW42) was purchased from National Center for Cell Sciences, Pune, India. The cells were maintained in T-75 cm² tissue culture flask with complete media i.e., DMEM (Dulbecco’s modified Eagle medium), 10% FBS (fetal bovine serum), with antibiotics and allowed to become 80% confluent. When the cells were grown to confluence, the medium was removed and washed once with PBS (phosphate buffered saline). Trypsin (0.25%)-EDTA solution was added and incubated for 3–5 min at 37 °C. Fresh medium (with serum) was added and cells were gently dispersed by a pipette. A known number of cells were dispersed in to new flasks or micro litre plates for further experiment. The cells were incubated at 37 °C and 5% CO₂ atmosphere.

2.9 MTT-cell proliferation assay

Cell growth inhibition was determined by MTT assay[24]. MTT [3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay is a simple nonradioactive colorimetric assay
to measure cell cytotoxicity, proliferation or viability. Cells were seeded on 96 well plates (5 000 cells/well) cultured for a day and then treated with different concentration of *A. purpurata* for 12, 24, 48 and 72 h at 37 °C in 5% CO₂. Controls well incubated on the medium were done in 96 well plates. At the end of the incubation, medium was removed and MTT (5 mg·mL⁻¹) was added and the cells were further incubated for 4 h after which the media was removed. DMSO was added in each well to solubilize the formazan crystals. The absorbance was read at a wave length 595 nm using a microtitre ELISA plate reader. Experiments for extract were carried out in triplicate including untreated cell control and blank cell–free control. Cell viability was expressed as percentage over the control.

2.10 Statistical analysis

The results (x ± s) of cell proliferation, invasion and ELISA assay were subjected to statistical analysis by student’s t-test. The level of significance was set at *P* < 0.05. All experiments were repeated twice using triplicates of sample.

3 Results and Discussion

![DPPH radical scavenging activity of *A. purpurata* was expressed in % inhibition. Radical scavenging was compared with BHT (butylated hydroxyl toluene) as standard](image1)

![Hydroxyl radical scavenging activity of *A. purpurata* was expressed in % inhibition. Radical scavenging was compared with Vit C as standard](image2)

![Superoxide radical scavenging assay](image3)

![Nitric oxide radical scavenging assay](image4)

![Hydrogen peroxide scavenging activity of *A. purpurata* was expressed in % inhibition. Radical scavenging was compared with Vit C as standard](image5)

Natural products play an important role in chemotherapy, having contributed considerably to approximately 60 available cancer chemotherapeutic drugs[25-26]. Agents capable of inhibiting cell proliferation, inducing apoptosis or modulating signal transduction are currently used for the treatment of cancer[27]. The use of multiple chemopreventive agents or
agents with multiple targets on cancer cells are considered to be more effective in cancer treatment[28]. Flavonoids are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity.

There are increasing suggestions by considerable evidence that the free radicals induce oxidative damage to biomolecules (lipids, proteins and nucleic acids), the damage which eventually causes atherosclerosis, ageing, cancer, diabetes mellitus, inflammation, AIDS and several degenerative diseases in humans[39].

In this research, the antioxidant and anticancer activity of A. purpurata is investigated. There are many methods to determine the antioxidant capacities which differ in terms of their determination principles and experimental conditions[30]. For measuring antioxidant activity, the parameters such as DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical scavenging activity nitric oxide radical scavenging activity, hydrogen peroxide radical scavenging activity and reducing power capacity. In radical scavenging activity, all the above six antioxidants show good scavenging activity with a concentrated dependent manner, the scavenging activity also increasing with the concentration of the extract.

DPPH radical scavenging is considered a good in vitro model widely used to assess antioxidant efficacy within a very short time. The DPPH free radical scavenging of antioxidants is due to their hydrogen donating ability, the plants with higher hydrogen donating capacity have shown higher DPPH free radical scavenging activity[31]. The highest scavenging activity on DPPH radical of A. purpurata is 68.42% for ethylacetate extract at the concentration of 500 µg mL⁻¹. The extract is close to that of BHT standard (88.37%) (Fig. 1).

Hydroxyl radicals are most reactive species, initiating the peroxidation of the cell membrane[39]. Hydroxyl radical is an extremely reactive species formed in biological systems. It is capable of damaging almost every molecule found in living cells[32]. This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. In addition, hydroxyl radical is considered to be one of the quick initiators of the lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids[33]. This study shows the highest scavenging activity on hydroxyl radical is 51.86% for extract at the concentration of 500 µg mL⁻¹. The extract is close to that of Vit C standard (82.67%) (Fig. 2).

Superoxide is the first reduction product of molecular oxygen, a highly toxic radical, the most abundantly produced in all aerobic cells by several enzymatic and non-enzymatic pathways, attacks a number of biological molecules and leads to unfavourable alterations of biomolecules including DNA[34]. The highest scavenging activity on superoxide radical is 55.7% for extract at the concentration of 500 µg mL⁻¹. The extract is similar to that of Vit C standard (55.7%) (Fig. 3).

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[35]. The study shows highest scavenging activity on nitricoxide radical is 59.87% for extract at the concentration of 500 µg mL⁻¹. The extract is close to that of Vit C standard (82.61%) (Fig. 4).

H₂O₂ is highly important because of its ability to penetrate biological membranes. H₂O₂ itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells[36]. The results showed that the ethylacetate extract of A. purpurata leaves had an effective H₂O₂ scavenging activity. The highest scavenging activity on hydrogen peroxide radical is 57.89% for extract at the concentration of 500 µg mL⁻¹. The extract is similar to that of Vit C standard (60.87%) (Fig. 5).
The reducing ability of a compound generally depends on the presence of reductants which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom \(^{[37]}\). The plant extract reduces the most Fe\(^{3+}\) ions, in a concentrated dependent-manner. The high reducing power is the high absorbance at 700 nm. Reducing power is compared with BHT (Fig. 6).

It was reported that plant-derived extracts containing antioxidant principle showed cytotoxicity toward tumour cells \(^{[38]}\). The in vitro screening of the ethylacetate extract of \(A.\) purpurata showed potential anticancer activity against the ovarian cancer cells. Cell viability results were compared with a known anticancer drug, Cisplatin at same concentration and time. IC\(_{50}\) for cisplatin and \(A.\) purpurata showed 50 and 130.20 µg·mL\(^{-1}\), respectively (Fig. 7).

The results obtained from the present study showed that the \(A.\) purpurata has moderately antioxidant and anticancer activity. The anticancer activity may be due to the presence of alkaloids, flavonoids and terpenoids present in the leaves of \(A.\) purpurata respectively. We hope that the intensive study on the out-coming active constituents of \(A.\) purpurata will lead to the discovery of a novel botanical-drug for chemoprevention.

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**References**


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