Leaf extract of *Alpinia purpurata* (Vieill.) K. Schum screened for its phytochemical constituents and antibacterial and anticancer activities

Chinthamony Arul Raj¹, Dominic Sophia¹, Paramasivam Ragavendran¹, Thangarajan Starlin², Muthian Ahalliya Rathí³, Velliyur Kanniappan Gopalakrishnan¹,²

1. Department of Biochemistry, Karppagam University, Coimbatore 641021, Tamilnadu, India
2. Department of Bioinformatics, Karppagam University, Coimbatore 641021, Tamilnadu, India
3. Department of Biochemistry, Sree Narayana Guru College, Coimbatore 641105, Tamilnadu, India

**OBJECTIVE:** The study was formulated with the objective to assess phytochemical constituents, antibacterial activity and anticancer activity of *Alpinia purpurata*.

**METHODS:** The leaves of *A. purpurata* were washed thoroughly by tap water, shade dried and powdered. The plant powder was extracted with successive solvent system. Phytochemical constituents were evaluated, antibacterial activity was carried out by disc diffusion method and anticancer activity of the ethyl acetate leaf extract was evaluated by using 3-(4,5 dimethylthiazol-2-yi)-2,5-diphenyltetrazolium bromide assay.

**RESULTS:** The ethyl acetate extract of *A. purpurata* showed most of the phytochemicals. The extract exhibited antibacterial activity with a zone of inhibition from 5 to 14 mm at various concentrations and the extract showed potential anticancer activity against PA1 ovarian cancer cell line at the 48 h with half maximal inhibitory concentration value of 110.25 μg/mL and exhibited a dose-dependent decrease in cell count for all the concentrations tested.

**CONCLUSION:** The present study scientifically proved that ethyl acetate leaf extract of *A. purpurata* is a good source of phytoconstituents, showing antibacterial and anticancer activities.

**KEYWORDS:** Alpinia; plant extracts; antibacterial agents; antineoplastic agents; in vitro

Plants still constitute one of the major sources of drugs in modern as well as traditional medicine throughout the world. Plants belonging to Zingiberaceae (Ginger family) are known for a number of medicinal properties. A spectrum of essential oils is present in the members of Zingiberaceae. Rhizome extracts of some members of the medicinal Zingiberales are widely used in dietary intake as...
well as in traditional systems of medicine. Alpinia is the largest genus in ginger family in which A. purpurata (Vieill.) K. Schum is a very popular garden plant in India. The rhizome has sharp odour, which could improve appetite, taste and voice. It is also used for headache, rheumatism, sore throat and renal disease. Phytochemical studies on A. purpurata revealed that it possesses flavonoids, rutin, kaempferol-3-rutinoside and kaempferol-3-oligomeric. One of the major biological properties of flavonoids is their antimicrobial activity and their main role in plants is to act as protective compounds against diseases caused by microorganisms such as fungi, bacteria and viruses.

Ovarian cancer is the fourth leading cause of cancer death and the most frequent cause of death from gynaecological malignancy. The annual worldwide incidence of ovarian cancer exceeds 140,000. Ovarian cancer rates vary enormously between countries and appears to relate to their respective reproductive patterns.

Many chemotherapeutic drugs eliminate cancer cells by inducing a genetically programmed form of cell death. 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. Therefore, the main objectives of this study were to screen the ethyl acetate leaf extract of A. purpurata for its phytochemical constituents, antibacterial activity and anticancer activity against the human ovarian cancer cell line (PA1).

1 Materials and methods

1.1 Plant material collection 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 was deposited in the laboratory for future reference (BSI/SC/5/23/10-11/Tech). The voucher specimen was deposited at the herbarium of Karpagam University, Coimbatore, India. The leaves of A. purpurata were washed thoroughly in tap water, shade dried and powdered.

The plant powder was extracted with successive solvent system, petroleum ether, chloroform, ethyl acetate, ethanol and water. Totally 100 g of plant powder was extracted in 500 mL of corresponding solvents for 24 h with occasional shaking at room temperature. The supernatant was collected and evaporated to make final volume, one fifth of the original volume. It was stored at 4°C in air-tight bottles for further studies. The dried extract thus obtained was used directly for various assays.

1.2 Phytochemical analysis Preliminary phytochemical screening of A. purpurata crude extract was estimated according to the method adopted by Paech et al. We took 1 g of each respective extracts separately and dissolved in 15 mL of corresponding solvents, then carried out the estimations.

1.3 Antibacterial activity Different amounts (5, 10, 15 and 20 mg) of ethyl acetate leaf extract of A. purpurata were taken and diluted with 2 mL of dimethyl sulphoxide (DMSO). The organisms used for the study are Bacillus cereus, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Salmonella paratyphi. The antibacterial activity test was done by disc diffusion method. The sterile discs were dipped in different concentration of the extracts and kept to dryness for 2 min, then the discs were placed over the swabbed nutrient agar media in the petri plates using flamed forceps, and the discs were gently pressed down to ensure complete contact of the disc with the agar surface. The plates were incubated at 37°C for 24 h and the inhibition zones were measured. Novomycin (20 μg per disc) was used as a reference standard. DMSO was used as a negative control. The American type culture collection and the microbial type culture collection bacterial strains were obtained from the Department of Microbiology, Karpagam University, Coimbatore, India.

1.4 Anticancer activity against PA1 ovarian cancer cell line

1.4.1 Maintenance of the cell line Human Ovarian Cancer cell line (PA1) was purchased from the National Center for Cell Sciences, Pune, India. The cells were maintained in T-75 cm² tissue culture flask with complete media, namely, Dulbecco’s modified Eagle’s medium (DMEM) and 10% fetal...
bovine serum (FBS), with antibiotics and allowed to become 80% confluent. When the cells grew to confluence, the medium was removed and washed once with phosphate buffered saline (PBS). Trypsin (0.25%) -ethylene diamine tetraacetic acid solution was added and the cells were incubated for 3 to 5 min at 37 °C. Fresh medium (with serum) was added and cells were gently dispersed by a pipette. A known number of 1 000 cells were dispersed into new flasks or micro litre plates for further experiment. The cells were incubated at 37 °C and 5% CO₂ atmosphere.

1.4.2 Cell proliferation assay Cell growth inhibition was determined by MTT assay[13]. 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 per well) and cultured for a day and then treated with different concentrations of ethyl acetate leaf extract of A. purpurata for 12, 24, 48 and 72 h at 37 °C in 5% CO₂. Control cells were incubated on the medium in 96-well plates. At the end of the incubation, medium was removed and MTT (5 mg/mL) was added and the cells were further incubated for 4 h after the media were removed. DMSO was added in each well to solubilize the formazan crystals. The absorbance was read at a wave length of 595 nm using a microtitre enzyme-linked immunosorbent assay 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.

1.5 Statistical analysis SPSS 10.0 software was used for statistical analysis. The results (mean ± standard deviation) of cell proliferation and invasion were subjected to statistical analysis by Student’s \( t \)-test to compare with the standard drug. The level of significance was set at \( P < 0.05 \). All experiments were repeated twice using triplicates of sample.

2 Results

2.1 Phytochemical screening The ethyl acetate leaf extracts of A. purpurata were screened for its phytochemical constituents. The ethyl acetate leaf extracts of A. purpurata showed the presence of most of the secondary metabolites from the five solvents checked. See Table 1.

2.2 Antibacterial activity Antibacterial activity was checked with ethyl acetate leaf extract of A. purpurata against five bacteria. Of the five organisms, S. aureus and K. pneumonia showed 9 mm zone of inhibition at a very small concentration (5 mg/mL). This was followed by other organisms evaluated. See Table 2.

2.3 Anticancer activity against ovarian cancer cell line The cells were incubated for 48 h with A. purpurata at different concentrations. Cell viability results were compared with a known anticancer drug, cisplatin at same concentration and time. There is no statistical significance between plant extract and ovarian cancer drug cisplatin. Half maximal inhibitory concentration (IC₅₀) value for cisplatin and A. purpurata showed 52.32 and 110.25 µg/mL, respectively. See Figure 1.

3 Discussion

Herbal products prepared either from single or multiple botanical ingredients are usually complex and variable in nature. Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value that have yet to be discovered. For these reason, A. purpurata (Vieill.) K. Schum, a medicinal plant belonging to family Zingeberaceae, was selected for the present study.

<table>
<thead>
<tr>
<th>Extract</th>
<th>AL</th>
<th>SA</th>
<th>TP</th>
<th>FL</th>
<th>ST</th>
<th>CG</th>
<th>OF</th>
<th>TN</th>
<th>AP</th>
<th>CH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Chloroform</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ethanol</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Water</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*“+” means present; “−” means absent. AL: alkaloids; SA: sapogenin; TP: tannin and phenolic compounds; FL: flavonoids; ST: steroids; CG: cardiotaurosides; OF: oils and fats; TN: terpenoids; AP: amino acids and proteins; CH: carbohydrates.*

<table>
<thead>
<tr>
<th>Tested organism</th>
<th>Antibiotic (Novomycin)</th>
<th>Negative control (DMSO)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(2.5 mg/mL)</td>
<td>(5 mg/mL)</td>
</tr>
<tr>
<td>B. cereus (MTCC 441)</td>
<td>20 mm</td>
<td>7 mm</td>
</tr>
<tr>
<td>S. aureus (MTCC 96)</td>
<td>21 mm</td>
<td>9 mm</td>
</tr>
<tr>
<td>E. coli (ATCC 25922)</td>
<td>20 mm</td>
<td>5 mm</td>
</tr>
<tr>
<td>K. pneumonia (MTCC 530)</td>
<td>23 mm</td>
<td>9 mm</td>
</tr>
<tr>
<td>S. paratypophil (MTCC 734)</td>
<td>23 mm</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*Data are presented as mean measurement of inhibition zone (mm). DMSO: dimethyl sulfoxide; MTCC: microbial type culture collection; ATCC: American type culture collection; N/A: no activity or no zone of inhibition.*
Phytochemical screening helps to reveal the chemical nature of the constituents of the plant extract and the one that predominates over the others. It may also be used to search for bioactive agents that could be used in the synthesis of very useful drugs\cite{11,15}. The phytochemical screening showed the presence of maximum secondary metabolites in ethyl acetate extract of \textit{A. purpurata}, although phytochemical screening of rhizome of \textit{A. purpurata} suggested that most of the phytochemicals in the crude extract of rhizomes are eluted in ethanol extract like carbohydrate, tannins, resins, proteins, alkaloids, flavonoids glycosides, phenols and saponins\cite{16}.

Bacterial and fungal infections are widespread throughout the world. The situation is more critical especially in the third-world countries. In most cases, lack of adequate sanitation and primary healthcare programs makes it difficult and expensive to combat diseases. A number of higher plants have been used for centuries as remedies for human diseases. This has encouraged scientists to screen higher plants for various biological activities including antibacterial and antifungal effect\cite{17,18}. About 40\% of pharmaceuticals are derived from natural sources (plants, animals, bacteria and fungi). Moreover, several natural products obtained from medicinal plants lead to the development of various pharmaceuticals and analogues or derivatives. Recently, focus on plant research has increased and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems\cite{19}.

The result obtained in the present study revealed that \textit{A. purpurata} possesses potential antibacterial activity against all the five tested bacterial organisms (\textit{B. cereus}, \textit{S. aureus}, \textit{E. coli}, \textit{K. pneumonia} and \textit{S. paratyphi}). \textit{A. purpurata} showed a broad spectrum of activity against all the bacterial strains at the tested concentrations of 5 to 20 mg/mL disc. Novomycin was used as the control and DMSO was used as the negative control. It is reported that antibacterial activity was checked by preparing sample dissolving with DMSO and using DMSO as a negative control, that there was no formation of zone in the negative control\cite{20}. Except \textit{S. paratyphi} all the four remaining organisms showed a zone of inhibition at 5 µg per disc concentration. \textit{A. purpurata} showed antibacterial activity against \textit{S. paratyphi} at concentrations of 15 and 20 mg per disc. \textit{A. purpurata} exhibited greater zone of inhibition for \textit{S. aureus} and \textit{K. pneumonia} (9 mm) in a 5 mg/mL concentration. This was followed by \textit{B. cereus} and \textit{E. coli}. It was reported that \textit{A. purpurata} oil may be regarded as active against most of the bacterial strains tested with the exception of \textit{Proteus} species, which grew even at the highest concentration of oil assayed (1 000 g/mL). The lowest minimal inhibitory concentration (MIC) values were recorded for the gram-positive species, indeed some \textit{S. aureus}, oxacillin-resistant \textit{S. aureus} strains were highly susceptible to \textit{A. purpurata} oil with MIC values < 10 g/mL. In contrast, the MIC values for gram-negative species were typically around 1 000 g/mL\cite{21}. The antimicrobial activity of plant extracts has been screened because of their great medicinal relevance. In recent years, infections have increased to a great extent and resistance against antibiotics has become an ever increasing therapeutic problem.

It was reported that plant-derived extracts containing antioxidant principle showed cytotoxicity toward tumor cells\cite{22}. The \textit{in vitro} screening of the ethyl acetate extract of \textit{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. Cisplatin is a most effective and widely used chemotherapeutic agent against human cancers\cite{23}, including ovarian cancer. IC_{50} value for cisplatin and \textit{A. purpurata} showed 52.32 and 110.25 µg/mL, respectively.

The results obtained from the present study revealed that the ethyl acetate extract of \textit{A. purpurata} showed the presence of most of the secondary metabolites in the plant leaves. The plant possesses moderate antibacterial and anticancer activities, which may be due to the presence of secondary metabolites in the leaves of \textit{A. purpurata}. We hope that intensive study on the outcomes active constituents of \textit{A. purpurata} will lead to the discovery of a novel botanical drug for chemoprevention.

4 Acknowledgements

The authors are thankful to our Chancellor, Advisor, Vice Chancellor and Registrar of Karpagam University for providing facilities and encouragement.

5 Competing interests

The authors declare that they have no competing interests.

REFERENCES

红姜叶提取物所含化学成分的抗肿瘤及抗菌作用

Chinthamony Arul Raj¹, Dominic Sophia¹, Paramasivam Ragavendra¹, Thangarajan Starlin¹, Muthian Ahalliya Rathi², Velliyur Kanniappan Gopakrishnan¹,²

1. Department of Biochemistry, Karpagam University, Coimbatore 641021, Tamilnadu, India
2. Department of Bioinformatics, Karpagam University, Coimbatore 641021, Tamilnadu, India
3. Department of Biochemistry, Sree Narayana Guru College, Coimbatore 641105, Tamilnadu, India

目的：研究红姜叶提取物中的化学成分的抗肿瘤及抗菌活性。

方法：采用水蒸气充分洗净红姜叶，晾干后制成粉末，用连续溶解系统提取其化学成分。对植物化学成分进行评估，用纸片扩散法检测植物提取物的抗菌活性。利用四甲基偶氮唑盐比色法检测植物提取物的乙酸乙酯提取物的活性。

结果：红姜叶经乙酸乙酯提取后，能最大限度地得到有效的植物化学成分。不同浓度的红姜叶植物提取物抑制细胞的区域为5～14 mm，并且在48 h内，植物提取物的半数抑制浓度为110.25 μg/mL，表现出抗PAI卵巢癌细胞株活性，且细胞数量呈剂量依赖性递减。

结论：红姜叶的乙酸乙酯提取物中的化学成分可有效抑制细菌活性，并具有抗肿瘤的作用。

关键词：红姜叶；植物提取物；抗菌药；抗肿瘤药；休外研究