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In-Vitro Anticancer Investigation of Alpinia Galanga (Linn.)

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Abstract:
The present study aimed to determine the in vitro anticancer activity of Alpinia galanga. Phytochemical screening of aqueous extracts from the rhizomes of Alpinia galanga was conducted using standard procedures. The total phenolic and flavonoid contents were measured using Folin-Ciocalteu and aluminum chloride reagents, respectively. Various cytotoxic assays (MTT) were performed on the extracts using standard methods. Preliminary phytochemical screening of Alpinia galanga revealed the presence of flavonoids, phenols, terpenoids, carbohydrates, and proteins. The results indicated that rhizome of aqueous extract of Alpinia galanga showed 88.36% cell viability whereas Alpinia calcarata showed 73.59% cell viability.. The results of this study indicate that the rhizome of Alpinia galanga is rich in phenols and flavonoids, which may be useful for the development of anticancer drugs. Alpinia galanga demonstrated higher antioxidant and anticancer activity.

Keywords: Alpinia galangal, Anticancer activity, MTT assay, Cytotoxicity, Cell Viability

Introduction

India boasts a vast wealth of medicinal and aromatic plants due to its rich plant diversity. This diversity is bolstered by India's unique biogeographical position, which supports all known types of ecosystems. The country exhibits all three levels of biodiversity: species diversity, genetic diversity, and habitat diversity. The Indian subcontinent's varied topography and altitudinal rang from sea level to the highest mountain ranges, an extensive coastal line, desert regions in the west, and a cool desert in the east contribute to its versatile plant diversity [1-3].

Traditional Indian medical systems, such as Ayurveda, Siddha, and Unani, utilize about 2,000 of the 17,000 flowering plants present in India. Additionally, traditional village physicians use around 4,500 to 5,000 plant

species, while villagers' oral traditions employ about 5,000 plants for medicinal purposes. Over the last decade, tribal and other traditional communities in India have used more than 8,000 wild plant species to treat various health ailments. Given that 80% of the global population relies on traditional medicines for primary healthcare, the importance of these practices in treating infectious diseases is evident. In rural India, 65% of the population depends on traditional drugs for primary healthcare needs. Effective conservation of medicinal plants requires understanding the status of existing medicinal plant resources [4-6].

Alpinia galangal (L.) (Family: Zingiberaceae) is another rhizomatous plant widely distributed in tropical areas and used medicinally in many countries. The rhizomes are utilized to treat rheumatism, bronchitis, diabetes mellitus, and loss of appetite. Various bioactive compounds, including Diarabinoside, β-steroldiglucosyl caprate, Galangoflavonoside, and 1-Acetoxychavicol acetate, have been isolated from Alpinia galanga. Different parts of the plant also exhibit numerous medicinal properties: leaf extract shows antibacterial activity and wound healing properties, and the seeds possess antibacterial activity. The aim of the present investigation was to determine the antioxidant and anticancer activity of Alpinia galangal [7-10].

Materials and Methods

Collection and Authentication of plant Materials

Fresh whole plant was collected from the Manoj Nursery, Opposite T.V. Tower Dubagga, Hardoi Road, Lucknow, India in the month of December. The plant was identified and authenticated by *Dr. D.C. Saini,* Senior Scientist, Birbal Sahni Institute of Palaeobotany, Lucknow, India with the voucher specimen No.13316.

Processing of plant material and preparation of crude extract

The root of plant is first screened and then heated by the steam process at a temperature higher than 100°C to stop Lipase hydrolysis in Aerial part before the completion of extraction process.

Preliminary phytochemical screening

Petroleum ether, methanolic and aqueous extracts of *Alpinia galanga* aerial parts were subjected to qualitative tests for the identification of various active constituents viz. Alkaloids, Carbohydrates, Glycosides, Phenolic compounds, Flavonoids, Protein and free amino acids, Saponins, Steroids, fixed oil, tannins and Phenolic, Inorganic chemicals etc.

Anticancer Activity

a. Aqueous Extract Preparation

Fresh whole plants of Alpinia galangal were procured from a local market in Mashhad, Iran. These fresh plants were initially sliced and then subjected to drying in a tray-dryer oven set at 50°C for a duration of 24 hours. Once

dried, the plants were finely ground into a powder using a blender. Subsequently, various concentrations of the Alpinia galangal extract ranging from 125 µg/ml to 1000 µg/ml were prepared for further experimentation [11].

b. Morphological Examination

AGS and L929 cells were sourced from the National Cell Bank of Iran (NCBI). These cells were cultured in a 1:1 mixture of Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution. The cultures were maintained in a humidified atmosphere and fed 2-3 times per week until they reached confluence. Upon reaching confluence, cell aggregates were treated with a 0.25% trypsin-EDTA solution. Mechanical dispersion of the EDTA-treated cells was carried out using a 10ml pipette. To inhibit trypsin activity, growth medium was added, and the cells were centrifuged at 1000 rpm for 5 minutes. The supernatant was then removed, and the cell pellet was seeded into seven 50cm2 flasks. After 24 hours, both types of cells were exposed to different concentrations of the extract in the flasks. Cellular morphology, including shape, granulation, and suspension, was observed under a light inverted microscope over a period of 24 to 72 hours [12].

c. Cell viability assay

The effect of aqueous extract of Alpinia galangal on AGS and L929 cells proliferation was determined. All five Alpinia galangal extracts, at the concentration of 0–1000 µg/mL, dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich), were added to the wells, 24h after seeding of 5 ×103 cells per well of 96-well plate. DMSO was used as a solvent control. After 24 and 48h of incubation, 20µL of 5mg/mL MTT (Sigma Chemical Company) solution was added to each well, and the plates were wrapped with aluminum foil and incubated for 4h at 37°C. The purple formazan product was dissolved by addition of 100µL of 100% DMSO to each well. The absorbance was monitored at 570nm (measurement) and 630nm (reference) using a 96- well plate reader (Bio-Rad, Hercules, Calif, USA). Data were collected for each four replicates and used to calculate the means and the standard deviations. Optical density of AGS and L929 cells incubated in the presence and absence of the different concentrations of Alpinia galangal was compared together and the percentage of surviving cells was determined using the following formula:[11, 12]

Surviving cells (%) in compared to the controls = OD of cells in each well X 100/Mean OD of control cell

Result and Discussion

Preliminary Phytochemical Investigation:

Various extracts i.e. petroleum ether, methanolic and aqueous were subjected to preliminary phytochemical screening through qualitative chemical tests. (Table 1)

Table 1: Preliminary qualitative analyses (aerial parts) of *Alpinia galanga* extracts for the presence of various chemical constituents:

Test Name	**Aerial parts**		
	Petroleum ether	**Methanolic**	**Water**
For Carbohydrate Molish's test	+	+	+
Fehling's test	-	+	+
Benedict's test	-	+	+
Barfoed test	-	-	-
Bial's orcinol test	-	-	-
Selwinoff's test	-	+	+
Test for Protein Biuret test	-	-	-
Millon's test	-	-	-
Xanthoprotein test for tyrosine and tryptophan	-	-	-
Amino Acid Ninhydrine test	-	+	-
For tyrosine	-	-	-
For cysteine	-	-	-
For Fats And Oils Filter paper test	+	+	-
$CuSO_4$ + NaOH (ethanolic solution)	+	+	-
Test for Steroids Salkowski test	-	-	-
Liebermann's Burchard	+	+	+
Liebermann's	+	-	-
Test for Glycosides For Cardiac Glycoside Baljet's test	-	+	+
Legal's test	-	+	+
Keller's killani test	+	+	-
Test for Anthraquinone Glycoside	-	+	+

Borntrager's test			
Modified Borntrager's test	+	+	+
Test for Flavonoids Shinoda test	-	+	+
Lead acetate	+	+	-
NaOH Test	+	+	+
Alkaloids Test Dragendorff's	-	-	-
Mayer's	-	+	-
Hager's	+	+	+
Wagner's	-	+	-
Murexide test for purine	-	-	-
Tannins and phenolic compound 5% $FeCl_3$	+	+	-
Lead acetate	-	+	-
Gelatin	-	-	-
Acetic acid	-	-	-
Potassium dichromate	-	-	-
Dil. Iodine	-	+	-
Dil. HNO_3	+	-	+
Dil. NH4OH and potassium ferricyanide	-	+	+
Silver mirror test	+	+	+
Dil. KMnO4	+	+	+

Where ' + ' = Present, ' - ' = absent.

The study revealed the presence of flavonoids, alkaloids, carbohydrates, tannins and glycosides fixed oil and fats as reported in table 6.5. (Above)

Anticancer Activity

Morphological Assay

After 72h, AGS and also L929 cells with low concentrations (125 and 250 µg/ml) of extract in sense of morphology were very similar to the control. Morphological effects of the extract on both cells started at concentration of 500µg/ml. A complete response from the extract was seen on both cells at concentrations 750 and 1000 µg/ml, so that cells appeared as round and suspended with increase granulation of cytoplasm when compared to the control. The granulation of cytoplasm also was seen on times 24 and 48 h after addition of extract. However, this effect was lower than 72h (data not shown).

MTT Assay

a. After 24hrs

After 24 hours, the percentage of living AGS cells showed no significant decrease compared to control at concentrations of 125 and 250 µg/ml. However, significant decreases were observed at the remaining concentrations ($p < 0.05$). Similarly, the percentage of surviving L929 cells at the extract concentration of 125 µg/ml was not significant, but it was significant at the other concentrations. Additionally, there was a direct correlation between increasing extract concentration and decreasing survival of AGS and L929 cells. The maximum decrease in the percentage of surviving cells for both cell types was observed at the concentration of 1000 µg/ml (Figure 1 and Table 2).

b. After 48hrs

As depicted in Figure 2 and table 3, there was a significant decrease in the percentage of living AGS and L929 cells at the last three concentrations of the extract compared to control cells ($p < 0.05$). Moreover, a clear correlation was observed between the increase in extract concentration and the survival of AGS and L929 cells. The maximum decrease in surviving AGS cells was observed at 250 µg/ml, while for L929 cells, it was at 500 µg/ml.

c. After 72hrs

These changes became more pronounced on the third day, with a significant decrease observed in the percentage of surviving AGS and L929 cells at concentrations of 500, 750, and 1000 µg/ml of the extract compared to control cells ($p < 0.05$). The most substantial decrease in surviving AGS cells occurred at 500 µg/ml, while for L929 cells, it was at 1000 µg/ml. Additionally, there was a clear correlation between the increase in extract concentration and the decrease in surviving AGS and L929 cells. The effect of the Alpinia galangal extract on the percentage of surviving AGS and L929 cells after 72 hours is depicted in Figure 3 and Table 4.

Table 2: The table showing the Concentration of Aqueous extract on viability of ASG and L929 Cell after 48 hrs

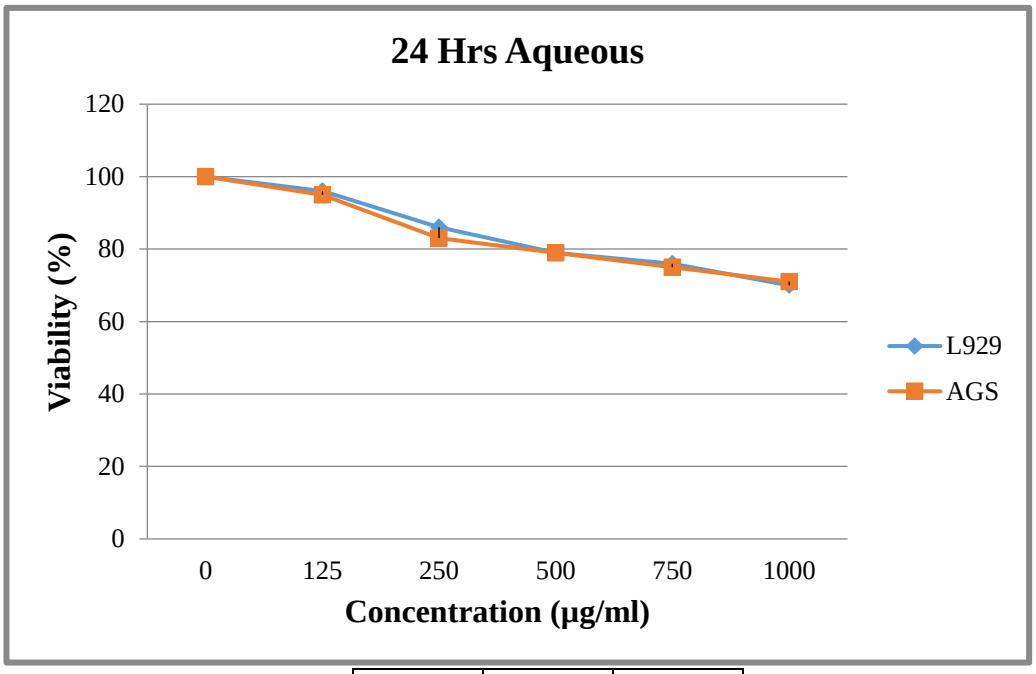

Figure 1: The effect of aqueous extract of Alpinia galangal (concentrations of 0 (control), 125, 250, 500, 750 and 1000 µg/ml) on viability of AGS and L929 cells after 24 hours

Table 3: The table showing the viability of ASG and L929 Cell after Concentration of Aqueous extract on 48 hrs.

Conc.	L929	AGS
0	100	100
125	96	95
250	86	83
500	79	79
750	76	75
1000	70	71

Conc.	L929	AGS
0	100	100
125	95	80
250	93	74
500	66	65
750	62	62
1000	60	60

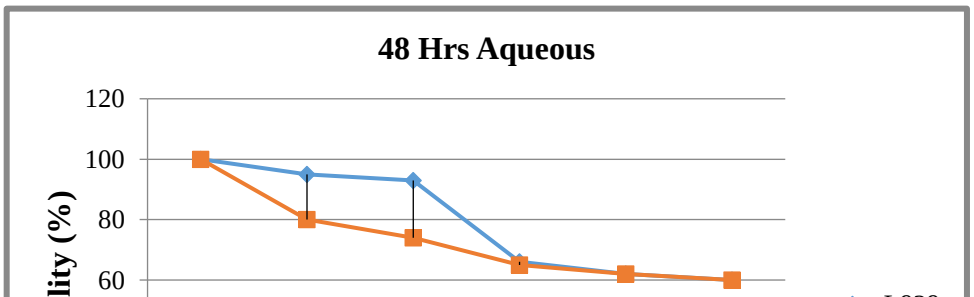

Figure 2: Effect of aqueous extract of Alpinia galangal (concentrations of 0 (control), 125, 250, 500, 750 and 1000 µg/ml) on viability of AGS and L929 cells after 48 hours.

Table 4: The table showing the Concentration of Aqueous extract on viability of ASG and L929 Cell after 72 hrs

Conc.	L929	AGS
0	100	100
125	80	95
250	78	82
500	75	60
750	62	58
1000	60	56

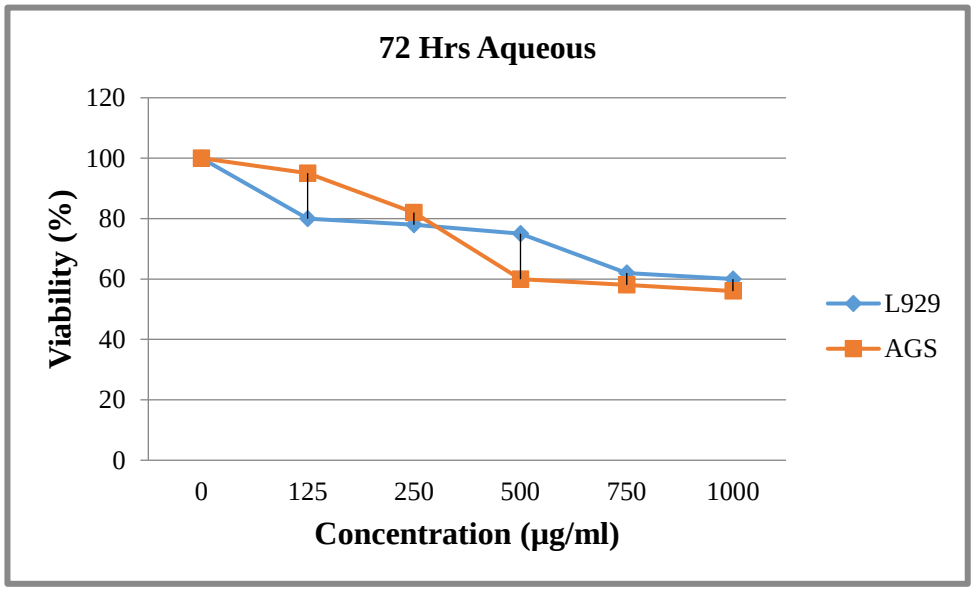

Figure 3: Effect of aqueous extract of Alpinia galangal (concentrations of 0 (control), 125, 250, 500, 750 and 1000 µg/ml) on viability of AGS and L929 cells after 72 hours.

Conclusion

Phytochemical screening of aqueous extracts Alpinia calcarata and Alpinia galanga rhizomes had revealed the presence of flavonoids, terpenoids, phenols, carbohydrates and proteins. The results of the present investigation indicate that Alpinia galanga rhizome extract exhibited the highest antioxidant activity in all the assays than Alpinia calcarata. The effect of aqueous extract of HepG2 cells are expressed as % cell viability. The results indicated that rhizome of aqueous extract of Alpinia galanga showed 88.36% cell viability whereas Alpinia calcarata showed 73.59% cell viability. Further investigation to be done for the identification of bioactive compounds from Alpinia galanga for the development of the anticancer drug.

References

1. Rabita, N., & Palanisamy, K. (2023). Comparative Phytochemical Analysis of Two Alpinia Species From Zingeberaceae Family. *bioRxiv*, 2023-10.
2. Devi, N. N., Ragunathan, R., & Baskaran, K. (2024). Phytochemical Profiling Using GCMS And Antioxidant Activity of Alpinia Purpurata Flower Extract. *Journal of Advanced Zoology*, *45*(3).
3. Afifi, H.M. *A phytochemical and biological study of major constituents in Alpinia officinarum Rhizomes* (Doctoral dissertation, King Abdulaziz University).
4. Niazvand, F., Ashtari, A., Chamkouri, N., & Azari, M. (2023). Hepatoprotective effects of Alpinia officinarum rhizome extract on cisplatin-induced hepatotoxicity in rats: A biochemical, histopathological and immunohistochemical study. *Journal of Trace Elements in Medicine and Biology*, *80*, 127306.
5. Johnson, J. B., Batley, R. J., Mani, J. S., du Preez, R., Trotter, T., Netzel, M. E., & Naiker, M. (2024). Phytochemical composition and biological activity of native Australian ginger (Alpinia caerulea). *Journal of Food Measurement and Characterization*, 1-13.
6. Adnan, Q., & Akhtar, N. (2023). Profiling of phytochemicals using LC-ESI-MS 2, in vitro, in vivo characterization and cosmeceutical effects of Alpinia galanga (wild) extract loaded emulgel. *Journal of Cosmetic Dermatology*, *22*(5).
7. Sharma, D., Jeet, K., & Kumar, S. (2024). A review of the pharmacological effects of Alpinia galanga Linn. and it's phytoconstituents. *Research Journal of Pharmacognosy and Phytochemistry*, *16*(1), 31-36.
8. Ahmad, A., Sakhawat Riaz, R. Farooq, M. Ahmed, and N. Hussain. "Alpinia officinarum (galangal): A beneficial plant." *J. Med. Public Health* 4 (2023): 1057.
9. Divya, S., Miniraj, N., Suma, A., Aneesha, A. K., & Gleena Mary, C. F. (2023). Flowering phenology in greater galangal (Alpinia galanga (L.) Wild. genotypes.
10. Liu, P., Wu, S. L., Wang, T., Zhang, X. M., & Geng, C. A. (2023). Four new phenolic compounds from the fruits of Alpinia galanga. *Phytochemistry Letters*, *55*, 75-79.

11. Wen, H., Kuang, Y., Lian, X., Li, H., Zhou, M., Tan, Y., ... & Xu, J. (2024). Physicochemical Characterization, Antioxidant and Anticancer Activity Evaluation of an Acidic Polysaccharide from Alpinia officinarum Hance. *Molecules*, *29*(8), 1810.
12. SP, N. K., & Maya, C. Anti-cancerous Activity of the Rhizome Extract of Alpinia galanga L. Willd. on the Human Liver Carcinoma Cells.