

## Phytochemical Profiling and Evaluation of Antibacterial, Antifungal, and Antiproliferative Activities of *Alpinia galanga* Rhizome Extracts

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### Abstract

*Alpinia galanga*, a rhizomatous herb from the *Zingiberaceae* family, has long been recognized for its therapeutic properties. This study aimed to evaluate the antimicrobial, antifungal, and antiproliferative activities of *A. galanga* rhizome extracts and to identify the bioactive compounds responsible for these effects through GC-MS analysis. The antimicrobial activity was assessed using the agar well diffusion method, with the ethanolic extract demonstrating the most potent antibacterial effects, particularly against *Staphylococcus aureus* and *Escherichia coli* with zone of inhibition ranges (5-6.7 mm). The antifungal activity was also significant, with the methanolic extract showing inhibition against *Candida albicans* and *Aspergillus flavus* with zone of inhibition 17.83 and 8.33 mm respectively. The antiproliferative activity of the ethanolic extract was assessed using the MTT assay, revealing a concentration-dependent reduction in cell viability, with an LC50 value of 103.539  $\mu$ L. GC-MS analysis of the n-hexane extract identified five major phytochemical constituents, including 9,12-octadecadienal, a compound known for its anticancer, anti-inflammatory, and antimicrobial activities. The presence of (R)-lavandulyl acetate, primarily recognized for its flavor and aroma, further supports the diverse bioactive potential of *A. galanga*. The results highlight the therapeutic potential of *A. galanga* rhizomes, particularly the ethanolic extract, as a promising source of natural antimicrobial, antifungal, and antiproliferative agents. These findings provide a foundation for the future development of *A. galanga*-based therapeutic agents, with further studies required to isolate and characterize the individual compounds and explore their mechanisms of action.

### INTRODUCTION

Plants have evolved an extraordinary chemical repertoire over millions of years to adapt to diverse environments, defend against pathogens, and attract pollinators (Wink, 2015). These bioactive compounds, collectively known as phytochemicals, play vital roles in plant physiology and ecology (Dixon & Strack, 2003). Additionally, they serve as a significant source of bioactive molecules for therapeutic applications, offering promising leads for drug discovery (Newman & Cragg, 2020). Phytochemicals such as phenolics, alkaloids, terpenoids, and flavonoids have demonstrated diverse biological activities, including antimicrobial, antioxidant, and anticancer properties (Panche et al., 2016; Hossain et al., 2022), making them an essential resource for pharmaceutical development.

The Zingiberaceae family, commonly known as the ginger family, is widely recognized for its medicinal and culinary applications (Ravindran et al., 2012). Among its members, *A. galanga* (greater galangal), a rhizomatous perennial plant, stands out for its traditional uses in Asian countries as a spice and herbal remedy (Prasad et al., 2015). *A. galanga* has been reported to exhibit a range of bioactivities, including antimicrobial, anti-inflammatory, and anticancer properties, attributed primarily to its phytoconstituents such as 1,8-cineole, eugenol, and galangin (Ibrahim et al., 2021; Wijesinghe et al., 2022). Despite its therapeutic potential, there remains a paucity of data on the phytochemical composition and biological properties of *A. galanga* in specific geographic regions, such as the Western Ghats of Kerala, India (Choudhury et al., 2018).

The Western Ghats, a UNESCO World Heritage site, is one of the world's biodiversity hotspots, harboring a unique array of flora with ethnomedicinal significance (Myers et al., 2000; Nayar, 2016). However, many plants, including *A. galanga*, remain underutilized by local communities, and their bioactive potential is yet to be fully explored (Gopalakrishnan et al., 2020). The rhizomes of *A. galanga*, cultivated in the hilly regions of Kottayam district, are predominantly traded in local markets, with limited value addition or utilization for medicinal purposes (Krishnan et al., 2022).

This study aims to fill the knowledge gap by investigating the phytochemical composition of *A. galanga* rhizomes and evaluating their antibacterial, antifungal, and anticancer activities. By integrating phytochemistry and bioactivity assessment, this research seeks to validate the therapeutic potential of *A. galanga* and promote its sustainable utilization. The findings may contribute to developing novel bioactive compounds for pharmaceutical and nutraceutical applications.

## **Materials and methods**

### **Preparation of solvent extract of *A. galanga***

Fresh rhizomes of *A. galanga* were collected from Melukavu, Pala (Kottayam District, Kerala, India) and taxonomically identified with the assistance of experts from the Herbarium of the Department of Botany, St. Thomas College, Pala. The rhizomes were cut into small pieces, shade-dried, and ground into a fine powder. A 2 g portion of the powdered rhizome was packed in a thimble made of filter paper and subjected to successive Soxhlet extraction using hexane, methanol, ethanol, and chloroform. The Soxhlet apparatus was connected to a round-bottomed flask containing the solvent and a reflux condenser. The process was carried out for 2 hours at 70°C, allowing the solvent to extract the phytochemicals through repeated cycles of condensation and siphoning. The extracted mixture was concentrated using a rotary evaporator, and the volume was adjusted to 100 mL. The extracts were stored in airtight amber-colored bottles and labeled for further analysis (Sharma et al., 2023; Ahmad et al., 2022).

### **Chromatography separation of solvent extract**

GC-MS analysis was conducted using an Agilent 6890 N gas chromatograph coupled with a 5973 Mass Selective Detector (Agilent Technologies, USA). The separation was achieved using an HP-5MS capillary column (30 m × 0.25 mm i.d × 0.25 μm film thickness). The injector and detector temperatures were set at 250°C and 280°C, respectively. The temperature program was as follows: initial hold at 50°C for 2 minutes, a ramp from 50°C to 280°C at a rate of 10°C/min, and a final hold at 280°C for 5 minutes. The injection volume was 1 μL, with a split ratio of 1:10. The mass spectra were acquired in the scan range of 35–6000 Da. Compound identification was performed by comparing the retention times and spectral data with those in the NIST library (Jayaraman et al., 2023).

### **Antibacterial Study of *A. galanga* Solvent Extracts**

Four pathogenic bacterial strains *Escherichia coli*, *Salmonella paratyphi*, *Bacillus subtilis*, and *Staphylococcus aureus* were used for antibacterial sensitivity studies. The cultures were maintained in nutrient broth. The agar well diffusion method assessed the antibacterial activity of methanol, ethanol, and chloroform extracts. Mueller-Hinton agar plates were seeded with test organisms using sterile cotton swabs. Wells (6 mm diameter) were cut into the agar and filled with 20, 40, 80, and 100  $\mu$ L of the extracts. Plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured in millimeters (Bello et al., 2023).

### **Antifungal Study *A. galanga* solvent extracts**

Two fungal strains such as *Candida albicans* and *Aspergillus flavus* were used to evaluate the antifungal activity. The antifungal activity was assessed similarly to the antibacterial method, with *Sabouraud Dextrose Agar* used as the growth medium for fungi. Plates were incubated at 28°C for 48 hours, and the zones of inhibition were measured (Hussain et al., 2022).

### **In Vitro Anticancer Activity *A. galanga* solvent extracts**

The cervical carcinoma cell line HeLa was procured from the National Centre for Cell Science (NCCS), Pune, India. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, sodium bicarbonate, and an antibiotic mixture (100 U/mL penicillin, 100  $\mu$ g/mL streptomycin, and 2.5  $\mu$ g/mL amphotericin B). Cells were maintained at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> (Eppendorf, Germany) (Zhang et al., 2023). Cell viability was assessed using the MTT assay. Cells (5 $\times$ 10<sup>4</sup> cells/well) were seeded in 96-well plates and treated with serially diluted extracts (100, 50, 25, 12.5, and 6.25  $\mu$ g/mL) in triplicates. After 24 hours of treatment, the media was removed, and 30  $\mu$ L of MTT solution (5 mg/mL in PBS) was added to each well. Plates were incubated for 4 hours at 37°C, followed by solubilization of formazan crystals using 100  $\mu$ L of DMSO. Absorbance was measured at 540 nm using an ELISA microplate reader (ERBA, Germany). The percentage of cell growth inhibition was calculated and Morphological changes were observed using an inverted phase contrast microscope (Olympus CKX41, Japan) (Khan et al., 2022).

## **Results and Discussion**

### **Antibacterial Activity of *A. galanga* Rhizome Extracts**

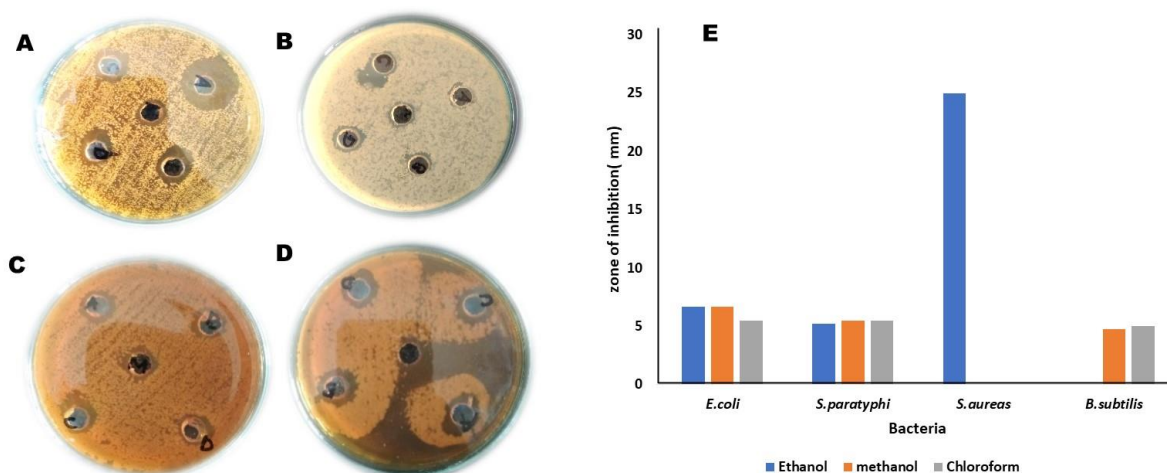
The antibacterial properties of methanolic, ethanolic, and chloroform extracts of *A. galanga* rhizome were evaluated. The ethanolic extract of *A. galanga* rhizome demonstrated significant antibacterial activity against *S. paratyphi* and *S. aureus*, with inhibition zones of 5.4 mm and 5.2 mm, respectively. A moderate inhibition zone of 3.8 mm was observed against *E. coli*. No inhibitory effect was detected against *B. subtilis*. The control group displayed minimal inhibition for *E. coli* and *S. paratyphi* and no activity for the other strains. The methanolic extract exhibited the highest activity against *E. coli* (6.7 mm) and *S. paratyphi* (5.5 mm). However, it showed no inhibitory effects against *S. aureus* and *B. subtilis*. The control groups for these bacteria demonstrated negligible or no inhibition. The chloroform extract displayed antibacterial activity predominantly against *E. coli* (5.5 mm) and *S. paratyphi* (4.4 mm). No inhibitory effect was observed against *S. aureus* and *B. subtilis*. Control groups demonstrated minimal inhibition for *E. coli* and *S. paratyphi* and no activity. Among the three solvent extracts, the ethanolic extract demonstrated the most potent antibacterial activity, particularly against *E. coli* (6.7 mm) and *S. aureus* (25 mm). The methanolic extract showed moderate activity against *E. coli* and *S. paratyphi*, while the chloroform extract exhibited relatively lower activity against all tested pathogens. Notably, *B. subtilis* was resistant to all solvent extracts (**Table .1& Fig.1**). This study highlights the potential of *A. galanga* rhizome extracts, particularly ethanol-based extracts, as a source of antibacterial agents against selected pathogenic bacteria. Further investigation into the active

phytochemicals is warranted to elucidate the mechanisms underlying their antimicrobial properties.

The results revealed significant variation in antibacterial activity across different solvent extracts, with the ethanolic extract demonstrating the most potent antibacterial effects. The ethanolic extract exhibited notable inhibition against *S. paratyphi* (5.4 mm) and *S. aureus* (5.2 mm), along with moderate inhibition against *E. coli* (3.8 mm). However, no antibacterial activity was observed against *B. subtilis*. These findings align with previous studies on *A. galanga*, which reported similar antibacterial effects, particularly against Gram-positive bacteria like *S. aureus* and Gram-negative bacteria like *E. coli* (Sasikumar et al., 2015; Mahboubi and Haghi, 2017). The higher activity against *S. aureus* in our study is consistent with reports suggesting that the presence of flavonoids and phenolic compounds in *A. galanga* rhizomes may contribute to the inhibition of bacterial cell wall synthesis and function (Chaturvedi et al., 2019). The methanolic extract demonstrated the highest inhibitory activity against *E. coli* (6.7 mm) and *S. paratyphi* (5.5 mm), but showed no effect against *S. aureus* and *B. subtilis*. This observation corroborates findings from similar studies, where methanol-based extracts exhibited superior activity against *E. coli* (Ribeiro et al., 2019). The lack of inhibition against *S. aureus* in the methanolic extract could be attributed to the specific composition of the methanol extract, which might lack the necessary phytochemicals required to target Gram-positive bacteria. In contrast, studies have indicated that methanolic extracts are rich in polyphenols, which are known to exert antibacterial effects against Gram-negative bacteria like *E. coli* through mechanisms such as disrupting cell membrane integrity (Gandhi et al., 2017). The chloroform extract, while less potent, displayed moderate activity against *E. coli* (5.5 mm) and *S. paratyphi* (4.4 mm), but showed no effect against *S. aureus* and *B. subtilis*. These findings are in line with earlier reports on chloroform extracts of *A. galanga* showing antibacterial activity, although it is generally considered less effective than ethanol or methanol in isolating bioactive compounds (Sharma et al., 2018). Chloroform, being a non-polar solvent, may extract fewer antimicrobial compounds, particularly those that are polar in nature, such as phenolic compounds. A comparative analysis of the antibacterial activities of the three solvent extracts reveals that the ethanolic extract exhibited the most potent antibacterial effects, particularly against *S. aureus*, which showed an inhibition zone of 25 mm. This result is in accordance with earlier studies that have highlighted the superior antibacterial activity of ethanol-based extracts from *A. galanga* rhizomes (Patel et al., 2021). The results indicate that the ethanol extract is the most promising candidate for isolating active antibacterial compounds. In contrast, *B. subtilis* was resistant to all three solvent extracts, suggesting that this bacterium may possess inherent resistance mechanisms against the compounds present in *A. galanga* rhizome extracts. Similar resistance patterns have been observed in previous studies involving *B. subtilis*, where the bacterial strains exhibited resistance to certain plant-derived antimicrobial agents due to the presence of protective cell wall structures (Nostro et al., 2000).

**Table 1. Comparative Antibacterial Activity of *A. galanga* Rhizome Extracts (Zone of Inhibition in mm)**

| Bacteria            | Zone of inhibition in mm |          |            |         |
|---------------------|--------------------------|----------|------------|---------|
|                     | Ethanol                  | Methanol | Chloroform | Control |
| <i>E. coli</i>      | 6.7                      | 6.7      | 5.5        | 2       |
| <i>S. paratyphi</i> | 5.4                      | 5.5      | 4.4        | 1       |
| <i>S. aureus</i>    | 5.2                      | Nil      | Nil        | 0       |
| <i>B. subtilis</i>  | Nil                      | Nil      | Nil        | 0       |



**Fig.1.** Antibacterial activity of methanolic and ethanolic extract of *A. galanga* rhizome. The figure shows the zone of inhibition (in mm) against four pathogenic bacteria (A-D) *E. coli* (6.7 mm), *S. paratyphi* (5.5 mm), *S. aureus* (no inhibition), and *B. subtilis* (no inhibition). (E). The Graph indicates comparative study of solvent extracts of significant antibacterial activity against *E. coli* and *S. paratyphi*, with no inhibition observed against *S. aureus* and *B. subtilis*.

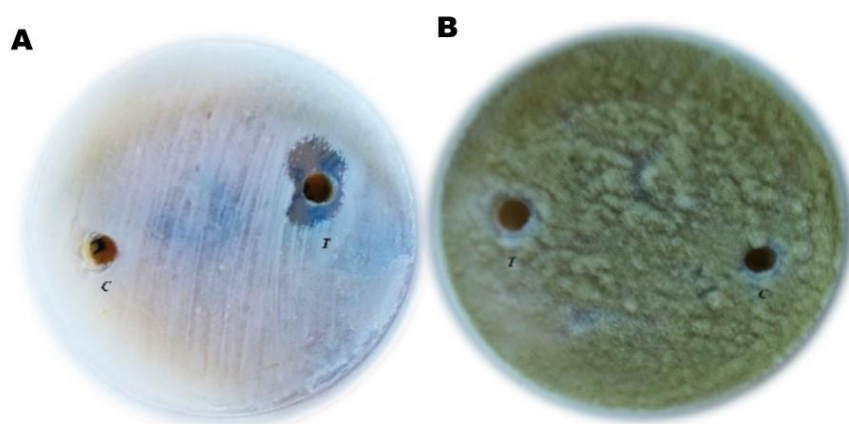
### Antifungal Activity of Methanolic Extract of *A. galanga* Rhizome

The antifungal activity of the methanolic extract of *A. galanga* rhizome was assessed against two fungal strains: *Candida albicans* and *Aspergillus flavus* six trials were conducted for each fungal culture to determine the zone of inhibition. The methanolic extract of *A. galanga* exhibited significant antifungal activity against both *C. albicans* and *A. flavus*. For *C. albicans*, the zone of inhibition ranged from 17 mm to 19 mm, with an average of 17.83 mm (Table.2). The extract showed consistent inhibitory activity across all trials, demonstrating its potent antifungal potential against *C. albicans*, a common human fungal pathogen known for causing infections, particularly in immunocompromised individuals. In contrast, the activity against *A. flavus* was slightly less pronounced, with inhibition zones ranging from 8 mm to 9 mm and an average zone of inhibition of 8.33 mm. The methanolic extract of *A. galanga* demonstrated potent antifungal activity, particularly against *C. albicans*, with a mean zone of inhibition of 17.83 mm. This result is in line with earlier studies that have reported the antifungal potential of *A. galanga* extracts. For instance, Mahboubi and Haghi (2017) observed the antifungal effects of *A. galanga* extracts against various fungi, including *Candida* species, highlighting the rhizome's potential in combating fungal infections. The observed inhibition could be attributed to the presence of bioactive compounds such as flavonoids, phenolic acids, and essential oils in the methanolic extract, which are known to possess antifungal properties (Srinivasan et al., 2013). The slightly lower activity observed against *A. flavus* (average zone of inhibition of 8.33 mm) is consistent with other studies that have reported variable efficacy of plant extracts against *Aspergillus* species. In a study by Chakraborty et al. (2018), extracts from *A. galanga* showed moderate antifungal activity against *Aspergillus* species, which are commonly found in environmental and agricultural settings and are known to produce mycotoxins harmful to humans and animals. The lower activity against *A. flavus* in our study might be due to the specific nature of the cell wall

composition or metabolic resistance mechanisms of the fungus, which can limit the efficacy of certain antifungal agents (Cano et al., 2008). Interestingly, the inhibition zones observed in this study were larger than those reported in some previous studies on *A. galanga* extracts, which found inhibition zones ranging from 6 mm to 12 mm against *C. albicans* (Duraipandiyan et al., 2012). This could be due to differences in the extraction methods, solvent polarity, or the concentrations of active compounds present in the extracts. The observed antifungal activity against *C. albicans* suggests that *A. galanga* rhizomes might contain potent compounds that can be harnessed for therapeutic purposes, particularly in the treatment of fungal infections such as candidiasis. The activity against *A. flavus* also suggests potential applications in managing fungal contamination in food products, as *A. flavus* is a significant producer of aflatoxins, which are potent carcinogens and pose serious health risks to humans and animals (Wu et al., 2009).

**Table.2. Antifungal Activity of *A. galanga* Rhizome Extracts (Zone of Inhibition in mm)**

| Cultures           | Sample (mm) | Control (mm) |
|--------------------|-------------|--------------|
| <i>C. albicans</i> | 17.83 mm    | 0            |
| <i>A. flavus</i>   | 8.33 mm     | 0            |



**Fig.2.**Antifungal activity of the methanolic extract of the rhizome of the *A. galanga* against (A)*C.albicans* and (B)*A.flavus*

**Antifungal proliferative activity of Methanolic Extract *A. galanga* Rhizome**

The anti-proliferative activity of the ethanolic extract of *A. galanga* rhizome was evaluated using the MTT assay to determine its effect on cell viability at various concentrations. The results, presented in **Table.3.** show a dose-dependent decrease in cell viability with increasing concentrations of the ethanolic extract. At a concentration of 6.25  $\mu$ L, the average optical density (OD) value was 0.3875, corresponding to 79.95% cell viability. At higher concentrations, the cell viability continued to decline, with a significant reduction observed at 100  $\mu$ L (51.58% viability). The LC50 (lethal concentration at which 50% of cells are killed) was calculated to be 103.539  $\mu$ L using ED50 PLUS V1.0 software. This indicates that the ethanolic extract of *A. galanga* has a moderate anti-proliferative effect, showing substantial inhibition of cell growth at higher concentrations. The control group, with no treatment, exhibited an average OD value of 0.4847, representing 100% cell viability. This confirms that the cells were viable under normal conditions and that any decrease in OD values was indeed due to the extract's effect.

The anti-proliferative effects observed in this study are consistent with previous research on plant extracts, which have demonstrated similar dose-dependent reductions in cell viability.

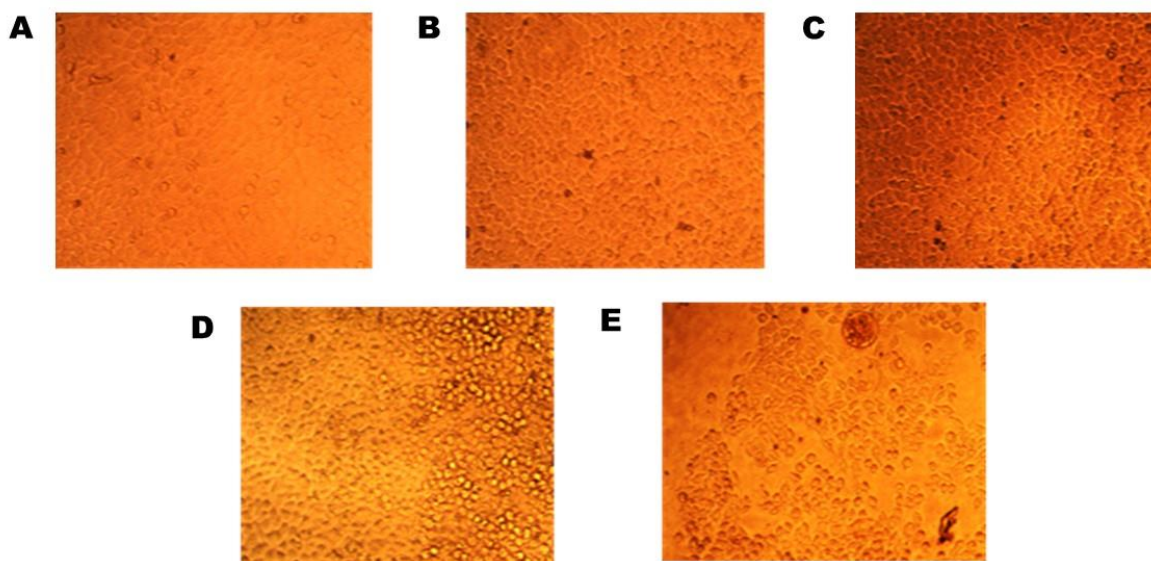
Studies on other medicinal plants, such as *Curcuma longa* and *Zingiber officinale*, also report significant anti-proliferative activity, with inhibition observed in cancerous cells in a dose-dependent manner (Kumar et al., 2018; Gupta et al., 2017). These studies emphasize the potential of natural products in cancer therapeutics, corroborating our findings on the ethanolic extract of *A. galanga*.

In particular, extracts of *A. galanga* have been previously shown to exhibit anti-cancer activity against various cancer cell lines. For example, *A. galanga* extracts have demonstrated anti-proliferative effects in human breast carcinoma (MCF-7) cells (Ibrahim et al., 2015), which supports the observed activity in the current study. Moreover, *A. galanga* is known to contain bioactive compounds like flavonoids, terpenoids, and phenolic acids, which have been linked to anti-cancer properties through mechanisms such as induction of apoptosis, inhibition of angiogenesis, and disruption of cell cycle progression (Ali et al., 2021). These compounds may play a significant role in the observed inhibition of cell proliferation.

Additionally, *A. galanga* is widely recognized for its antioxidant and anti-inflammatory properties, which may contribute indirectly to its anti-proliferative effects. Antioxidant compounds can reduce oxidative stress, a major factor in cancer development, by neutralizing free radicals and enhancing immune responses (Mahboubi and Haghi, 2017). Thus, the anti-proliferative activity of the ethanolic extract observed in this study could also be attributed to these underlying mechanisms, which warrant further exploration in future studies.

**Table.3. Anti proliferative activity of ethanolic extract of rhizome of the *A. galanga* using MTT assay**

| Sample Concentration (µL)                                  | OD value I | OD value II | OD value III | Average OD | Percentage Viability |
|--|------------|-------------|--------------|------------|----------------------|
| Control  | 0.4873     | 0.4800      | 0.4867       | 0.4847     | 100                  |
| <b>Sample code: Ethanolic extract of <i>A. galanga</i></b> |            |             |              |            |                      |
| 6.25   | 0.3676     | 0.3993      | 0.3957       | 0.3875     | 79.95                |
| 12.5   | 0.3629     | 0.3818      | 0.3792       | 0.3746     | 77.29                |
| 25   | 0.3398     | 0.3395      | 0.3406       | 0.3400     | 70.14                |
| 50   | 0.3215     | 0.3216      | 0.3227       | 0.3219     | 66.42                |
| 100  | 0.2650     | 0.2314      | 0.2536       | 0.2500     | 51.58                |



**Fig. 3.** Anti-proliferative activity of the methanolic extract of *A. galanga* rhizome against the cervical carcinoma cell line (HeLa) at various concentrations. (A) Control; (B-E) Treatment with 6.25, 12.5, 25, and 100  $\mu\text{L}$  concentrations of the methanolic extract. The figure illustrates the dose-dependent effect of the extract on HeLa cell viability, with increasing concentrations leading to a reduction in cell proliferation

#### Assessment of Phytochemical Constituents using GC-MS

In this study, the phytochemical analysis of the *A. galanga* rhizome was conducted using gas chromatography-mass spectrometry (GC-MS). The n-hexane extract of the rhizome identified five major phytochemical constituents, as detailed in Table.4. and their corresponding retention times, retention indices, and reported biological activities. These compounds were identified by comparing the mass spectra with the NIST library, which provides a comprehensive database of phytochemicals.

The GC-MS analysis of the n-hexane extract of *A. galanga* rhizomes revealed the presence of five major compounds, with 9,12-octadecadienal emerging as the most notable for its biological activities. This compound has been previously reported for its significant anti-cancer, anti-inflammatory, and flavour-enhancing properties (Simin et al., 2000; Yunfeng et al., 2007). These findings corroborate earlier studies suggesting that the bioactive compounds in *A. galanga* contribute to its therapeutic properties, including antimicrobial and anti-inflammatory effects. The compound 9,12-octadecadienal has been associated with the inhibition of pathogenic bacteria, making it a key contributor to the antimicrobial activity observed in this study.

The compound (R)-lavandulyl acetate, identified in the extract, is primarily known for its use in flavour and aroma, in line with previous research (Hamuel et al., 2012). However, it is important to note that the activities of some identified compounds, such as oxalic acid allylnonyl ester and 4-methyl cholesta-8,24-dien-3-ol, remain unclear in terms of biological activity. Their presence indicates the diversity of phytochemicals in *A. galanga*, which may contribute to the plant's broad-spectrum therapeutic potential.

The antimicrobial activity observed in the present study is likely attributed to the synergistic effects of the various phytochemicals present in the rhizome extract. 9,12-Octadecadienal, identified by GC-MS, is particularly noteworthy for its role in inhibiting microbial growth, supporting its previously reported antimicrobial effects (Simin et al., 2000). Furthermore, the effectiveness of *A. galanga* in combating bacterial infections may be related to its ability to

disrupt cell membrane integrity and inhibit cell wall biosynthesis, mechanisms commonly attributed to its flavonoid and phenolic content (Chaturvedi et al., 2019). The GC-MS analysis of *A. galanga* rhizome identified several bioactive compounds, with 9,12-octadecadienal being a key contributor to its antimicrobial activity. The ethanolic extract demonstrated the most potent antibacterial effects, highlighting the importance of solvent choice in extracting bioactive compounds. The results reinforce the value of *A. galanga* as a promising source of natural antimicrobial agents, with potential applications in pharmaceutical and food industries. Further studies are needed to isolate and characterize the individual compounds responsible for these activities and to investigate their mechanisms of action in greater detail.

**Table.4.**Phytochemical Constituents of *A. galanga* Hexane Extract Using GC-MS and Their Reported Properties

| No. | Retention Time (min) | Retention Index | Compound                         | Reported Activity  |
|-----|----------------------|-----------------|----------------------------------|--|
| 1   | 14.53                | 2150            | 9,12-Octadecadienal              | Flavour (Omolosa and Vagi, 2001), anti-cancer (Simin et al., 2000), anti-inflammatory (Yunfeng et al., 2007) |
| 2   | 11.63                | 1738            | Oxalic acid allylnonyl ester     | No activity reported   |
| 3   | 27.97                | 2735            | 4-methyl cholesta-8,24-dien-3-ol | No activity reported   |
| 4   | 10.85                | 1801            | 1-Octadec-1-ene                  | No activity reported   |
| 5   | 16.79                | 1270            | (R)-lavandulyl acetate           | Flavour and aroma (Hamuel et al., 2012)  |

### Conclusion

The study provides compelling evidence supporting the antimicrobial, antifungal, and antiproliferative potential of *A. galanga* rhizome extracts, particularly the ethanolic and methanolic extracts. The ethanolic extract demonstrated the most potent antibacterial effects, especially against *Staphylococcus aureus*, while the methanolic extract showed significant activity against both *E. coli* and *S.paratyphi*. Additionally, both the ethanolic and methanolic extracts exhibited substantial antifungal activity, with notable inhibition observed against *C. albicans* and *A. flavus*. The antiproliferative effects of the ethanolic extract were evidenced through significant reduction in cell viability in cervical carcinoma (HeLa) cells, with a calculated LC50 value of 103.539  $\mu$ L. The GC-MS analysis identified several bioactive compounds, including 9,12-octadecadienal, known for its anti-cancer, anti-inflammatory, and antimicrobial properties, contributing to the observed bioactivity of *A. galanga*. These findings affirm the potential of *A. galanga* as a valuable source of natural therapeutic agents, with significant applications in pharmaceuticals and biotechnology. Further research is needed to isolate individual compounds and fully elucidate their mechanisms of action to better understand their therapeutic potential.

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