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Bioprospecting the Anticancer Efficacy of *Garcinia anomala* from North-East India in Ovarian Cancer Cells – An *In Vitro* Study

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Abstract

Background: Ovarian cancer is a type of gynaecological cancer with a worldwide prevalence affecting women aged above 50. Despite several advances made in chemotherapy and surgery, the average time of clinical exemption is approximately two years, and the 5-year survival rate is 45%. But even after survival, they have long-term side effects. So, there is an urgent need for natural anticancer drugs. In this context, *Garcinia spp.* is one such plant that is explored for its anticancer properties. However, the endemic species *Garcinia anomala* from North-East India has not been studied for anticancer efficacy. Thereby present study aimed to understand the anticancer efficacy of hexane and methanol extracts of *Garcinia anomola* short mamilla plant available in the North-East region of India with a major emphasis on antioxidant potential. Further, the best extract with higher antioxidant activity was used to evaluate the anticancer potential through modulation of redox status. **Methods:** Total phenolic content in extract samples was determined by the Folin-Ciocalteu method. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay was used to assess the antioxidant activities of extracts. For *in vitro* evaluation of the anticancer potential of the extract, ovarian teratocarcinoma PA1 cells were used as a model to study the dose-dependent effect of the extract. Various assays, such as wound healing assay and transcriptional analysis of genes, such as Bcl-2, PCNA, and TP53, were performed. For the redox parameter, DCFDA (2',7'-Dichlorofluoroscin Diacetate) assay was used. **Results:** The results showed that *Garcinia anomola* methanol extract (EB) exhibited higher antioxidant activity in comparison to the hexane extract (EA). A cytotoxicity assay was performed to find the effective IC_{50}. From this assay, the effective dose concentration confirmed for EA was 40 µg/mL, EB was 20 µg/mL, and the time point selected was 24 hrs. As the IC_{50} for EB was less than EA, EB was chosen for further assays. From the wound healing assay, the migration rate obtained was 0.967±0.041 µm/hr. The methanol extract was found to affect key cell cycle checkpoint genes at the transcript level. **Conclusion:** These results indicate that the anticancer potential is mediated through the modulation of antioxidant status. Thus, *Garcinia anomala* short mamilla extract from northeast India has good efficacy as an anticancer agent, which can be considered as a future potent drug candidate for ovarian cancer.

Keywords: Antioxidant, Anticancer, *Garcinia,* Phytochemicals, Transcript Analysis.

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1. Introduction

Cancer, a chronic disease, is caused by mutation in cellular DNA. Physical and chemical mutagenic agents in the environment or toxic products of the cell itself can also contribute to its development[1]. One of the causes of cancer is also oxidative stress resulting from the balance between the formation and neutralization of prooxidants[2]. Being one of the leading causes of death this has a major impact on society. Ovarian cancer is one of the most dangerous gynaecological diseases[3]. Ovarian carcinoma, which ranks fifth in cancer deaths among women, occurs mainly in post-menopausal women and accounts for more deaths than any other cancer of the female reproductive system[4]. Due to a lack of proper symptoms, most women are diagnosed only at an advanced stage of ovarian cancer, leading to high mortality[5]. One of the most common treatments for cancer is chemotherapy, but it has its side effects[6]. This directs us to quest for newer alternatives in the field of cancer treatment. Hence, plants, being an integral part of ancient Indian medicine, gained ground. According to the World Health Organisation's official report, 80% of the world population still relies on phytotherapy[7]. In this regard, several plants were identified with anti-cancerous properties. The Clusiaceae family is known as a rich source of bioactive compounds. This family contains around 1000 species under many genera, one of which is *Garcinia*.

Garcinia, a genus of the Clusiaceae family, is native to Asia, America, Australia, and Southern Africa. In India, it is mainly found in North-Eastern and Southern regions. Reports have shown that the genus *Garcinia* possesses many medicinal properties like antibacterial, anti-immunosuppressant, antifungal, antioxidant, anti-inflammatory, and anticancer activity[8]. The leaves and fruit facilitate blood circulation, as an expectorant for the treatment of coughs and indigestion, and as a laxative, while the roots are used to treat fever[9]. Phytochemical studies have revealed the presence of several bioactive secondary metabolites such as triterpenes, lactones, phenolic compounds, mainly xanthones, benzophenones, and bioflavonoids[11]. Prenylated benzophenones and xanthones isolated from *Garcinia* are potent cytotoxic agents against several human cancer cell lines in both *in vitro* and *in vivo* studies, exhibiting growth inhibition and apoptosis induction[12], they also have HIV–inhibitory activity[11]. The antioxidant activity of natural compounds is generally regarded as a good indicator of their anticancer efficacy[13]. There is ample evidence in the literature to suggest a potent antioxidant activity of garcinol, a polyisoprenylated benzophenone derivative extracted from *Garcinia*[14-16]. These data strongly support the view that polyisoprenylated benzophenones present in *Garcinia* genera have interesting biological activities with potential therapeutic applications. Also, sparse data on anticancer efficacy is available for *Garcinia anomala*. However, the potential of their phytocomponents in dealing with ovarian cancer is unknown. Thereby, the goal of the study was to understand the role of *Garcinia anomola* short mamilla extract containing isolated phytocomponents as an anticancer agent using an *in vitro* model of PA1 ovarian cell line (as a prototype of ovarian cancer).

2. Materials and Methods

2.1 Plant Collection and Preparation of Extract

Garcinia anomola fruits from the North-Eastern states of India were collected, identified and authenticated by The Energy and Resources Institute (TERI), Guwahati, Assam. These fruits were processed and subjected to extraction for isolation of active compounds, polyisoprenylated benzophenones, at the Indian Council of Agricultural Research (ICAR) - Directorate of Medicinal and Aromatic Plants Research (DMAPR), Anand, Gujarat. The extracts were prepared by subjecting dried fruit powder through extraction using solvents of varying polarities (dielectric constant at 25^{0}C of *n*-Hexane is 1.88 and Methanol is 32.7)[17].

2.2 Phytochemical Analysis of the Extracts

2.2.1 Determination of Total Flavonoid Content

The aluminium chloride colorimetric method was used for the determination of the total flavonoid content of the sample[18]. For total flavonoid determination, quercetin was used to make the standard calibration curve. Stock quercetin solution was prepared by dissolving 1.0 mg quercetin in 1.0 mL 80% methanol, then the standard solutions of quercetin were prepared by serial dilutions using methanol (10–100 µg/mL). An amount of 0.5 mL diluted standard quercetin solutions or extracts were separately mixed with 1.5 mL of 95% methanol. The double diluted sample of quercetin in the amount of 0.57

mL was taken along with 0.57 mL of 95% methanol and 28.6 uL of 10% aluminium chloride. To this 28. 6uL of 1M potassium acetate was added followed by distilled water to make up the sample volume to 2 mL. After mixing, the solution was incubated for 30 min at room temperature in the dark. The absorbance of the reaction mixtures was measured against a blank at 415 nm wavelength in a UV-Vis spectrophotometer (Cary 50 Bio UV-Vis Spectrophotometer, Agilent). The concentration of total flavonoid content in the test samples was calculated from the calibration plot and expressed as mg quercetin equivalent (QE)/g of dried plant material. All determinations were carried out in duplicates.

2.2.2 Determination of Total Phenol Content

The Folin Ciocalteu method was used to check the total phenol content present in the methanolic and hexane extract of *Garcinia anomola* short mamilla[43]. For the standard calibration curve, gallic acid was used. About 1mg gallic acid was dissolved in 1 mL of 95% methanol, then the standard solution was prepared by taking serial dilutions(10-100ug/mL) From each dilution prepared about 150 uL was taken along with about 1.2mL of 5% sodium carbonate and 600 uL Folin Ciocalteu reagent was added. After mixing it using a vortex the solution was incubated in the dark for 30 mins at room temperature. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer.

2.3 Free Radical Scavenging Activity by 1, 1 Diphenyl 2, Picryl Hydrazyl (DPPH) Assay

DPPH (1, 1 Diphenyl 2, Picryl Hydrazyl): 1 mM solution of DPPH in 100% methanol was prepared[19-21]. One mL of this solution + one mL of the fraction dissolved in methanol at different concentrations 1-10 µg/mL, the mixture was shaken and allowed to stand at room temperature for 30 min, and the absorbance was measured at 517 nm using a spectrophotometer. The % scavenging activity at different concentrations was determined and compared with that of ascorbic acid and quercetin, which was used as the standard.

2.4 Cell Line and Culture

To investigate the anti-cancer activity of methanolic and hexane extract of *Garcinia anomola* short mamilla, the extracts were tested on the PA1 (ovarian teratocarcinoma) cell line with a doubling time of 36 hrs. The cells were procured from the National Centre for Cell Science (NCCS), Pune, Maharashtra, India. The cells were grown in MEM media supplemented with 10% Fetal Bovine Serum, both from Himedia, along with antibiotic-Pen/Strep (*Penicillin* and *Streptomycin*) and maintained at 37°C in a humidified incubator with 5% CO_2 supply. Trypsin was purchased from GIBCO and was used to trypsinize and split the cells.

2.5 Drug Preparation

A stock solution of *Garcinia anomola* short mamilla hexane and methanol extracts (1mg/mL) was prepared in 0.1% DMSO. Different dilutions of EA and EB were made in sterile MEM. The stock solution was further diluted in incomplete MEM for experiments.

2.6 ROS Detection by DCFDA Assay

DCFDA (2,7- Dichlorofluoroscin Diacetate) is a widely used, cell-permeable redox-sensitive fluorescent probe that is oxidized by ROS to yield the highly fluorescent product 2,7-dichlorofluorescein[22,23]. DCFDA was purchased from Sigma. Its 10 mM stock solution was prepared and stored for further use at -20° C for one month. About 10^6 cells were seeded in 6 well plates with complete MEM media (Himedia) and maintained at 37°C in a humidified incubator with a 5% CO_2 supply for 24 hrs. Media was aspirated from each well and DPBS wash was given. For the treatment of cells 25 µm, 50 µm, and 100 µm of DCFDA were prepared in the dark from stock solution of 10mM solution and was added in 2mL media. Cells were further incubated in the dark at 37°C for 30 mins in a humidified incubator with a 5% CO_2 supply. After incubation, media containing DCFDA was aspirated and 2mL DPBS wash buffer was added to each well-containing cells. The cells were observed using a fluorescent microscope. The presence of ROS was confirmed from the green fluorescence, and images were obtained using a confocal microscope, Nikon-Ti2E live imaging system.

Several assays were performed to evaluate the anticancer potential. The initial goal was to find the IC_{50} concentration of the extract.

2.7 Evaluation of Cytotoxicity of the Extract by MTT Assay

Cytotoxicity induced by the extract on the cell line was assessed by performing MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay[24]. Ten thousand cells were seeded per well in 96 well plates

containing MEM media and 10% FBS and 1% Pen strep and plates were incubated overnight in a 5% CO_2 incubator. After 24 hours the cells were subjected to extract treatment at doses of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µg/mL and Taxol (5mg/mL) at 10 µg/mL (positive control). The plates were incubated for 24 h,48 h and 72 h. At the commencement of the drug treatment period, the plates were removed from the incubator, and the media containing the drug was disposed of. Ten microliters of MTT (5 mg/mL) reagent, prepared in phosphate buffer saline (1X), pH 7.4, was added to each well with fresh media to a final concentration of 0.25 mg/mL and incubated for 3 h at 37°C in the dark. Following colour development, the media was removed, and formazan crystals were then solubilized with an inorganic solvent (e.g., DMSO) and kept for 1 hr incubation at 37°C. Absorbance was measured at 570 nm (with background wavelength 620 nm) in a 96-well plate reader. Absorbance from the culture medium without cells was considered blank and was subtracted. Cells treated with DMSO were used as vehicle control. The 50% inhibition concentration of the drugs (IC_{50} values) was calculated for all the 3 time points of treatment.

2.8 Cell Wound Healing Capacity Assay with the Best Effective IC_{50}

About 1×10^5 cells were seeded in each well of 6-well plates, which were incubated at 37°C in a humidified incubator with 5% CO_2 supply until the plate was 80% confluent. When this confluency was attained the used media was removed and fresh media containing the treatment drug (IC_{50}) was added. With a sterile 200 µL tip, a straight scratch was made in each well. The image at 0 h was captured and further, the plate was incubated at 37°C in a humidified incubator with 5% CO_2 supply for 24 h, 48 h, and 72 h, and an image of evidence of cell migration at each time point was captured for all the 6 wells.

2.9 RNA Isolation and Gene Expression Analysis for Key Cell Cycle Genes

Total RNA was obtained using RNAiso Plus reagent as per the manufacturer's instructions, from both control and extract-treated cells (10^6). The quantification was performed using a spectrophotometer at a wavelength of 260 nm. RNA stability was assessed by electrophoresis in an ethidium bromide-stained 1.5% agarose gel. Purity was checked by taking ratio at OD260/OD280, and samples showing a value less than 1.8 were discarded. The reverse transcription reaction to cDNA was performed using the high-capacity cDNA Reverse Transcription kit (Takara kit). PCR was performed using Veriti 5 using Sigma-Aldrich PCR master mix. Thermal cycling conditions included initial denaturation in one cycle of 1 min at 94°C, followed by 35 cycles of 45s

Table 1. List of primers used for transcription analysis

Gene	Primer sequence	Annealing temperature
GAPDH	**FP:**CAAGGTCATCCATGACAACTTTG	58.3°
	RP:GTCCACCACCCTGTTGCTGTAG	58.0°
Bcl-2	**FP:**TGATTGAAGACACCCCCTCG	58.35°
	RP:GCCCAGACTCACATCACCAAG	57.78°
TP53	**FP:**AGTCAGATCCTAGCGTCGAG	54.7°
	RP:TCAGGAAGTAGTTTCCATAGG	55.1°
PCNA	**FP:**CTCTTCCCTTACGCAAGTCTC	55.0°
	RP:GCGGGAAGGAGGAAAGTCTA	56.3°

Table 2. Total phenol and flavonoid content for hexane and methanol extractst

***G. anomola* short mamila**	**Extract**	**Total phenolic content (mg GAE/g)**	**Total flavonoid content (mg QE/g)**
	Hexane (EA)	3.886 ± 0.235	2.74 ± 0.743
	Methanol (EB)	4.135 ± 0.313	2.813 ± 0.195

All the values are represented as Mean ± SEM. (N=4)

at 94°C, 45s at 58°C and 45s at 72°C for annealing of primers and amplification. Expression analysis for Bcl-2 (B-cell lymphoma 2), TP53 (Tumor protein P53), PCNA (Proliferating cell nuclear antigen) and internal control GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) was checked. The list of primer details is given below:

3. Results

3.1 Phytochemical Composition of the Extracts

3.1.1 Total Phenol and Flavonoid Content

The total phenol content of different extracts was estimated using the Folin–Ciocalteu method. *p*-Coumaric acid was used as a standard. The results were expressed as milligrams of GAE per gram of the extracts. Total flavonoid content was estimated using aluminium chloride colorimetric assay quercetin as the standard. The results were expressed as milligrams of QE per gram of the extracts. The hexane extract showed a high concentration of phenol while the methanol extract showed a high amount of flavonoid.

3.1.2 Free Radical Scavenging Activity by 1, 1 Diphenyl 2, Picryl Hydrazyl (DPPH)

The free radical scavenging activity of the extracts was assessed using 1, 1 Diphenyl 2, Picryl Hydrazyl (DPPH) assay. The results are expressed in percentage values of inhibition of radicals. The Hexane and Methanol extracts showed good scavenging activity at approximately 74.3% and 75.35%, respectively.

3.1.3 ROS Detection Revealed in 2,7- Dichlorofluoroscin Diacetate Assay

DCFDA is oxidized by ROS producing green fluorescent product 2,7- Dichlorofluoroscin Diacetate. The cancer cells when provided anticancer treatment showed the presence of high levels of ROS. The same was observed upon the extract treatment. The fluorescence was seen suggesting that the extracts have anticancer potential by modulating redox status in PA1 cells. High fluorescence was seen in extracts- and Taxol-treated cells compared to untreated cells. This indicates that the extract is effective in increasing cellular oxidative stress, thus damaging the cell.

Table 3. DPPH radical scavenging activity of hexane extract EA and methanol extract EB

Sample	Concentration (µg)	%RSA
Quercetin	20	93.7 ± 1.032
Hexane extract	20	73.4 ± 2.786
Methanol extract	20	81.5 ± 3.061

All the values are represented as Mean ± SEM. (N=3) values

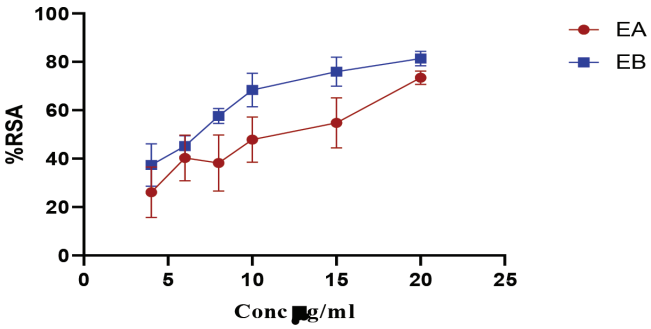

Figure 1. Graph showing free radical scavenging activities of hexane (EA) and methanol (EB) extracts.

3.2 Anticancer Activity

3.2.1 Cytotoxicity of the extracts as revealed in MTT Assay

Cell viability assay performed adopting MTT assay clearly showed dose and time-dependent activity of the extracts against the cancer cell PA1. At 30 µg/mL and 20 µg/mL concentrations, the IC_{50} was achieved, respectively for hexane and methanol extracts. From the results, it can be observed that the Methanol Extract (EB) has more potent anticancer efficacy than the Hexane Extract (EA), as the IC_{50} value was obtained at respective lower concentrations. Hence, methanol extract was chosen for further analysis.

3.2.2 Cell Wound Healing Capacity

From the above IC_{50} values, it is evident that methanolic extract was more potent than hexane extract. Therefore, further experiments were designed with methanol extract. It was observed that there was a decrease in migration rate for cells treated with the extract when compared with the negative and positive controls. The results also indicate that the extract had similar efficacy as the traditional cancer drug Taxol.

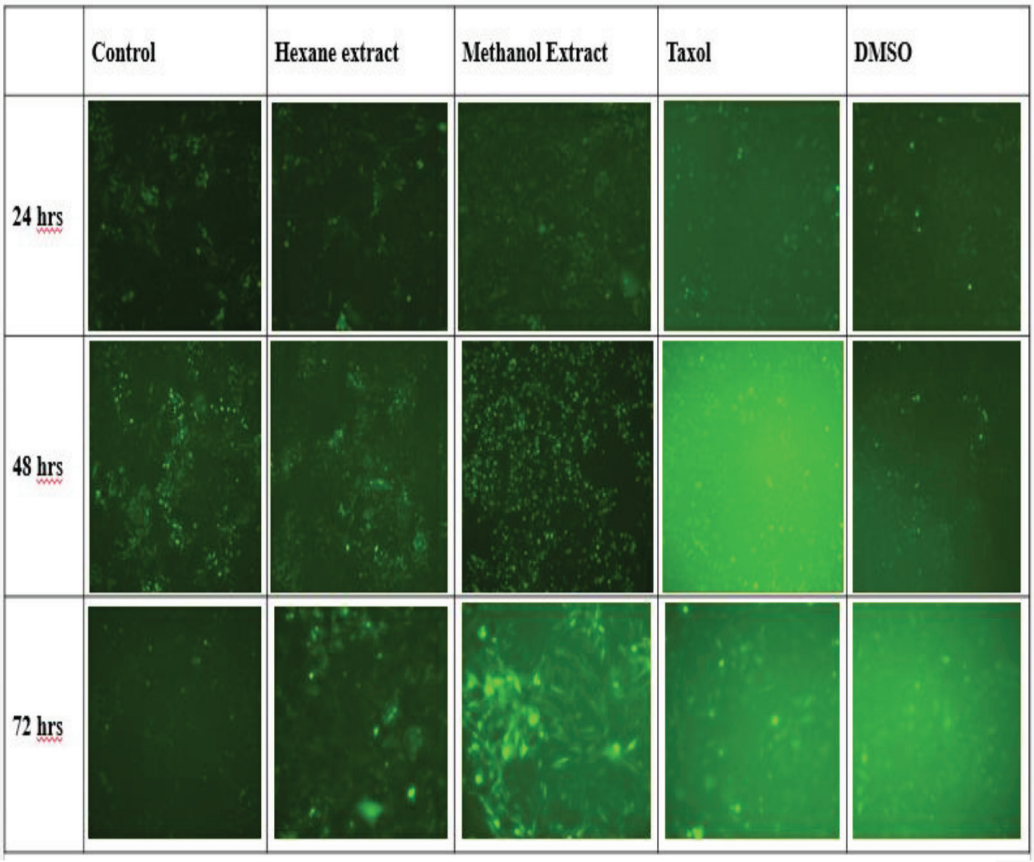

Figure 2. Cellular ROS detected by DCFDA staining. Fluorescence images of extract-treated cells at 100x magnification..

3.2.3 RNA Isolation and Expression of Key Cell Cycle Genes

Transcript analysis started with the isolation of RNA using the Trizol method. The RNA isolated was checked for purity by a 260/280 ratio, observed by agarose gel electrophoresis. cDNA was prepared from RNA. Using the primer expression of the key cancer genes PCNA (Proliferating Cell Nucleus Antigen) and TP53 (Tumour Protein) were checked. GAPDH was used as an internal control. From the data, it was observed that expression of Bcl-2, which is an apoptotic marker, and PCNA, a cell proliferating gene, which is usually high in cancer cells were significantly reduced when the cells were treated with the extract. On the other hand, the TP53 gene coding for tumor-suppressing protein, inducing cell cycle arrest and apoptosis, is high in the extract-treated cells.

4. Discussion

The most common cancers are those originating from the gastrointestinal tract, breast, and gynaecological cancers. There are five main gynaecological cancers, of which ovarian cancer is more widespread. Various synthetic drugs have been discovered for therapeutic uses, but their long-term use produces side effects. So, there is an urgent need for natural anticancer drugs. In this context, the present study aimed to study the efficacy of different polarity-based extracts of *Garcinia spp.* obtained from North East India.

The plant *G. anomola* was collected and extraction of phytocomponents was done using both nonpolar (i.e. hexane) and polar (i.e. methanol) solvents. The plant extract exhibits good antioxidant activity. Several studies have been reported previously, wherein the molecules possessing antioxidant activities can slow or prevent the development of cancer[25]. Cheok *et al.* reported that among solvents of various dielectric constants (ethanol, methanol, isopropanol, and ethyl acetate), the solvent extracting the highest amount of phenol from *Garcinia* is methanol which is in line with the current study[26]. The phytochemicals related

to phenols and flavonoids were measured. The total flavonoid content and total phenol content in the extract range from 2.74 ± 0.743 to 2.813 ± 0.195 mg/g QE and from 3.886 ± 0.235 to 4.135 ± 0.313 mg/g GAE, respectively. Total phenol content (%) in fruit extracts of eight *Garcinia* species were as follows: *G. gummi-gutta* (3.26), *G. indica* (5.01), *G. mangostana* (2.33), *G. xanthochymus* (4.43), *G. subelliptica* (3.14), *G. kydia* (4.32), *G. lanceaefolia* (3.03) and *G. pedunculata* (2.43)[27]. Our result of total phenol content was in agreement with this report.[27] In these studies antioxidant activity is studied by DPPH free radical scavenging assay. The DPPH assay is a frequently used technique to determine the radical scavenging capacity of plant-based extracts because of its quick and responsive features, which involve simple conventional laboratory equipment. Methanol extract exhibited high DPPH scavenging activity (81.5 ± 3.061) in comparison to hexane extract (73.4 ± **2.786**). Ambarwati *et al.*[28] reported that the % inhibition of methanol extract at a concentration of 100 µg/mL was 95.68%, higher than the % inhibition of stem bark ethyl acetate extract (93.77%) and *G. latissima* Miq. stem bark n-hexane extract (25.36%), indicating that the methanol extract had the highest antioxidant activity compared to the other two extracts. The result of the present study is in agreement with this report, as methanol extract has better antioxidant potential than hexane extract. From our data, we infer that the antioxidant activity of methanol extract is due to the presence of polyphenols like flavonoids. Also, Karimi *et al.*[29] proposed that plant fruits contain a variety of polyphenolics and phenolic derivatives. These phenolic compounds could be potential antioxidant sources[30].

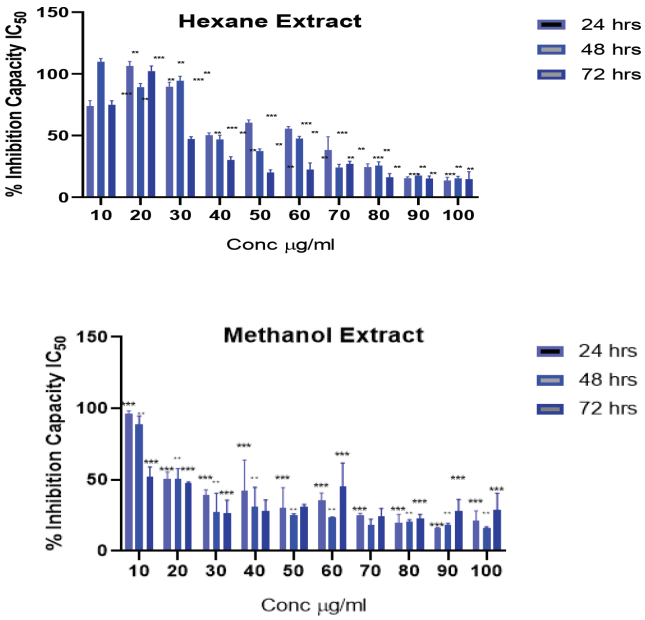

Figure 3. Dose and time effect of the two extracts on PA1 cell viability.
N=5. Error bars represent Mean ± SEM for 24 h,; P = 0.0008; 48 h, P = 0.0017; 72 h, P = 0.004. *P<0.05; **P<0.01; ***P<0.001

Anticancer and antioxidant assays were performed on PA1 ovarian teratocarcinoma cells using the extracts. Increased ROS contributes to tumour formation due to several factors including oxidative DNA damage; however, there are also reports about anticancer drugs inducing ROS and this plays an important role in their cancer chemotherapeutic activity[31,32]. Similar results were observed for the DCFDA assay performed to study cellular ROS. In the DCFDA assay, more fluorescence was seen in the extract- and Taxol-treated cells when compared to untreated cells indicating the presence of more free radicals. A cytotoxicity test with an MTT assay was performed to find the IC_{50}. Kumar *et al.,* reported that the methanol extract of the fruit rinds of *Garcinia indica* showed potent cytotoxic activity against three human cancer cell lines, colon (COLO-320-DM), breast (MCF-7) and liver (WRL-68), as determined by the MTT assay[33]. A cytotoxicity test with MTT assay was performed to find the IC_{50}. The present data showed that methanol extract was more potent in inhibiting cell proliferation as the IC_{50} value for methanol extract (20 µg/mL) was lower than hexane extract (30 µg/mL). This result agrees with Kumar *et al.*[33]. Further data obtained from phytochemical analysis of antioxidant activity and cytotoxicity analysis imply that methanol extract has better efficacy than hexane

Table 4. Mean migration rate of control and extract treated cells.

Groups	Control	Taxol (positive control)	EB	DMSO (negative control)
Migration rate (µm/hr)	2.277 ± 0.150	0.95 ± 0.053	1.597 ± 0.166	2.957 ± 0.52

All values are represented as Mean ± SEM. (N=3)

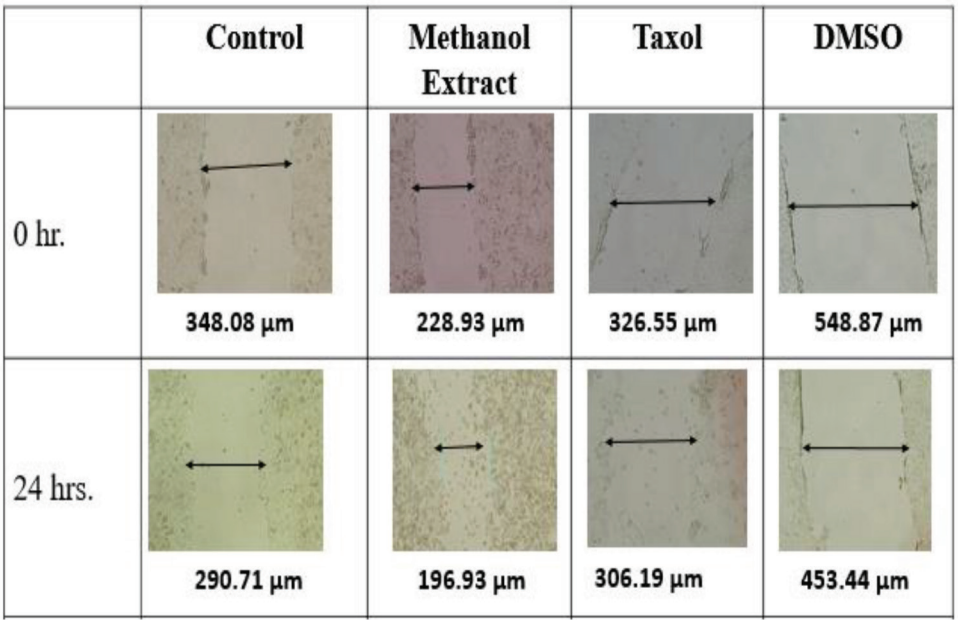

Figure 4. Migration rate of cells exposed to methanol extract.

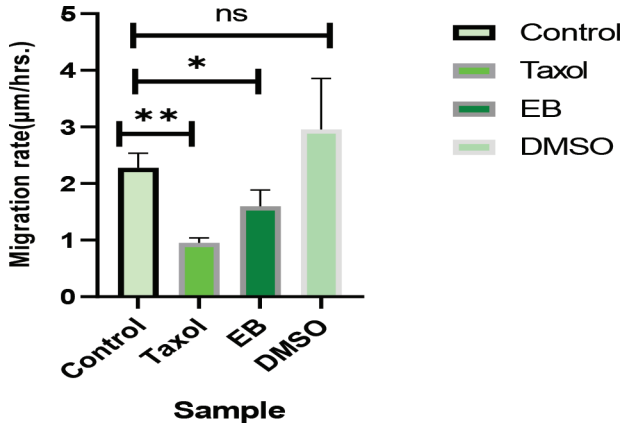

Figure 5. Mean migration rate of control and extract treated cells. N=3. All values are Means ± SEM. Taxol. p= 0.0011; EB p=0.0381; *P<0.05; **P<0.01.

Table 5. Quantification of RNA in µg/mL after treating the cells with the extracts.

Groups	Control	Taxol	EB	DMSO
Conc.µg/µL	0.78	2.00	1.18	1.29

extract[33]. A decrease in migration rate was observed when cells were treated with methanol extract when compared with the negative and positive controls. The migration rate obtained for methanol extract (1.125 ± 0.166) treated cell is almost similar to the standard anticancer compound Taxol (0.95 ± 0.053).

The transcript analysis of GAPDH (control), PCNA, TP53, and Bcl-2 was carried out. Proliferating Cell Nuclear Antigen (PCNA) is a marker for cell proliferation as it is essential for cell replication[34]. p53 acts as a guardian of the genome by facilitating cell cycle arrest, differentiation, and apoptosis, thereby decreasing the accumulation of mutant cell populations[35]. The Bcl-2 family of proteins plays a vital role in regulating apoptosis[36]. Paul *et al.* reported that expression of PCNA and Bcl-2 decreased upon treatment of root extract of *Polygala senega*, and increased expression of tumour suppressor gene p53 was observed[37]. In a similar line of study, Subapriya *et al.* showed that treatment of ethanolic leaf extract downregulated the expression of PCNA and Bcl-2 and upregulated the expression of TP53[38]. The results of the current study show that PCNA and Bcl-2 markers of cell proliferation were elevated in the PA1 cell line, but their transcript levels were down-regulated upon extract treatment. However, an upregulation was observed for the TP53 gene responsible for cell cycle arrest after extract treatment, suggesting the role of methanol extract as a transcription modulator. However, an in-depth molecular study needs to be done to carry this concept forward.

All the biochemical, cytotoxic, and transcript analyses point out that the extract has the potential to modulate ovarian cancer. This modulation could be correlated with the presence of phytochemicals like phenols and benzophenones. In this context, Ignas and Vilma

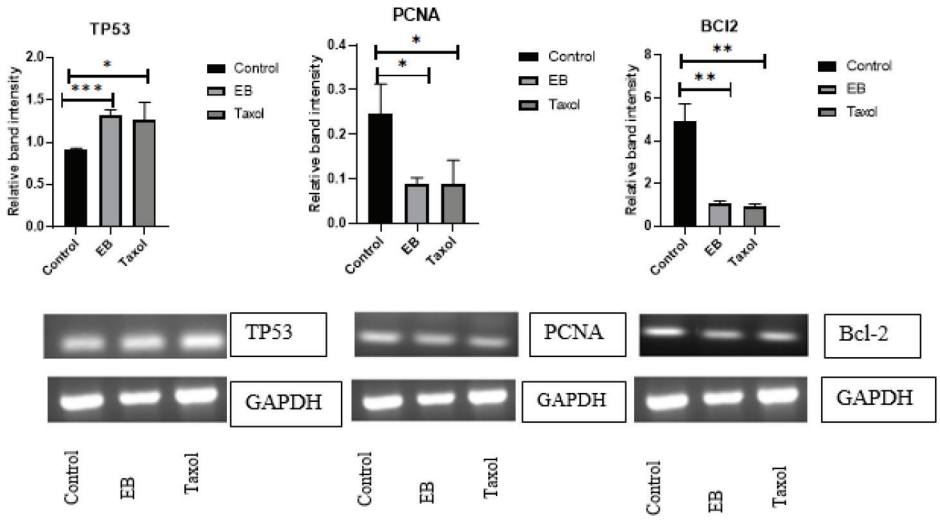

Figure 6. Transcript analysis of key cell cycle regulatory genes (TP53, PCNA, Bcl-2 and GAPDH). N=3. All values are represented as Means ± SEM. Taxol p= 0.0011; EB p=0.0381. ***p<0.001; **p<0.01; *p<0.056.

reported that the anticancer effect of trihydroxyflavones, a polyphenol, against A549 and U87 cells could be related to their antioxidant activity, and anti-proliferative effect that directly correlates with DPPH radical scavenging activity[39]. Katsube *et al.,* compared the antiproliferative activity of the ethanol extract of 10 edible berries on HL-60 human leukemia cells and HCT-116 cells and showed that bilberry extract was the most effective[40]. Ross *et al.* showed that the anti-proliferative activity of the raspberry extract in human cervical cancer (Hela) cells was predominantly associated with ellagitannins[41]. A positive correlation has been shown between antioxidant activity and anticancer activity of *Trigonella foenum-graecum* (Fenugreek), *Cassia acutifolia* (Senna), and *Rhazya stricta* (Harmal)[42]. With the above findings, it is interesting that polyphenols could be an active component to modulate the cancerous property through the redox modulation pathway. This is the first study to indicate that benzophenones from *Garcinia anomala* could be an alternative herbal cancer drug.

5. Conclusion

In the present study, the extract of *Garcinia anomala* short mamilla, collected from Northeast India, has been characterized for its anticancer efficacy along with antioxidant potential using the PA1 (ovarian teratocarcinoma) cell line. The plant extract characterized for its phytochemicals showed the presence of a higher amount of phenols and flavonoids which is known to have high antioxidant potential as indicated by DPPH assay. When these extracts were subjected to DCFDA for cellular ROS detection, the results were similar to the potent anticancer drug Taxol. Along with antioxidant potential, it showed good anticancer efficacy. The extract showed satisfactory cytotoxic activity against ovarian cancer cells. The administration of the extract could also effectively modulate the key cell cycle genes associated with cancer. Thus, this study suggests that *Garcinia anomala* short mamilla, a northeast endemic species, holds significant potential as a taxol mimic, necessitating '*in-vivo*' validation.

6. Acknowledgment

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7. References

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