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Original Article

From Biowastes to Potent Anticancer Leads Evaluation from Peel of *Zingiber officinale* Linn.

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ABSTRACT

Objectives: *Zingiber officinale* Linn. is a rhizome commonly consumed as a culinary purpose, medicine or spice in India. The aim of this study was to evaluate potent anticancer leads from ginger rhizome peel. **Methods:** Cytotoxicity study was carried out by using brine shrimp, Antimitotic study was carried out through onion root method, Anti-proliferating study was carried out using *in vitro* cell line study and at last the cell viability study was carried out through yeast model using quantitative and qualitative methods. **Results:** LC_{50} of phenolic group contain fraction (PHGF) of Petroleum ether fraction (PEF) was found to be 18 µg/ml, which was threefold higher than that of synthetic standard podophyllotoxin, i.e., 50 µg/ml. 2 isolated compounds (6-shogaol and 6-gingerol) have good HSF1 inhibition activity and creating apoptosis. 6-shogaol showed good Green fluorescent protein (GFP) at 10µg/ml and 6-gingerol compound good GFP at only 5 µg/ml. In MTT assay cell viability was showing ±54% in ginger peel extract sample dose of 100µg/ml. When the pet ether fraction of ginger peel extract was tested, result showed the ±96 % cell death in dose of 100µg/ml. We observed cell viability of c.90%, 60% and 94% in the case of the wild-type strain. **Conclusion:** This study revealed the strong antiproliferative potentials of Petroleum ether fraction (PEF) of extract of Ginger peel. It was also found to be moderately safe for consumption and thus could serve as a source of candidate for the development of new antiproliferative and antimicrobial drugs.

Key words: Anticancer, Antiproliferative, Anti-mitotic, Yeast model, *Zingiber officinale* Linn.

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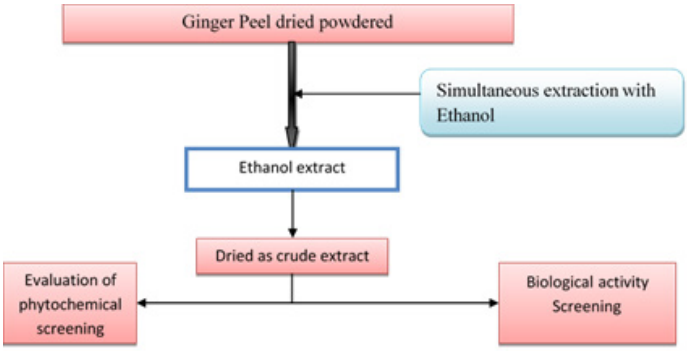
INTRODUCTION

Zingiber officinale Linn, Zingiberaceae (Ginger) is an extensively used in various foods and potions around the human kind and is utilized in traditional Indian and Chinese medicine. For centuries, ginger has been used in traditional Indian Ayurvedic medicine as Ginger in many cases, like as powdered rhizome, extract of rhizome, dried or steamed rhizomes, fresh rhizomes for the treatment of life expectancy, potency, memory, immunity, oxidant stress, skin complexion, gastrointestinal constipation, improves pronunciation mistakes, heart problems, common cold disorders, inflammation and helminthiasis. But only ginger peel efficacy in human health is not checked previously in traditional medicine. Ginger peel is wasted by people in various ways (like Food Processing industry, household culinary) and it increases environmental pollution by producing greenhouse gases.[1] The most significant greenhouse gas produced from bio-waste is methane. It is released during the breakdown of organic matter in landfills. Several scientific studies have revealed that the peels of ginger contain a wide range of active ingredients that are responsible for its antioxidant, antimicrobial, anti-inflammatory, anticancer and spasmolytic and diuretic properties. The evaluating of certain polyphenols (such as flavonoids, phenolic acids, terpenoids and others) and their derivatives are mainly considerable for these potential health benefits.

Extraction is an essential first step for the isolation and refinement of these bio-active components of plant materials. Generally, the conventional extraction procedures (such as solvent extraction, steam distillation, etc.) are often limited by the elongated extraction time, environmental pollution, low yield and mass transfer resistances caused by the contribution of more than one phase in the system. To provide a useful alternative to the traditional extraction methods, new physical practices such microwave extraction processes and ultrasound have been applied to disrupt the cell wall and discharge active components during product extraction. Shogaol and Gingerol are known as the major phenolic oil of ginger oil especially in *Zingiber officinale* Linn. oil showing anticancer effects.

In recent years, extensive research has been done on cancer and depression, the two prime causes of mortality and morbidity in people, however there are two main problems: the first is the etiology and the second is the treatment of these diseases.[2] indicated that cancer and depression are two independent risk factors for mortality rate in humans; hence, their effects on all-cause of mortality are additive, not synergistic.

Cervical and lung cancers are the principal causes of cancer-related death. Cervical cancer is caused by chronic infection with a range of high-risk Human Papilloma Virus (HPV) leading to an estimated 274,000 death globally every year. Moreover, lung cancer is a highly aggressive, progressive and heterogeneous malignant disease predominantly results from smoking tobacco with few options of treatment. For the treatment, chemotherapy with drugs such as Cisplatin and Iressa and psychotherapy with antidepressant drugs like fluoxetine, sertraline and citalopram are widely used. However, these drugs have several side effects leading to further research for treatment with herbal medicines to reduce adverse side effects. Insufficient attention has so far been devoted to *Zingiber officinale* Linn. Therefore, the goal of this study was to investigate the potential effects of *Zingiber officinale* Linn. on human colon cancer cell



Flowchart for Experimental design.

growth in *in vitro* cell culture. My objective is to find potent anticancer leads from Biowastes of *Zingiber officinale* Linn. peels.

MATERIALS AND METHODS

Experimental design

Chemicals

From Sigma Aldrich (Bangalore, India) purchased bacterial strains like *Staphylococcus aureus* (*S. aureus*, ATCC 6538), *L. monocytogenes* (*L. monocytogenes* ATCC 19115), *Salmonella typhi* (*Salmonella typhi*. ATCC 19430), *Escherichia coli* (*E. coli* ATCC 8099), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) reagent, Cyclophosphamide, dimethyl sulfoxide (DMSO), Norfloxacin TZ, podophyllotoxin, doxorubicin. Wild yeast, ethylenediaminetetraacetic acid (EDTA), Dulbecco's modified Eagle's medium (DMEM), propidium iodide (PI), fatal bovine serum (FBS) Media and trypsin were obtained from Himedia Ltd., (Mumbai, (India)). Other chemicals and solvents were obtained from Merck India, Loba Chemicals (India) and SD Fine Chemicals (Mumbai, India).

Plant material

Ginger was purchased from local market of Kolkata and the outer dry and semi-dry layers and the apical trimmings of yellowish brown-skin ginger (*Zingiber officinale* Linn.) peel were collected immediately after processing from household cooking (Kolkata, India).[3] The peels were dried in hot air oven thoroughly and ground with a mixture grinder, sieved by various mesh sized sieve and stored for the further experiments.

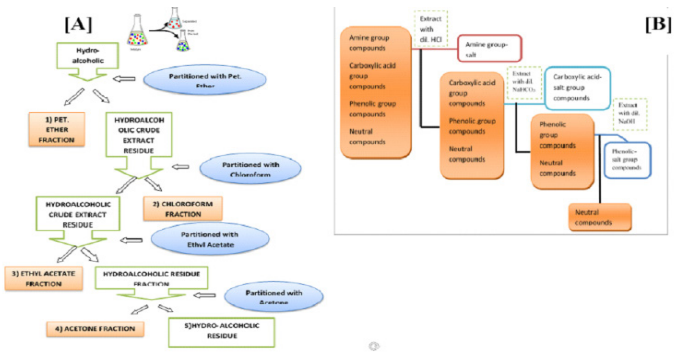


Figure 1: [A] Extraction and Fractionations flow diagram, [B] Further fractionations from previous fractions.

Extraction and fractionation

As per (Figure 1 [A] and [B]), the dried ginger peel (2 Kg) were ground and carried on microwave assisted extraction, Sonicated assisted extraction and cold macerated extraction (three types of extraction for optimization of chemical constituents in extract) with ethanol for 72 h.[3-5] The Ethanol extract was filtered and the filtrate was evaporated under reduced pressure at 40°C which yielded the crude extract (481 gm).[6,7] A portion of the crude extract (400 g) was successively fractioned to yield Pet ether (260 g), chloroform (40.6 g), Ethyl Acetate (24.2 g) and aqueous-methanol (156 g) fractions. Pet ether fraction was further fractionated by dil. HCl, Sodium bicarbonate and sodium hydroxide for getting pure chemical group fractions. From total (200 g) pet ether fraction we got Amine group of compounds (21g), Carboxylic acid group contain fraction (8 g), phenolic group contain fraction (PHGF) (167 g) and neutral ionic compound fraction (4 g).

Phytochemical analysis

The qualitative and the quantitative analyses of ginger peel's constituents were carried out by the methodology described in the book[8] by using TLC and test tube chemical analysis methods.

Antifungal activity

Antifungal activity of the ethanolic extract and subsequent fractions was measured by employing the agar tube dilution method against the selected pathogens. The molds and yeasts used in this study included *Aspergillus flavus* (ATCC32611, human pathogen), *Candida albicans* (ATCC2091, human pathogen) and *Candida glabrata* (ATCC90030, human pathogen) using the methods.[3,9-11] The strains were provided by Akansha Skin and hair Herbal Ltd. Industrial Culture Collection (Kolkata, India). Dilutions were made by the final concentration of the extracts, subsequent fractions and the standard drug to 200 µg/ml of SDA (Sabouraud Dextrose Agar). Miconazole (an antifungal medication used to treat ring worm, pityriasis versicolor and yeast infections of the skin or vagina) was used as the standard drug and the experiments were carried out in triplicates.

Antibacterial activity

Antibacterial activity of the ginger peel crude extract and subsequent factions were assessed against various Gram-positive and Gram-negative human pathogens by the agar disk diffusion protocol. Gram-positive bacteria (*S. aureus* and *L. monocytogenes*)[3,9-11] and Gram-negative bacteria (*Salmonella* spp. and *Escherichia coli*) were used for this bioassay using the methods. These specific micro-organisms were selected due to the well-known causes of these pathogens to food borne diseases. *Staphylococcus aureus* (*S. aureus*, ATCC 6538), *L. monocytogenes* (*L. monocytogenes* ATCC 19115), *Salmonella typhi*. (*Salmonella typhi*. ATCC 19430) and *Escherichia coli* (*E. coli* ATCC 8099) were provided by Akansha Skin and hair Herbal Ltd., Industrial Culture Collection (Kolkata, India). Norflox TZ was used as the standard antibacterial agent.

Cytotoxicity assay

A cytotoxicity assay was carried out by brine shrimp lethality bioassay. In this bioassay, the eggs of the brine shrimp a food for tropical fishes were collected from local pet fish store as and placed to hatch in a chamber with brine solution.[12,13] After 48 h, eggs hatched and the larvae (nauplii) were observed. The larvae were collected and transferred into the test tubes. 10 no.s larvae were transferred in each test tube containing different concentrations (1, 10, 100, 1000 µg/ml) of the extracts and fractions, dose selected as per randomised study. Drug exposure were given to the larvae for 24 hr, then the numbers of dead larvae were counted and

this data was used for estimate LD_{50} (Median Lethal Concentration 50).[14] Podophyllotoxin was used as standard drug in this bioassay.

Antimitotic assay

Onion root tip method was used for Antimitotic activity study.[15,16] Approximately equal sized onion bulbs (*Allium cepa* L.) near about (40±10 g) were obtained from the local vegetable market at Kolkata, West Bengal, India. The onion bulbs were grown in dark place for 48 h over 50 ml of purified water at ambient temperature for allowing the roots to be grown approximately 3 cm. The bulbs with root tips developed up to 2-3 cm were selected for the study. The onion rooted bulbs were divided into five groups each group containing 3 bulbs. The first group provided as control. Immediately, the bulbs were placed in containers filled with test samples (one bulb in each) and control (purified water). Whole material was incubated at 22 ± 3°C for 72 h and away from the direct sunlight. The length of the roots grown during incubation period of 72hr and grown. The root number and the mitotic index were recorded. The root tips (2-3 mm) were collected and were fixed in the fixing solution of acetic acid and ethanol (3:1). Onion root tip squash preparation was made for microscopic studies by stained with a mixture of acetocarmine and 1 N HCl (9:1). Using high resolution (100x) bright field light microscopy, each root tip, the numbers of mitotic cells and total meristematic cells were counted manually. Good quality onions were rooted in water and the onion roots were treated with different concentrations of the extracts and various fractions and standard drug cyclophosphamide for 24 h. The following formula has been used for calculated of Mitotic index:

$$\text{Mitotic index} = \frac{\text{number of dividing cells}}{\text{number of non-dividing cells}}$$

Antiproliferation assay

a) Cell Culture

HCT116 cells (Human Colon cancer cell line)[16-18] were cultured according to the protocol provided by the depositor. HCT116 cells were grown in 10% MEM supplemented with FCS at 37°C under a humidified atmosphere of 5% CO_2. Gently gave passaging by using serological pipette. After proper passaging split the cells into 1:2, 1:3 ratio for cytotoxicity studies.

b) HSF1 (HEAT SHOCK FACTOR 1) inhibition assay

In order to study the HSF1 inhibition of a new drug, it is important to determine the inhibition concentration of the drug.[16,17] GFP study by fluorescence microscope helps to detect the HSF1 inhibition of the drug. The concentration non-toxic to the cells is chosen for HSF1 inhibition assay. After 48hr of the addition of drug, cell death and GFP (Green Fluorescent Protein) was estimated. After 24hr addition of drugs heat shock incubation was given for 1 hr at 39°C. The result is confirmed by less GFP.

c) Heat shock:

After 24 hrs incubation the cytotoxicity was observed and plates were incubated for 1 hrs at 39°C in heat shock incubator.[16,17] After 1 hr heat shock the plates were further incubated for 24hrs at 37°C in 5% CO_2 environment and observed for GFP by fluorescence microscope.

d) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Antiproliferation assay was carried out via MTT assay.[16,17] Cell proliferation, which imitates the normal function of mitochondria and cell viability. HCT116 cells were seeded at flat-bottomed 96-well culture plate with a ratio of 5 × 104 cells/well in DMEM. The cells were washed after 24 hrs and cultured in a culture medium with different concentrations of Ginger peel extracts and various fractions (25 to 200 µg/ml, for 48 h and 72 h). Non-FBS culture medium encompassing 10% MTT was added to each well of a microtitre plate and after that the samples were incubated for 4 h at 37°C. After 4 h culture medium was removed and DMSO was added. Then the absorbance was read at 570 nm (ELISA reader). The noted absorbance of control cells (DMSO treated) was considered as 100%. The following formula has been used for calculating the percentage cell viability:

$$\frac{\text{Mean value of [(OD in control group – OD in treated group)}}{\text{OD in control group}} \text{ x } 100$$

Cell viability activity screening by yeast model:

i) Introduction of cell viability assay by yeast model

The yeast *Saccharomyces cerevisiae* is a very beneficial model organism for studies of cellular response to numerous types of stresses. Fortitude of cell viability is one of the most frequently used methods in a study of cyto- or genotoxicity under dissimilar kinds of chemical, physical, or environmental factors. The analysis of the feasibility parameter is also very significant for industrial processes where micro-organisms are used. Generally, cell viability is defined as a % of live cells in a whole population.

ii) Yeast strains and growth conditions

The yeast strains were used mainly wild-type SP4 MATα leu1 arg4[19,20] Yeast was grown in a standard liquid YPD medium using of a rotary shaker at 150 r.p.m. A solid YPD medium yeast cells cultured at a temperature of 28°C.

iii) Cell viability assay method

To determine cell viability, yeast cells culture was centrifuged, washed with sterile water and suspended to the final density of 10^8 cells mL^{-1} in 100 mM phosphate buffer at pH 7.0, comprising 0.1% glucose, 1 mM EDTA, 10 mM H_2O_2 and 0.105 mM menadione, 0.4 mM allyl alcohol.[21,22] Then the 1h incubation period, the yeast cells were pelleted with centrifugation, after that washed twice with sterile water. Suspended in a buffer solution.

To determine the cell viability and vitality, the methods were used:

1. Spotting test: Cells were suspended in sterile buffer solution. Diluted to give 10^7, 10^6, 10^5 mL^{-1}. Samples (5 µL) of each suspension were inoculated on solid YPD medium. After that incubated at 28°C for 48 h. At last the colony growth was inspected.[22-24]

2. Colony-forming units: Cells were suspended in sterile buffer solution and diluted to a final concentration of 10^3 cells mL^{-1}. A sample of 100 µL of the suspension was inoculated on solid YPD medium. Incubated at 28°C for further 48 h. then the colony forming unit was counted.[24-27]

3. Staining with Methylene blue: Cells were suspended in PBS and cell suspension was mixed with 100 µL methylene blue and incubated for 5 min at room temperature.[28-30] Then the viability was examined under binocular microscope (QUASMO) with attached digital camera at 40x from at least 200 cells in one biological replicate. The dead cells were blue and viable cells were colourless in nature. The quantitative results were presented as mean ± SD.

Statistical analysis

The values were voiced as Mean ± standard error of mean (SEM). Analysing the data by using one-way analysis of variance (ANOVA) using Graph-Pad Prism software. $p < 0.05$ were considered as statistically significant.

RESULTS

Through extraction, fractionations, isolations and purifications processes got GPEE = Ginger Peel Ethanol Extract, PEF = Pet Ether Fraction, CF = Chloroform Fraction, EAF = Ethyl Acetate Fraction, AMF = Aquas Methanol Fraction, AGF = Amino Group Fraction, CGF = Carboxylic Group Fraction, PHGF = Phenolic Group Fraction, NCF = Neutral Compound Fraction, two types of pure compounds 6-shogaol and 6-gingerol from ginger rhizome peels.

Phytochemical Screening

The results of phytochemical analysis showed the presence of alkaloids (34.6%), flavonoids (4.8%), glycosides (2.4%), triterpenoids (22.2%) and phenolic content (36%) in Ginger peel extract and alkaloids (0%), flavonoids (1.8%), glycosides (1.0%), triterpenoids (12.4%) and phenolic content (84.8%) in PEF.

Antifungal activity

The results obtained for the antifungal activity of the extract and subsequent fractions derived from *Zingiber officinale* Linn. peel against the tested pathogens. The most prominent antifungal activity was observed against *Candida glabrata*. The crude extract showed 85% inhibition against this pathogen while pet ether fraction (PEF). Phenolic group fraction and carbonic group fractions inhibited the growth of *Candida glabrata* by 93 ± 2%, 98 ± 2% and 82 ± 4, respectively; $p < 0.05$ (Figure 2[A]). Pet ether, phenolic group fraction and carbonic group fractions showed antifungal activity against *Candida albicans* and inhibited its growth by 91 ± 2%, 98 ± 2% and 84 ± 2%, respectively; $p < 0.05$. The other active fractions in this bioactivity were pet ether, phenolic group fraction and carbonic group fractions against A. flavus (80 ± 10%, 86± 10% and 78±10% respectively; $p < 0.05$) (Figure 2[A]).

Antibacterial activity

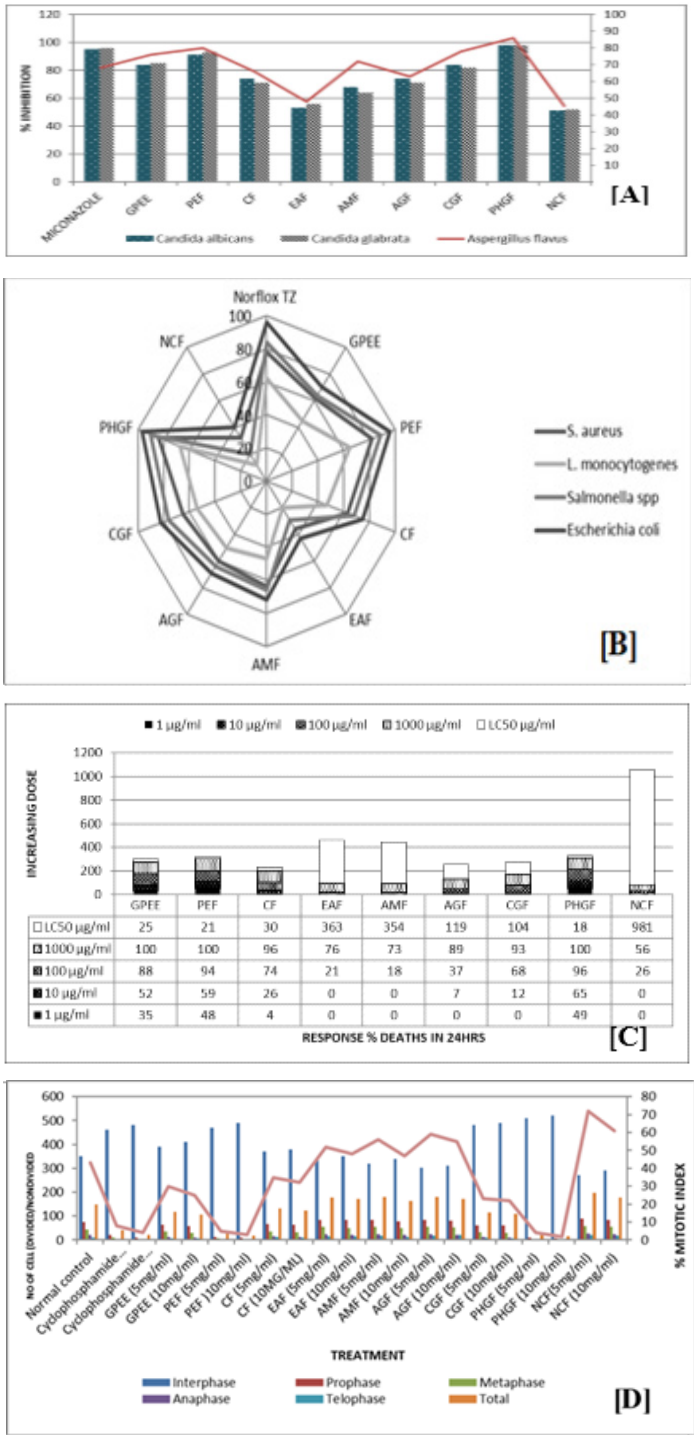
The extract and subsequent fractions derived from *Zingiber officinale* Linn. peel displayed a strong antibacterial activity against the tested organisms. The crude extract pet ether, phenolic group fraction and carbonic group fractions inhibited the growth of *S. aurias* only by 62%, 82% and 84% as compared to standard drug (Norflox TZ). The crude extract pet ether, phenolic group fraction and carbonic group fractions inhibited the growth of *E. coli* by 76%, 96% and 97% as compared to standard drug (Norflox TZ). in (Figure 2[B]).

Cytotoxicity assay

The outcomes of cytotoxicity assay, carried out in triplicates of GPEE = Ginger Peel Ethanol Extract, PEF = Pet Ether Fraction, CF = Chloroform Fraction, EAF = Ethyl Acetate Fraction, AMF = Aquas Methanol Fraction, AGF = Amino Group Fraction, CGF = Carboxylic Group Fraction, PHGF = Phenolic Group Fraction, NCF = Neutral Compound Fraction, revealed that PHGF and PEF triggered an increase in percentage death of shrimps in a dose-dependent manner (Figure 2[C]). The LC_{50} of PHGF was found to be 18 µg/ml, which was threefold higher than that of synthetic standard podophyllotoxin, i.e., 50 µg/ml, while PEF showed little bit lesser response (21 µg/ml) in comparison to PHEF but which also two and half fold higher than standard.

Antimitotic activity

PHGF showed significant reduction in the mitotic index of onion root tips with increase in concentration (Figure 2[D]). Antimitotic activity was found to be significant at 10 mg/ml and 5mg/ml of PHGF (0.02 ± 0.02; $p < 0.05$) when equated with the control mitotic index (0.43 ± 0.02) and was also more than that of standard cyclophosphamide (0.04 ± 0.01; $p < 0.05$).



	GPEE	PEF	CF	EAF	AMF	AGF	CGF	PHGF	NCF
LC50 µg/ml	25	21	30	363	354	119	104	18	981
1000 µg/ml	100	100	96	76	73	89	93	100	56
100 µg/ml	88	94	74	21	18	37	68	96	26
10 µg/ml	52	59	26	0	0	7	12	65	0
1 µg/ml	35	48	4	0	0	0	0	49	0

Figure 2: [A] Antifungal activity study and comparison. [B] Antibacterial activity study and comparison. [C] Cytotoxicity assay. [D] Antimitotic activity. GPEE = GINGER PEEL ETHANOL EXTRACT, PEF = PET ETHER FRACTION, CF = CHLOROFORM FRACTION, EAF = ETHYL ACETATE FRACTION, AMF = AQUAS METHANOL FRACTION, AGF = AMINO GROUR FRACTION, CGF = CARBOXILIC GROUP FRACTION, PHGF = PHENOLIC GROUP FRACTION, NCF = NUTRAL COMPOUND FRACTION

Antiproliferative activity

a) HSF1 inhibition assay

After 24hr of heat shock took the results of fractions of ginger peel extract activity on HCT116 cell. It showed very good GFP reading. From this

reading I understood that pet ether extract of ginger peels extract and its Pet ether fraction and also its 2 isolated compounds (6-shogaol and 6-gingerol) have good HSF1 inhibition activity and creating apoptosis. 6-shogaol showed good GFP at 10µg/ml and 6-gingerol compound good GFP at only 5 µg/ml (Figure 3[A]).

b) MTT Assay

In MTT assay cell viability was showing ±54% in ginger peel extract sample dose of 100µg/ml. When the pet ether fraction of ginger peel extract was tested, result showed the ±96 % cell death in dose of 100µg/ml (Figure 3[B]).

Cell viability activity screening by yeast model

The results found by these two methods are not the same. In the case of the spotting test, 1h incubation of cells with 12.5 µg/ml pet ether fractions causes 50% inhibition of growth. An assessment of these results with those obtained by the CFU method shows a significant alteration. In the case of the CFU method, it was noted a complete growth reduction of the wild type strain 99%. The differences so observed in the results obtained. These two methods may be explained in two ways (Figure 4):

A) It may reduce direct contact with oxidants and facilitate growth of some cells which are protected by other cells.

B) Out of all the cells, the observed growth was marked in the test even if it consisted in growth of few cells. We observed cell viability of 90%, 60% and 94% in the case of the wild-type strain. Our results by using methylene blue dye showed that after exposure to oxidants, there are few cells that are unable to reproduce but they are still alive in nature (Figure 4).

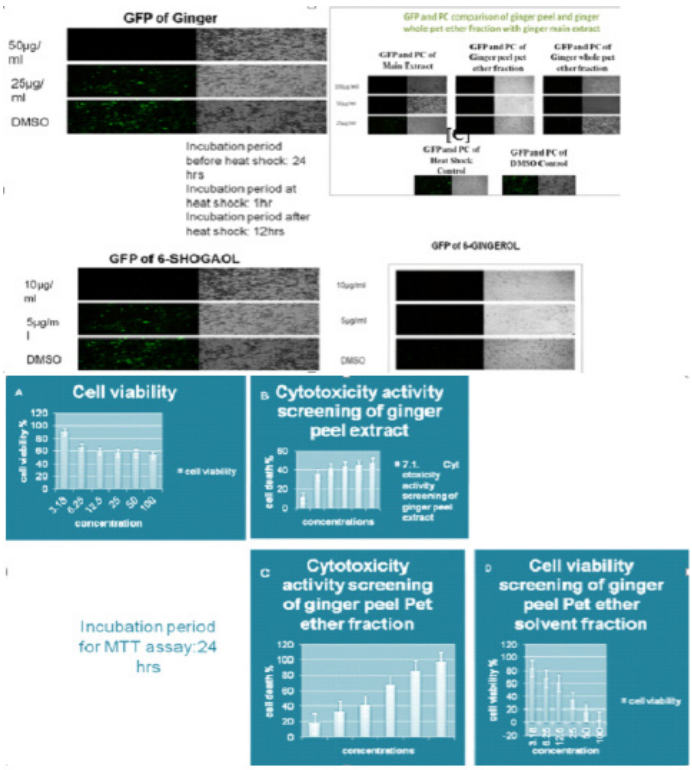


Figure 3: [A] GFP of Ginger rhizome extract, GFP and PC comparison of ginger peel and ginger rhizome pet ether fraction with ginger main extract. GFP of 6-shogaol, GFP of 6-Gingerol. [B] Cell viability and cytotoxicity activity screening of ginger rhizome peel extract and fractions cell death vs concentrations in MTT assay.

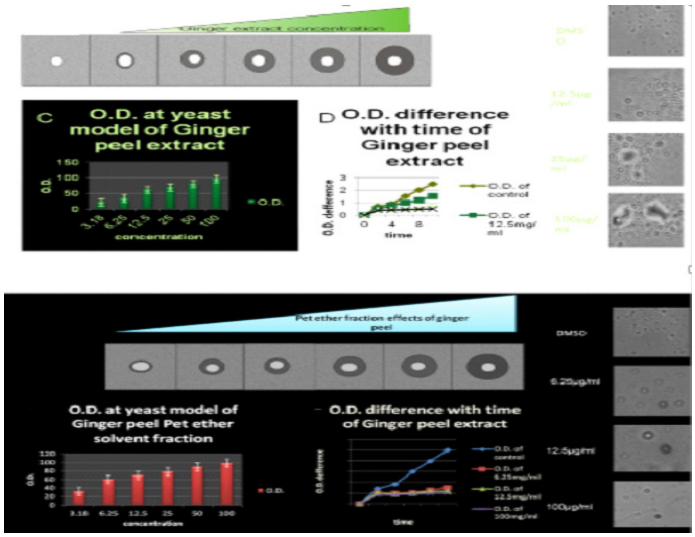


Figure 4: O.D. at yeast model with time variations screening of ginger rhizome peel extract and fractions O.D. vs concentration and O.D. vs time. pet ether fractions effect on yeast model.

DISCUSSION

The results of phytochemical analysis showed the presence of alkaloids (34.6%), flavonoids (4.8%), glycosides (2.4%), triterpenoids (22.2%) and phenolic content (36%) in Ginger peel extract and alkaloids (0%), flavonoids (1.8%), glycosides (1.0%), triterpenoids (12.4%) and phenolic content (84.8%) in PEF. Good results obtained for the antifungal activity[3,10] of the extract and subsequent fractions derived from *Zingiber officinale* Linn. peel against the tested pathogens, which are showing good antifungal activity in the study. Also the extract and subsequent fractions derived from *Zingiber officinale* Linn. peel displayed a strong antibacterial activity[3] against the tested organisms. The LC_{50} of PHGF was found to be 18 µg/ml, which was threefold higher than that of synthetic standard podophyllotoxin, i.e., 50 µg/ml, while PEF showed little bit lesser response (21 µg/ml) in comparison to PHEF but which also two and half fold higher than standard.[12,13] PHGF showed significant reduction in the mitotic index of onion root tips with increase in concentration. Antimitotic activity was found to be significant at 10 mg/ml and 5mg/ml of PHGF (0.02 ± 0.02; $P < 0.05$) when equated with the control mitotic index (0.43 ± 0.02),[15,16] and was also more than that of standard cyclophosphamide (0.04 ± 0.01; $P < 0.05$). pet ether extract of ginger peels extract and its Pet ether fraction and also its 2 isolated compounds (6-shogaol and 6-gingerol) have good HSF1 inhibition activity and creating apoptosis.[16,17] 6-shogaol showed good GFP at 10µg/ml and 6-gingerol compound good GFP at only 5 µg/ml. In MTT assay cell viability was showing ±54% in ginger peel extract sample dose of 100µg/ml. When the pet ether fraction of ginger peel extract was tested, result showed the ±96 % cell death in dose of 100µg/ml. We observed cell viability of 90%, 60% and 94% in the case of the wild-type yeast strain. In consideration of the two presented methods fail to control whether the cells incapable to reproduce are dead, an alternative method was adopted, time-lapse photo microscopy of separate yeast cells[19,20] in the presence of the vital dye Methylene blue is a dye absorbed by cells; metabolically lively cells are able to pump it out and endure colourless, (Figure 4) but dead cells are stained blue. Cells unable to reproduce were often mistakenly regarded as dead. These differences of colours were observable only after 8 h of culture, whereas they were not visible after 24 h. Cells bright to replicate and their offspring produce a colony, thereby completely masking the occurrence of the nondividing and

dead cells. A high number of dead cells was noted in the circumstance of incubation of the yeast cells with pet ether fractions, while a lower number was noted in the circumstance of incubation with another fractions. This method even nevertheless not a quantitative one, has an absolute advantage in comparison to the CFU method being simple and fast, permitting the dead cells to be distinguished and counted and also permitting differentiation between the alive cells that are able and unable to reproduce.

In comparison to the cell viability extent process based on the cell growth, the procedures based on colorimetric or fluorescent dyes are much quicker and give more quantifiable results by using methylene blue. The use of these dyes allows for analysis of individual yeast cells which penetrates into every cell. Living cells enzymatically decrease the dye to a colourless product and become unstained, but the dead cells are stained blue. We observed cell viability of 90%, 60% and 94% in the case of the wild-type strain.[21,22,24] Our results by using methylene blue dye showed that after exposure to oxidants, there are few cells that are unable to reproduce but they are still alive in nature (Figure 4).

CONCLUSION

So, evaluating all these above results, we can easily get a conclusion that Pet ether fraction and 2 isolated compounds (6-shogaol and 6-gingerol) from Pet ether fraction have good anticancer drug potentiality which fraction was isolated from biowaste peel of *Zingiber officinale* Linn.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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