Fermentative preparation of functional drink from *Punica granatum* using lactic acid bacteria and exploring its anti-tumor potential

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Fermentative preparation of functional drink from *Punica granatum* using lactic acid bacteria and exploring its anti-tumor potential

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Abstract. In the present research work probiotic pomegranate juice production by fermentation was carried out using two different strains such as *Lactobacillus plantarum VITES07 and Lactobacillus acidophilus NCIM2903* (Lactic acid bacteria). Fermented pomegranate juice was carried out at room temperature for 72h. During the fermentation period at regular intervals viable cells was determined. Efficiency of the fermented juice was analysed for 4 weeks under refrigerated condition at 4˚C. Total phenolics, sugar concentration, antioxidant potential, and antibacterial activity were determined. Organic acid concentration was determined by HPLC with retention time of a compound at 9.1 can be suspected to be Kaempferol hexoside and functional group was determined by FTIR also LCMS analysis was carried out to enumerate the chemical composition of the fermented juice.

1. Introduction

Pomegranate (*Punica granatum*) is a native fruit of Persia. The name *Punica granatum* is derived from Medieval Latin pomum meaning apple and *granatum* meaning seeded. Pomegranate is one of the fruit which is a source of vitamins and other supplements. The juice of pomegranate consists of more fibers and dietary supplements when compared to the fruit. But one of the disadvantages of the juice is not being able to store for long period of time without addition of preservatives. The key role of food industry is to develop food for consumer and their well-being. Many Food industries target on functional foods. This has increased the production and consumption of functional foods. Functional food is one where new supplements are added to a food and the new product has an additional function. Consumption of functional foods when added with active components like probiotics which prevents disease. Probiotic food is defined as which contains live microorganisms that promote health of the host. Probiotics may influence the host by expanding its intestinal microbial population beyond the amount already existing, therefore perhaps inhibiting pathogens [4,6]. Probiotics are increasingly used as functional beverages. According to scientific research, the maintenance of a healthy gut micro flora may provide protection against gastrointestinal disorder including infections and inflammatory disorder of the bowel [4]. Many researchers recommended that fruit juices contains suitable and number of components for the cultivation of probiotic bacteria [6]. Fruit juices has major role in market sector as functional drink [16]. Fruit juices such as cabbage, beetroot, tomato and carrot as raw materials are used for the production of probiotic beverages. *L. casei, L. acidophilus* and *L. plantarum,* are generally used in probiotic bacterial cultures. The main feature of Lactic acid bacteria is to increase the shelf life of fruit juices such as Pomegranate (*Punica granatum*) which has health-promoting properties with antiviral, anti-atherosclerotic antimicrobial, anti-mutagenic, anticancer, antioxidant, and anti-inflammatory agent effects [15]. The

fermentation two different strains of probiotic lactic acid bacteria was selected *(L. plantarum, L. acidophilus*) [5]. The raw pomegranate juice constitute of water with 85.4% and equal amounts of total sugars, total soluble solids (TSS), phenolics, reducing sugars, ascorbic acid, proteins, and thiols have been reported which are the enrich sources of antioxidants. On molar basis antioxidants levels are high, rather than other antioxidants like coenzyme Q-10, vitamin E, alpha-lipoic acid, and vitamin C [2]. Antioxidant have a free radical scavenging properties, chelating the metal ions and decomposing the peroxides. Polyphenols is a type of antioxidant containing phenolic substructure. The main source of polyphenols is a dietary source that has been determined. Therefore, the antioxidant activity can be determined by different chemical tests [9]. An organic acid level in juice determines taste, spoilage of juice or freshness due to the organic acid levels that is present in each type of fruit juice this makes difference between fermented juices and raw juices. Similarly, sugar concentration (glucose and fructose) are important markers to determine sugar level in fruit juices.

The aim of this current study was to define the growth curve in the pomegranate fermented juice using selected probiotic Lactic acid bacteria and the study also includes to determine their viability during cold storage conditions and also to analyze the Antioxidant activity, Antibacterial activity, along with total phenolics, reducing sugar contents, the organic acid determination by HPLC, the functional group analysis by FTIR and anti-tumorous property of raw and fermented pomegranate juices for comparative study.

2. Methodology

2.1. Chemicals

Chemicals used were of analytical grade. Ascorbic acid, Gallic acid, Citric acid, Oxalic acid, Folin-Ciocalteu reagent, Di Nitro Salicylic Acid were procured from Sigma company, Man Rogosa Sharpe broth, Muller Hinton agar, Nutrient broth, Sodium hydroxide, Methanol, Potassium per sulfate, Sodium carbonate, , Glucose, Fructose, Rochelle salt, DPPH, , Sodium sulfate were supplied by Hi Media Lab., Mumbai, India.

2.2. Preparation of Pomegranate juice

Pomegranate fruit was bought from the local market of Vellore, washed and peeled. The juice was prepared with only pericarp and distilled water. The juice was checked for initial brix using a Refractometer. The juice was then transferred to a sterile conical flask and pasteurized at 80^{o}C in a water bath for 5-10 minutes.

2.3. Preparation of co-culture

Probiotic lactic acid bacteria (*Lactobacillus plantarum* VITES07 and *Lactobacillus acidophilus* NCIM2903) were inoculated in MRS broth and were incubated at 37°C for 24h. Then, the broth was centrifuged at 4000g, for10 minutes at 4°C and the pellet was suspended in the pomegranate juice.

2.4. Fermentation of pomegranate juice

Initial pH of the juice was measured. Pasteurized juice were inoculated with the pellet of the centrifuged MRS broth under sterile condition and the juice was kept for fermentation at room temperature for 3 days and after completion of fermentation it was stored at 4^{o}C. During fermentation period the pH and viability of the organism was checked at 10^{-7} cfu/ml by using MRS agar and spread plate technique.

2.5. Determination of reducing sugar by DNSA method

The reducing sugar concentration in the raw pomegranate juice and fermented pomegranate juice was determined by DNSA method by taking working standard of glucose concentrations (0.4 to 2 mg/ ml). To all the test tubes 3 ml of DNSA reagent was added and incubated in water bath for 6 minutes and the absorbance was measured at 540 nm using a colorimeter.

2.6. Determination of Antioxidant activity by DPPH (1,1-Diphenyl picryldihydrazine) method

About 2 ml of DPPH was added to each test tube. 2 ml of raw pomegranate juice and 2ml of fermented pomegranate juice were considered as test samples. For positive control 2 ml of ascorbic acid were added. Then, the tubes were incubated at 37°C for 20 minutes. The absorbance value was measured at 515 nm. [5]

2.7. Estimation of poly phenol content by folin-ciocalteu method

Gallic acid standard was prepared from 50µg - 500µg/ ml, the volume in each test tube was made up to 1ml. 0.125 ml of half strength folin - ciocalteu reagent was added to all tubes including test sample. Test sample was prepared by diluting fermented pomegranate juice for ten dilution factor. The test tubes were incubated at room temperature in dark for 8 minutes. After 8 minutes 300µl of sodium carbonate (25 %) was added to all the test tubes. The absorbance was measured at 764 nm [7].

2.8. Determination of Antibacterial activity by agar well diffusion method

Agar well diffusion method was denoted for the determination of antibacterial activity [20] using Muller Hinton Agar. On the solidified agar bacterial culture of *Staphylococcus aureus, Listeria monocytogens MTCC657, Salmonella typhi NCIM05* was swabbed. The wells were made using cork borer and different concentrations of fermented pomegranate juice and raw pomegranate juice was added to different wells ranging from 20µl -140µl. Control was maintained. Then the plates were incubated at 37°C for 24h. The zone of clearance was measured [12].

2.9. Fourier Transform Infrared Spectroscopy analysis

The FTIR spectrophotometer, denotes the structural information about the different vibrational modes. Fermented pomegranate juice was inserted in KBr matrix and pellets were prepared by using hydraulic press. The FTIR spectra were recorded in the range of frequency 400-4000 cm^{-1} at the resolution of 4 cm^{-1}(company shimadzu and model- ir affinity-1).

2.10. Analysis by High Performance Liquid Chromatography

Quantitative analysis was carried out by HPLC with a K2600- UV Visible detector. A column separation of C_{18} with 2.25 mM sulphuric acid as the mobile phase and 20µl of injection volume sample of at flow rate of 0.2 ml/min. organic acid were reported by using standards. (Knauer, Germany)

2.11. Hemolytic activity

Blood agar well diffusion was done to test hemolytic activity. To the sterilized nutrient agar media prepared, 2.5% of blood was added when temperature of the media was around 40°C it was mixed properly and poured in to a petriplate for solidification. After solidification wells were made on the media using an 8mm cork borer and to the wells different concentrations of fermented pomegranate juice were added and the plates were placed in the refrigerator for one hour for juice to settle. The plates were incubated at 37°C for 24 hours. The plates was observed for hemolytic activity.

2.12. Antitumor activity

The cells were developed in a 96-well plate in Duelbacco Minimum Eagle'sl Medium (DMEM) (HiMedia) upgraded with 10% fetal cow-like serum (Gibco Laboratories) and anti-infection agents (streptomycin, penicillin-G, kanamycin, amphotericin B) [13, 14]. Around 25 µL cell suspension (5 x 103 cells/well) was seeded in each 96 well plate and brooded at 37°C for 48 h in 5% CO2 for the blended monolayer development. The monolayer of cells in the plate was presented to various weakenings of the aged pomegranate juice. The cell suitability was watched utilizing MTT test (MTT - 5 mg/mL and 10% DMSO). This tetrazolium salt is metabolically diminished by practical cells to create a blue insoluble Formozan item measured at 570nm in spectrophotometer [14]. Controls were kept up all through the trial (untreated wells as cell control and diluents regarded wells as diluent control). The examine were performed in the copies. The mean of the cell suitability esteems was contrasted with the control to characterize the impact of the concentrate on cells and level of cell practicality was plotted against convergence of the aged pomegranate juice.The antitumor measures were executed on Human liver tumor cell lines (HepG2) acquired from National community for cell science, Pune, India. The cells was additionally kept up and passaged in Cell culture lab. The measure was performed in copies for each examples. The convergence of test tests extended from 50-500 µg/ml. The mean of the cell feasibility esteems was contrasted with the control to characterize the impact of the concentrate on cells. A diagram was plotted against the rate cell practicality Vs weakening of the concentrate. The most minimal grouping of concentrate was poisonous to liver disease cells was recorded as the powerful medication fixation with contrast with positive control, for example, Doxorubicin with 5mg/ml focus.

3. Results and discussion

3.1. Viability of Lactic acid bacteria

The achievability of the Lactic corrosive microscopic organisms in the juice were observed to be for 10 days. The bacterial cfu/ml rate is as specified in table 1 According to Mousavi et al., 2010 [14] microbial populace of *L. Plantarum* and *L. acidophilus* was diminished amid the primary seven day stretch of chilly stockpiling at 4˚C and following 2 weeks practicality was lost. The reason detailed was because of absence of their capacity to get by in the unpleasant state of low pH and high causticity of the pomegranate juice and furthermore moderately because of low ecological temperature.

Table 1. The viability of bacterial co culture during fermentation

Organism	Time (h)	Cfu/ml
Lactobacillus acidophilus	0^{th}h	$2.75x10^{-5}$
	2h	$3.24x10^{-5}$
Lactobacillus plantarum	48h	5.80×10^{-5}
	72h	$6.75x10^{-5}$

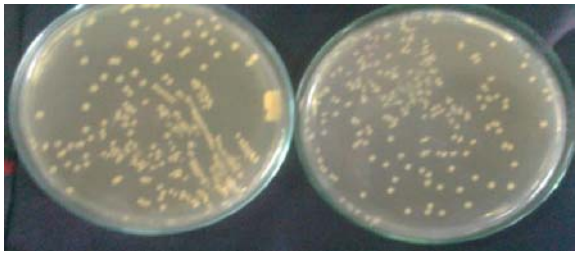


Figure1. The *Lactobacillus* viability count method by spread plate method at 10^{-7} cfu/ml

3.2. Determination of reducing sugar by DNSA method

The concentration of reducing sugar in fermented pomegranate juice was found to be 84.943µg/ml. The initial concentration of raw pomegranate juice was found to be of 106.58µg/ml. which can be reported that the sugar present in the juice has been utilized by the Lactic acid bacteria (co culture) during fermentation process.

3.3. Antioxidant activity by DPPH method

Antioxidant has scavenging properties. Antioxidant property of raw pomegranate juice was found to be 63% and for fermented pomegranate juice it was found to be 79.84%. The value observed at 515 nm for antioxidants is as following in Figure 2. As earlier reported using eight different pomegranate juice which has antioxidant activity ranging from 73.0–91.8%.

Formula

$$\% \text{ DPPH activity} = \frac{\text{Control} - \text{test}}{\text{Control}} \text{X } 100$$

For raw juice

$$\% \text{ DPPH activity} = \frac{2.041 - 0.52702}{2.041} \text{X } 100$$

% DPPH activity = 63 %

For fermented juice

$$\% \text{ DPPH activity} = \frac{2.041 - 0.448391}{2.041} \text{X } 100$$

% DPPH activity = 79.84 %

From the above calculation it can be observed that there is increase in 16.84% of antioxidant level after fermentation of pomegranate juice.

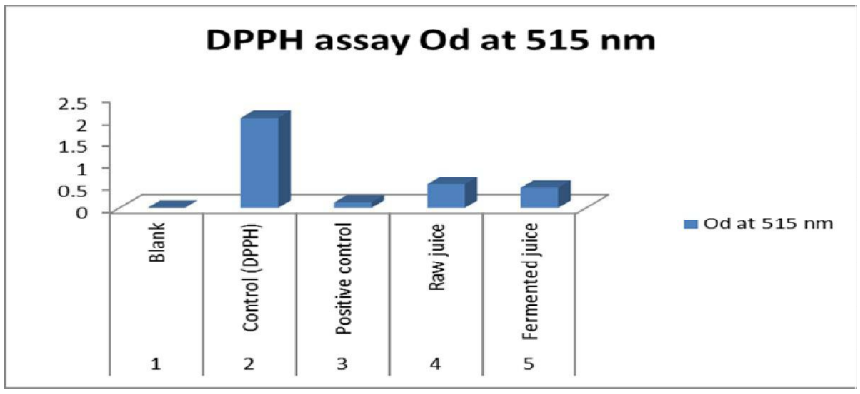


Figure 2. Estimation of DPPH activity

3.4. Estimation of Poly phenols by Folin's ciocalteu method

The concentration of poly phenols in fermented pomegranate juice was estimated to be 160µg/ml and for raw pomegranate juice it was estimated to be 75µg/ml. which when calculated to 1litre it was reported to be of 1.6 X 10^{-5} mg/l for fermented pomegranate juice where as for raw pomegranate juice it was reported to be 7.5 X 10^{-5} mg/l. which shows 85% of increase in poly phenol content of the juice after fermentation process.

3.5. HPLC analysis

From the chromatogram by comparing with standard chromatogram it was found that there are some components were found in the fermented juice which was not found in raw pomegranate juice which can be reported that Lactic acid bacteria (co-culture) inoculated to the raw pomegranate juice plays an important role for producing new components. The raw pomegranate juice chromatogram was reported with three peaks, which on further fermentation and analysis of sample at 24h interval it was reported with additional six peaks when compared to raw pomegranate juice chromatogram. The pomegranate juice was fermented for 72h to complete fermentation process and the chromatogram obtained for 72h fermented pomegranate juice was reported to have an increase in number of peaks with additional five peaks. The increase in number of peaks can be suggested due to fermentation of *Lactic acid* bacteria. The retention time of a compound at 9.1 can be suspected to be Kaempferol hexoside it has been reported by Pedro Mena. Kaempferol hexoside is suggested to have an antitumor potential which is produced during fermentation process as a byproduct by *Lactic acid* bacteria (co culture) which is said to have potential to inhibit the growth of tumor cells cytotoxic activity where fermented pomegranate juice inhibits the growth of cancer cell lines which is reported in table when compared with doxorubicin which is an anthracycline antibiotic.

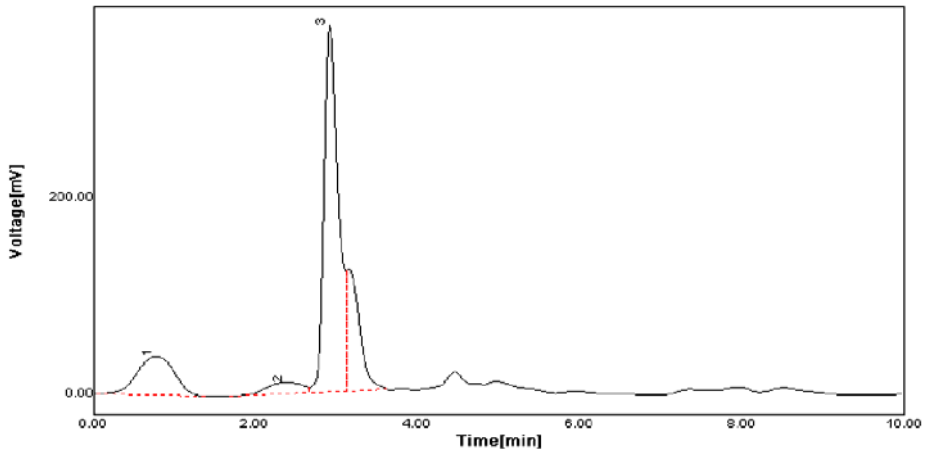


Figure 3. HPLC analysis for raw pomegranate juice

Table 2. Components present in the raw pomegranate juice

Peak No.	RT[min]	Area[mV*s]	Height[mV]
1	0.7667	1264.7806	39.5866
2	2.3833	348.3755	11.5880
3	2.9000	4635.1553	372.5527
Sum		6248.3115	423.7274

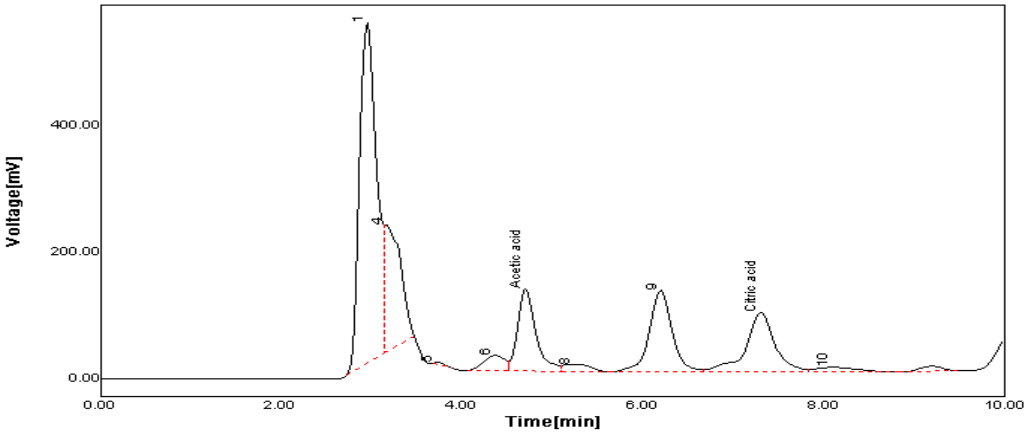


Figure 4. HPLC analysis for fermented pomegranate juice 24h

Table 3. Components present in 24 hour fermented pomegranate juice

Peak No.	RT[min]	Area[mV*s]	Height[mV]
1	2.9500	7110.9956	537.1661
2	3.1667	2424.8381	202.0718
3	3.7167	21.6437	3.1277
4	4.3667	399.6543	24.3519
5	4.7000	1804.2274	130.3569
6	5.2333	244.1536	11.8582
7	6.2000	2292.1296	128.8823
8	7.3000	2091.1265	94.2627
9	8.0833	251.7563	7.6712
Sum		16640.5254	1139.7489

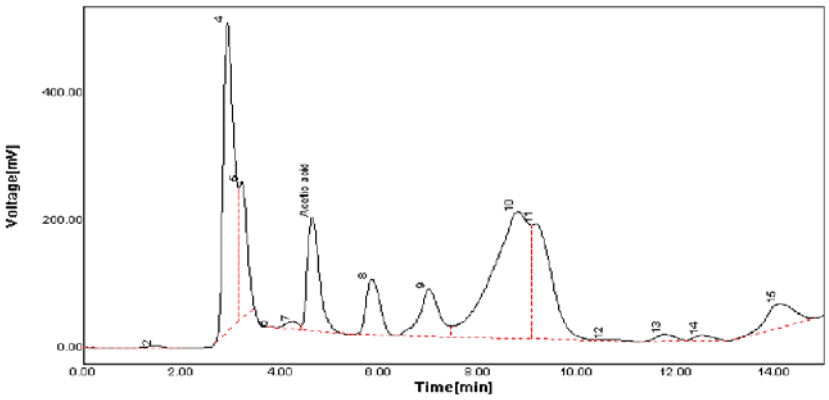


Figure 5. Fermented pomegranate juice after 72 hour

Table 4. Components present in 72 hour fermented juice sample

Peak No.	**RT[min]**	**Area[mV*s]**	**Height[mV]**
1	1.4500	117.3689	3.9265
2	2.9167	7432.1611	483.8552
3	3.2167	2334.1394	215.1001
4	3.8000	5.5075	0.7320
5	4.2500	215.6858	12.0234
6	4.6333	3012.2520	177.2495
7	5.8333	1687.4169	87.2656
8	6.9833	2065.0854	74.1280
9	8.7833	10189.2666	198.2570
10	9.1667	5031.5381	180.1830
11	10.6000	71.9985	2.4105
12	11.7667	277.9182	10.7578
13	12.5333	209.9920	7.8356
14	14.0833	1503.2129	37.7287
Sum		34153.5430	1491.4528

3.6. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of raw pomegranate juice and fermented pomegranate juice were analysed. FTIR spectra of raw pomegranate juice showed OH ,C=C stretching were observed at 3275.13 cm^{-1} and1631.78 cm^{-1} where as in case of 24 hr fermented juice showed methyl C-H asymmetric/symmetric and cyclic ethers, large rings, C-O stretching at 2945.30 cm^{-1} and1080.14 cm^{-1} .After 48 hr fermentation showed C=C compound,Alkyne C-H bend,OH stretching at 989.48 cm^{-1} ,653.87cm^{-1} ,3304.06 cm^{-1}. FTIR spectra of 72 hr fermented juice showed open chain imino (-C=N-) C-O stretch,trans C-H out of plane bend, secondary amine stretch at 1631.78 cm^{-1} ,1080.14 cm^{-1} ,989.48 cm^{-1} ,1145.72 cm^{-1}. It was concluded that due to fermentation process by co-culture of *Lacto bacilli* lead to formation of new products which was identified by using an external data (Figure 6).

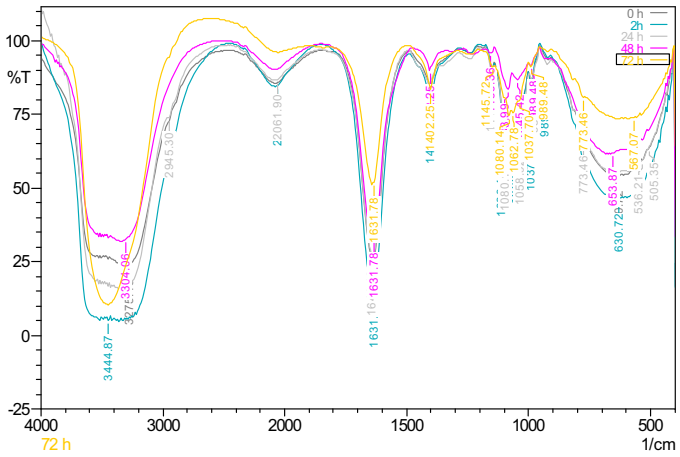


Figure 6. Raw pomegranate juice densitogram 24h, 48h, 72h

Table 5. FTIR analysis of pomegranate juice

Group frequency (cm^{-1})	**Functional group/assignment**
3275.13	Hydroxy group, H- bonded OH stretch
1631.78	Alkenyl C = C stretch
1402.25	Phenol or tertiary alcohol
1147.65	cyclic ethers, large rings, C-O stretch
1080.14	primary amine, CN stretch
1058.92	aliphatic phosphates (P-O-C stretch)

1037.70	organic siloxane or silicone (Si-O-Si)
987.55	aromatic ring aryl 1,3 distribution (meta position)
630.72	alkynes C-H bend
2945.30	methyl C-H asymmetric/symmetric stretch
2061.90	transition metal carbonyls
1641.42	secondary amine, NH bend
1408.04	vinyl C-H in plane bend
1149.57	secondary amine, CN stretch
1080.14	cyclic ethers, large rings, C-O stretch
1058.92	skeletal C-C vibrations
987.55	trans C-H out of plane
773.46	aliphatic bromo compounds
536.21	C-Br alkyl compound
505.35	C-I alkyl compound
3304.06	hydroxy group, H- bonded OH stretch

1631.78	open chain imino (-C=N-)
1402.25	C-Cl or C-Br aromatic compound
1155.36	secondary alcohol stretch
1083.99	alkyl substituted ether, C-O stretch
1045.42	aliphatic phosphates (P-O-C stretch)
989.48	C=C compound
653.87	alkyne C-H bend
4000- 3000	not determined
1631.78	open chain imino (-C=N-)
1402.25	C-Cl or C-Br aromatic compound
1145.72	secondary amine stretch

1080.14	cyclic ethers, large rings, C-O stretch
1062.78	skeletal C-C vibrations
1037.70	organic siloxane or silicone (Si-O-Si)
989.48	trans C-H out of plane bend
773.46	aliphatic bromo compounds C-Br stretch
567.07	C-Br alkyl

3.7. Antibacterial assay

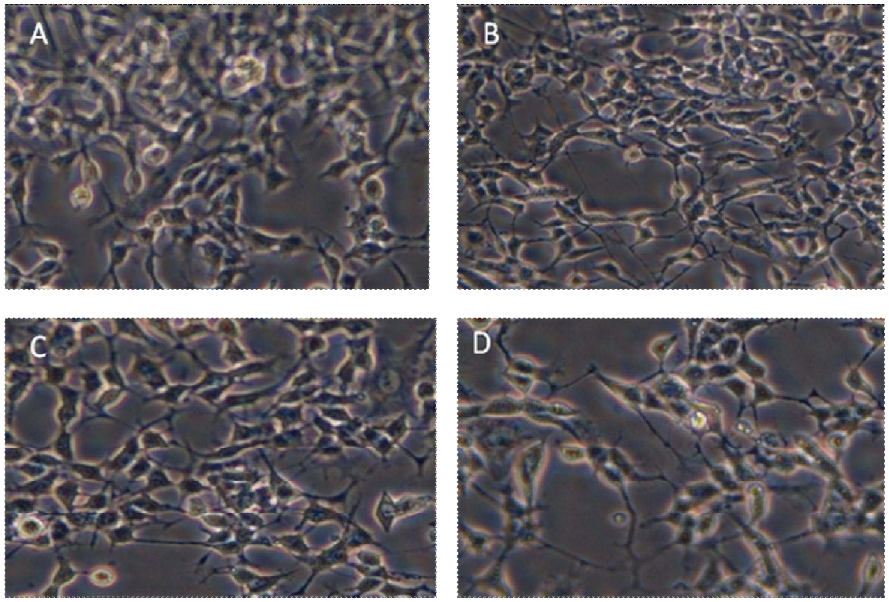
The antimicrobial activity of pomegranate were previously studied. It is reported that the bark, fruits, flowers and leaves of pomegranate are broadly used as phytotherapeutic agents in Brazil [12]. It has been reported from earlier works that alcoholic extracts of pomegranate juice is responsible for antibacterial property. Prashanth et al., 2015 [21] also reported that methanolic extracts of *Punica granatum* juice showed antibacterial activity against all the bacteria tested. In some paper they reported that the antibacterial property of pomegranate juice varies with variety depended on the contents of citric acid, phenolic compounds and pigments. Some research papers reported that solvent extract done by acetone when evaluated against Gram positive and Gram negative showed highest antibacterial activity when compared with methanolic extracts and aqueous extract. Results obtained in our study proved that it had less antibacterial activity when compared with methanolic extracts and acetone extracts. The diameter of zone of inhibition observed with solvent extract was reported to be of 18-25 mm in our study it was observed to be 1.65- 2.5cm.

Table 6. Antibacterial assay of *Salmonella typhi (NCIM05)* and *Listeria monocytogenes (MTCC657)*

Organism	Concentration µl	Zone of inhibition
Listeria monocytogenes(MTCC657)	80	1.65
	100	1.75
	120	2
	140	2.15
Salmonella typhi(NCIM05)	100	2.1
	120	2.1
	140	2.5

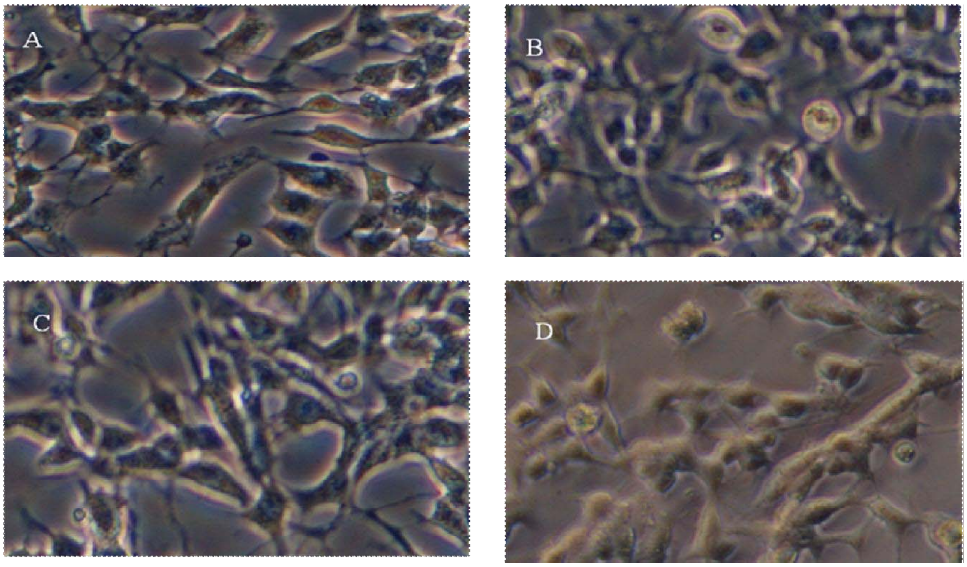
3.8. Hemolytic activity

Hemolysis was observed on the blood agar plate for fermented pomegranate juice, but not for raw pomegranate juice. This report that fermentation of pomegranate juice consists of a component for hemolytic activity. As positive results were observed for hemolytic activity, it was further subjected to cytotoxicity test for various cell lines. As it was reported that presence of hydroxylcinnamic acid present in juice decreases the cancer cell metastasis through down regulation of metalloproteinase expression [7]. Epicatechins and Catechins have Reversal of P-glycoprotein mediated multidrug resistance.



A-Pomegranate 6 µl treated cell, **B**-Pomegranate 12.5 µl treated cell, **C**-Pomegranate 25 µl treated cells, **D**-Pomegranate 50 µl treated cells

Figure 7. Fermented pomegranate juice to check its antitumor potential



A-doxorubicin treated cells 6µl, **B**-doxorubicin treated cells 12.5µl, **C**-doxorubicin treated cells 25µl, **D**-doxorubicin treated cells 50µl.

Figure 8. Doxorubicin treated cells to check its antitumor potential

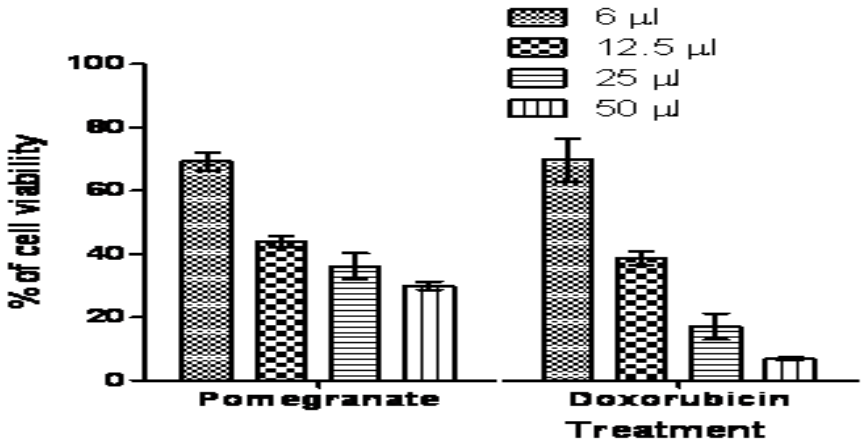


Figure 9. Graph representing viability of liver cancer cells against fermented pomegranate juice and doxorubicin.

3.9. Anti-tumor potential

The antitumor potential of fermented pomegranate juice was measured by various concentrations of toxicity. The well with 6µl showed 30.705% of lysis whereas 12.5µl of juice showed 55.995% of cell lysis. 25µl of juice showed 29.74% of lysis and 50µl of juice showed 70.08% of cell lysis. This shows that increase in the concentration of pomegranate juice is directly propotional to its cell lysis activity. The components present in the fermented juice are responsible for anti-tumor potential [3].

Gallic corrosive causes hindrance of development and apoptotic demise of human DU-145 prostate malignancy cells [16]. Cell aggravation diminish the pancreatic stellate [17]. Apoptosis advance the G0/G1 to the S stage, and COX in HL-60 leukemia cells. Flavonols, for example, kaempferol express the disease rot factor-interleukin-1_ quality articulation in tumor cells [1]. Acts synergistically with quercetin to hinder bosom tumor cell expansion [1]. Catechin is a flavan – 3-Ol, a sort of common phenol and cancer prevention agent, which is a plant optional metabolite. In trans setup it is catechin and in cis arrangement it is epicatechin. Catechin has been discovered the most effective forager among various classes of flavonoids. It is accounted for that catechin hinders intestinal tumor arrangement in mice [13], restrains the oxidation of low thickness lipoprotein [11], brooding trials with catechin demonstrated aversion of human plasma oxidation [10]. It is valuable in decrease of histamine related neighborhood insusceptible reaction as catechin is a histidine decarboxylase inhibitor. Epicatechin and catechin are additionally specific monoamine oxidase inhibitors (MAOIs) of sort MAO-B. They can be utilized to decrease side effects of Parkinson's and Alzheimer's patients [17]

Table 7. Toxicity in MCF-7 cells

Concentration	6µl	12.5µl	25µl	50µl
Antitumor compound Fermented pomegranate juice	69.205	44.005	72.26	29.92
Doxorubicin	69.745	38.595	17.02	6.845

Table 8. IC_{50} concentration

Test samples	IC50 in µl
Fermented pomegranate juice	11.12
Doxorubicin	8.63

4. Conclusion

From the various tests done it is reported that probiotic drink prepared from pomegranate juice by fermentation with co culture of Lactic acid bacteria has higher antioxidant level more than black tea and wine which on daily consuming leads to a healthy life style. The antioxidants present in the juice have various applications such as used to treat neurodegenerative diseases, cancer, coronary heart diseases. The system included is that cancer prevention agent hinders the oxidation of different particles. Oxidation is a procedure which includes compound response which exchanges electron from oxygen to hydrogen. Oxidation responses create free radicals. While these radicals begins chain responses. At the point when the chain response happens in a cell, it makes harm or demise the cell. Cancer prevention agent stops the harm to the cell by ending these chain responses by expelling the free radical intermediates, and hinders other oxidation responses. This leads to the survival of healthy cells without any damage. Various types of organic acids were determined by HPLC process such as formic acid which has an antibacterial and also inhibits the conversion of lactic acid to butyric acid during fermentation. It can support fermentation at low temperatures and reducing the nutritional loss. Oxalic acid helps in fermentation process with the help of lactate dehydrogenase enzyme which converts pyruvate in to lactate leading for fermentation lactate dehydrogenase enzyme also has an inhibitory effect on tumor formation and growth (le anne). Tartaric acid acts as an antioxidant. Gallic acid is also a source of antioxidant and also known to have antifungal and antiviral properties. It also has showed cytotoxicity against cancer cells (phytochemicals.info). Citric acid has a dominant effect as preservative and also flavor enhancing. Vanillic acid is an dihyddroxybenzoic acid which acts as flavor enhancer it is an intermediate compound of ferulic acid (chemicall). Malic acid is an organic compound which increases sour taste of the juice and also helps in malolactic fermentation process. To conclude, the present research work describes unreported lactic acid bacterial fermentation (co- culture method) of *Punica granatum* with high antioxidant potential, polyphenol content, antibacterial and antitumor activity.

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