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PUNICA GRANATUM L. WINE - HEALTH BENEFITS

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ABSTRACT: *Punica granatum L.* is the reddish colour fruit, commonly known as Pomegranate. It is known to have many health and therapeutic benefits which can be used as agent. The pomegranate juice is also used to prepare wine. The present study was carried out to determine the optimum fermentation parameters of pomegranate wine preparation. The antimicrobial and antioxidant activity of various wine preparations were also evaluated for their health benefits. The wines were prepared using various brix values under both aerobic and anaerobic conditions. The antimicrobial and antioxidant activity which was carried out using the agar cup method and DPPH assay respectively. The best wine was found to have 15.3% alcohol prepared with 0°B and aerobic conditions in 8 days. The antimicrobial and antioxidant activity of the wines prepared were higher than the fresh *P. granatum* juice. Therefore, we suggest that pomegranate wine is a better option of fruit juice storage having better antimicrobial and antioxidant properties.

INTRODUCTION: *Punica granatum L.* (Pomegranate) is a small tree of height around 5 to 8 m, cultivated in China, the Himalayas in Northern India, USA, and Mediterranean countries like Spain, Turkey, Egypt, *etc* [1]. More than 600 varieties of pomegranate are present throughout the world. Among those which fit for human consumption are much larger, about the size of a big red apple. This fruit is divided into several anatomical compartments including seed, juice, peel, leaf, flower, bark, and root with each having interesting pharmacological and toxicological activities. Edible fruit is a berry of rounded hexagonal shape, with around 5-12 cm in diameter and 200 g in weight.

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The fruit of the Pomegranate has been used as a traditional remedy against acidosis, dysentery, microbial infections, diarrhea, helminth infection, hemorrhage and respiratory pathologies [2]. Pomegranate seeds have also been shown to contain the estrogenic compounds: estrone and estradiol [2]. Furthermore, the dried pericarp and the juice of the fruit are considered beneficial for treatment of colic, colitis, menorrhagia, oxyuriasis, headache, diuretic, anthocyanin, piles, allergic dermatitis, and treatment of oral diseases [3].

Researchers have pinpointed a few direct beneficiaries of pomegranate intake: reduced cholesterol level, reduced plaques in arteries, lowering high blood pressure, prevent or reduce tumors in organs such as prostate. Pomegranate's high folic acid contents also make it an ideal supplement for woman for pregnancy and even skin beautification [2]. The high demand for pomegranate juice is the result of studies that have shown the health benefits of biological compounds,

specifically, phytochemicals, in pomegranates. The primary phytochemicals in pomegranates are the polyphenols, including anthocyanin pigments, flavonol glycosides, procyanidins, phenolic acids and ellagic acid derivatives [4]. Delphinidin-3,5-diglucoside is known as a major anthocyanin in pomegranate juice [5]. Anthocyanins possess antidiabetic, anticancer, anti-inflammatory, antimicrobial, and anti-obesity effects, as well as prevent cardiovascular diseases (CVDs) [6]. Pomegranate juice and peel also have catechins which have very high antioxidant and anti-inflammatory activity [6]. Ellagitannin is a type of tannins found in both pomegranate juice and peel is widely used in plastic surgeries [7]. Some other important compounds in pomegranates such as ellagic acid and ursolic acid also have antidiabetic properties [8].

The seed coats of this fruit have been processed and used as juice and wine as it contains a good amount of sugar [9]. Pomegranate wine is the product of anaerobic fermentation by yeast [10]. The use of baker's yeast *S. cerevisiae* in the making of fruit wine is one of the easiest and cheapest methods. There have been various studies on the methods and the factors affecting the pomegranate wine production using wine yeast [11, 12, 13, 14, 15].

Production of wines having medicinal and nutraceutical compounds would attract the consumers in terms of their health benefits. The extraction of these components from pomegranate juice into wine, offers a highly valued health drink. There are studies which says that, the pomegranate has a good antioxidant [16] and antibacterial property [17]. Thus, in this regard, alcoholic fermentation of pomegranate wine to extract the compounds into pomegranate wine and the effect of different concentrations of sugar on extractability of pomegranate compounds is attempted along with its antimicrobial and antioxidant activities was evaluated.

MATERIALS AND METHODS:

Sample Collection: Fresh pomegranate fruits were purchased from a local food market. The fruits were sectioned, and the seed coats were removed with a small stainless-steel knife. Seed coats from several fruits were thus collected. The seed coats were then blended using a blender and the juice obtained was filtered to remove debris. The filtered pomegranate juice was further used for wine preparation.

Wine Fermentation: Four flasks each containing 500 mL of pomegranate juice were set for wine production. 125 g of commercially available sugar was added in two flasks i.e. 25ºB (brix). While other two flasks contained 0ºB. In all the four flasks, 0.150 g of dried baker's yeast (commercial) i.e. S. cerevisiae was added. The anaerobic fermentation was carried out in an airtight glass container. While aerobic fermentation was carried out in glass flasks. Each of the fermentation type had two flasks: each having 0º B and 25º B sugar **Table 1**. The fermentation process was carried out for 8 days.

TABLE 1: TABLE FOR FERMENTATION FLASKS

Sr. no.	Concentration of flask (º B in terms of sugar)	Volume of fruit juice (in ml)	Amount of sugar (in grams)	Amount of yeast (in grams)
1	0º B Aerobic	500	0	0.150
2	25º B Aerobic	500	125	0.150
3	0º B Anaerobic	500	0	0.150
4	25º B Anaerobic	500	125	0.150

Alcohol Estimation: The alcohol estimation was measured using Friedemann and Klaas method [18]. In this method, each wine sample is added in 5ml Eppendorf tube and the tubes were placed with the thread and cello tape in the middle of conical flask containing 1ml of acid dichromate and incubated for 24 h. After the incubation period, wine sample was discarded and the 100ml of distilled water and 1 ml of potassium iodide (KI) was added in each flask. Resulting solution was titrated against sodium thiosulphate ($Na_2S_2O_3$).

The change in colour to faded colour was noted as first reading followed by two more readings for each flask. The blank was a solution of 10ml acid dichromate solutions, 10 ml distilled water and 1 ml potassium iodide.

The alcohol content was calculated using the formula mentioned below,

Volume of S_2O_3 = C.B.R (Blank) – C.B.R (Sample)

Conc. of ethanol = $\frac{(3x \text{ conc. } K_2Cr_2O_7 \text{ x Vol. } K_2Cr_2O_7) - 1/6}{(\text{conc. } S_2O_3 \text{ x Vol. } S_2O_3)}$

Volume of ethanol

Mass in grams = No. of moles x Molar mass

Density = Mass / Volume

Percentage of alcohol = Volume / 100 x 100

Antimicrobial Activity: The test organisms used to study the anti-microbial properties are *Escherichia coli, Staphylococcus aureus, Salmonella typhi* and *Bacillus subtilus.* All the cultures obtained from the Department of the Life Sciences, University of Mumbai. The antimicrobial assay was carried out byKirby- Bauermethod with some modifications. Ampicillin (100 mg/ml) was used as positive control and sterile distilled water as negative. 100µl each of five wine samples, pomegranate juice, Ampicillin and distilled water were added in the agar cups. The plates were incubated at 37ºC for 24 h. After 24h, zone of inhibition was measured in mm.

Antioxidant Activity: Antioxidant activity was performed with the DPPH free radical assay according to Brand-Williams *et al.* method [19] with some modifications. Ascorbic Acid (1000µg/ml) was used as a standard in the range of 100µg/ml to 1000µg/ml with 100µg/ml interval.

The 1ml extracts from each wine sample (0º B aerobic and anaerobic wine sample and 25º B aerobic and anaerobic wine samples) were treated with 1ml ethanolic DPPH solution. The samples were incubated for 30 min at 37°C. The color change was observed (from deep violet to light yellow) which was sphectrophotometrically analyzed at 517 nm. The ethanol was used as blank, and mixture of ethanol and DPPH was served control.

The concentration of the antioxidant activity was calculated using the standard graph from the slope, as it is difficult to calculate IC_{50} value as the sample is in liquid form; hence the percent inhibition was determined.

The following formula was used to calculate percentage inhibition:

% Inhibition = (Absorbance of control –Absorbance of sample) / (Absorbance of control) × 100

RESULTS:

Pomegranate Wine: All the four flasks i.e., 0º B aerobic, 25º B aerobic, 0º B anaerobic and 25º B anaerobic flasks were well fermented after 8 days of fermentation. The alcohol produced was determined by its fragrance. The Aerobic wine preparations were gave the best result of wine preparation as compared with anaerobic one. Also the preparation with 25º B sugar gave the best results along with good amount of alcohol (14.99%), aroma and colour of wine.

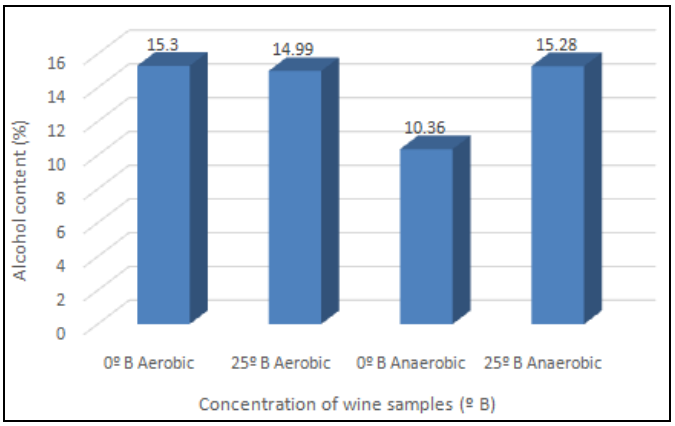

FIG. 1: ALCOHOL CONTENT OF WINES

Alcohol Estimation: The alcohol content was estimated by potassium dichromate method. When acid dichromate reacts with ethanol, it changes colour from yellow to blue. This colour change was observed in all the four flasks indicating the presence of alcohol in wine samples. The maximum alcohol content observed in 0º B aerobic (15.3%) and 25º B anaerobic (15.28%) whereas 0º B anaerobic has minimum alcohol content of 10.36% **Fig. 1**. It is observed that aerobic fermentation gave better alcohol content as compared to anaerobic fermentation.

Antimicrobial Activity: The positive results were observed in gram negative organisms as well as in gram positive organisms i.e. *E. coli, S. typhi and B. subtilus, S. aureus* respectively. Except 25º B Anaerobic wine sample showed negative results for gram positive organisms i.e. *B. subtilis* and *S. aureus.*

The best results were observed in the 25º B Aerobic wine sample followed by all the wine samples except 25% anaerobic wine sample. The highest zone of inhibition was 17mm shown by 25º B Aerobic wine sample but all the zone of inhibition were less than the zone of inhibition given by the positive control Ampicillin. 25% anaerobic wine sample was not showed antimicrobial activity **Table 2.**

The wine samples showed the zone of clearance against the gram negative organisms. In case of gram positive organism only the 25% anaerobic wine sample showed the negative results and rest of the other samples showed the zone of clearance **Fig. 2.** The results were compared with positive and negative control

TABLE 2: ZONE OF INHIBITION IN (NM) AGAINST MICROORGANISMS

Organism	Samples	Zone of inhibition (in mm)
Staphylococcus aureus	0º B Aerobic	13.5
	25º B Aerobic	17
	0º B Anaerobic	14.5
	25º B Anaerobic	0
	PG juice	10
	Ampicillin	32
Bacillus subtilis	0º B Aerobic	13
	25º B Aerobic	16
	0º B Anaerobic	15
	25º B Anaerobic	0
	PG juice	11
	Ampicillin	33
Escherichia coli	0º B Aerobic	12.5
	25º B Aerobic	15
	0º B Anaerobic	11
	25º B Anaerobic	12
	PG juice	11
	Ampicillin	35
Salmonella typhi	0º B Aerobic	14
	25º B Aerobic	16
	0º B Anaerobic	10.5
	25º B Anaerobic	11
	PG juice	9
	Ampicillin	35

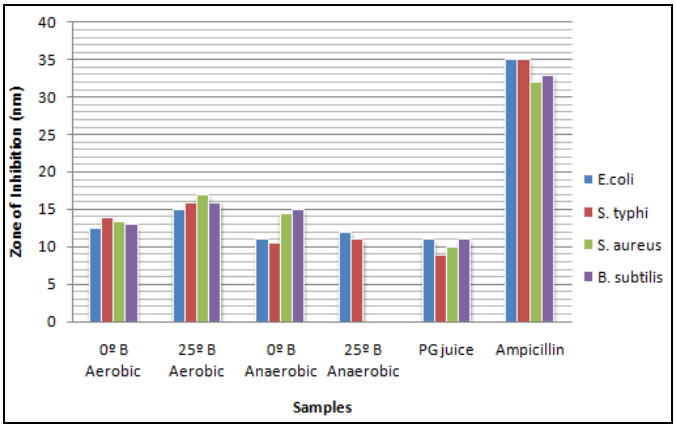

FIG. 2: ANTIMICROBIAL ACTIVITY OF WINES

Antioxidant Activity: The percentage inhibition is a measure of the antioxidant activity is a measure of DPPH scavenging free radical reaction of Ascorbic acid (standard) **Table 3**. The wine samples show positive antioxidant activity.

The concentration of 0ºB aerobic, 25º B aerobic, 0º B anaerobic and 25º B anaerobic wine samples and Pomegranate juice in the respective preparations was as follows: 110 µg/ml, 940 µg/ml, 170 µg/ml, 660 µg/ml and 100 µg/ml respectively determined from the slope of the standard plot of ascorbic acid inhibition **Fig. 3.**

It is comparable with the standard. The results show that 25º B aerobic wine sample had the highest inhibitory activity (82.09%). Other wine samples 0ºB aerobic, 0º B anaerobic and 25º B anaerobic had intermediate activity of 75.44%, 76.47%, 80.56% whereasPomegranate juicesample of had least inhibitory activity of 75.19% **Table 4**, **Fig. 4**. This indicates that Pomegranate wine samples have an anti-oxidant activity comparable with standard Ascorbic acid.

TABLE 3: ANTIOXIDANT ACTIVITY OF THE STANDARD I.E. ASCORBIC ACID

Sr. no.	Concentration (in µg/ml)	Absorbance at 517nm	% Inhibition
1.	100	0.098	74.93
2.	200	0.090	76.98
3.	300	0.080	79.53
4.	400	0.079	79.79
5.	500	0.078	80.05
6.	600	0.077	80.30
7.	700	0.075	80.81
8.	800	0.074	81.07
9.	900	0.071	81.84
10.	1000	0.068	82.60
11.	Control	0.391	0

TABLE 4: ANTIOXIDANT ACTIVITY OF THE SAMPLES

Sr. no.	Samples	Concentration (ml)	Absorbance at 517nm	% inhibition
1	0º B Aerobic	1	0.096	75.44
2	25º B Aerobic	1	0.070	82.09
3	0º B Anaerobic	1	0.092	76.47
4	25º B Anaerobic	1	0.076	80.56
5	PG juice	1	0.097	75.19

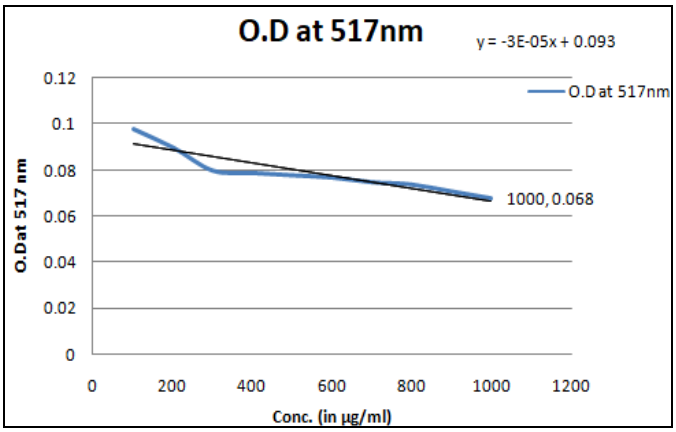

FIG 3: STANDARD GRAPH OF ASCORBIC ACID

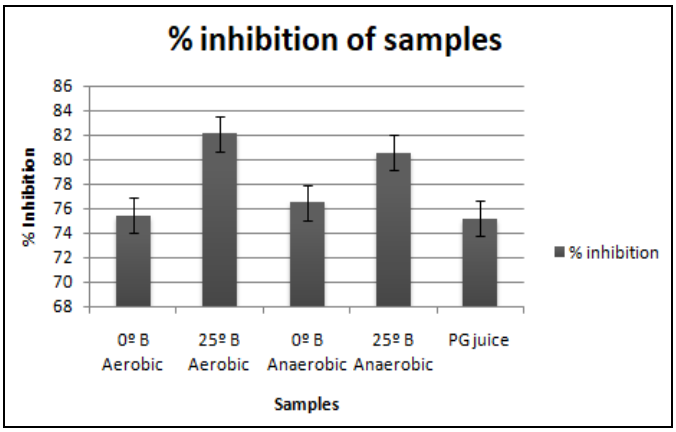

FIG. 4: PERCENTAGE INHIBITION OF WINES

DISCUSSION:

Pomegranate Wine: The Aerobic wine preparations were gave the best result of wine preparation as compared with anaerobic one. Also the preparation with 25º B sugar gave the best results along with good amount of alcohol (14.99%), aroma and colour of wine. The alcohol percentage of wines prepared was around 14-15% which is perfect for wine [20].

The fruits with high sugar content can be used for wine making using the easily available Baker's yeast and this was successfully practiced. Also, wine making using Baker's yeast was from practiced many since ancient times, and wine making from pomegranate juice using Baker's yeast was successfully tested [21, 22]. Pomegranate wine has several health benefits and it is easy for storage too. As the senesce of fruit occurs and the fruit juices may get contaminated leading to spoilage after certain period of time, wine is the best way for storage. As the old the wine gets, the quality of it improves. So, the wine from pomegranate juice is the best option to preserve the fruit and that too without adding any preservatives or additives.

Alcohol Estimation: The alcohol produced in wine preparation process was estimated by using [18]. The wine preparations were verified for amount of alcohol produced. All the wine preparations gave the results in the range of 14-15% except for 0º B Anaerobic wine preparation. The 14-15% of alcohol in wine is said to be ideal range of alcohol content in wine [20]. Therefore, in the present study the alcohol content of the pomegranate wine is in ideal range.

Antimicrobial Activity: The wine samples showed better antimicrobial activity as compared to Pomegranate juice. The wine samples gave a zone of clearance which was less than the positive control Ampicillin **Table 2** and it was approximately 65-70 % of the antibacterial activity of the positive control.

25ºB Anaerobic wine samples did not show antimicrobial activity against Gram positive organisms *S.aureus, B. subtilus* as compared to standard broad spectrum antibiotic Ampicillin. The results indicate the presence of antimicrobial activity in the wine preparation.

Ahmed and Beg [23] reported antimicrobial activity of alcoholic extracts of pomegranate fruit. The methanolic extracts of pomegranate fruit rind indicated presence of antimicrobial activity [24]. Methanol, ethanol and water extracts from pomegranate have shown similar antimicrobial activity [25]. It has been reported that the antibacterial action of pomegranate juice varied with variety and it depended on the phytochemical contents of the fruit like phenolic compounds, pigments and citric acid [26]. Dahham *et al* [27] demonstrated antimicrobial activity of pomegranate seeds against *Bacillus subtilis*, *Escherichia coli* and *Saccharomyces cerevisiae*. Pomegranate fruit peel compound punicalagin is reported to have antimicrobial activity against *S. aureus* and *P. aeruginosa* [4]. The wine samples showed better results than the pomegranate juice which contributes to its beneficial factor and reasons for its consumption.

Antioxidant Activity: The wine preparations show positive antioxidant activity. The concentration of wine samples of 0ºB aerobic, 25º B aerobic, 0º B anaerobic and 25º B anaerobic and Pomegranate juice was found to be 110 µg/ml, 940 µg/ml, 170 µg/ml, 660 µg/ml and 100 µg/ml respectively from the slope of the standard plot of ascorbic acid inhibition. The wine samples showed higher antioxidant activity than pomegranate juice [28]. The phenolic compounds found in fresh fruit juice are generally glycosylated with sugar that on fermentation of the juice and sugar consumption by microorganism undergo deglycosylation and release of free hydroxyl groups and relevant aglycones[15] which might be contributed to the improved antioxidant properties of the fermented wines. These samples which show higher antioxidant activity can be used in medicines, as the antioxidants are the one that prevents the oxidation of the chemicals and formation of free radicals which may damage the cell, cellular components and different metabolic reactions.

CONCLUSION: In the present study, the pomegranate wine and juice have shown antimicrobial and antioxidant properties when compared with standards. These properties can be utilized in health industry for production of different drugs which will make the drug cheaper as well as which has easily available raw material. Also it was found that pomegranate wine is more effective as compared to pomegranate juice, so to consumption of pomegranate wine in moderate amount can be useful for health.

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Author Contributions:

Madhura Joglekar: Investigation, Methodology, Data curation, Original draft preparation.

Neha Pandey: Investigation, Methodology, Data curation, Original draft preparation.

Suruchi Jamkhedkar*:* Conceptualization, Visualization, Supervision, Original draft preparation.

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