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Production, optimization, characterization and evaluation of antimicrobial activities in *Hibiscus rosa-sinensis* wine

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Abstract
Wine has been considered as safe and healthy drink, besides an important adjunct to the diet. The present work was carried out to develop process methodology for the production of Herbal wine from *Hibiscus rosa-sinensis*. The prepared *Hibiscus* based herbal wines were evaluated for their effect against common food borne pathogens. *Hibiscus* petals extract supplemented with sugar proved to be a good medium for the growth of different Strains of *Saccharomyces cerevisiae* for making the *Hibiscus* wine. The wine was found to be similar to any other wine in terms of its composition and sensory quantities. The wine exhibited bactericidal activity against common food borne pathogens (*Bacillus subtilis, S. typhimurium, S. aureus, E. coli*). Effect of different yeast strains of *Saccharomyces cerevisiae* var. *ellipsoideus*, viz. MTCC 178, MTCC 180, MTCC 786 Strains on the enological and sensory characteristics of *Hibiscus* wines was determined. Different yeast strains influenced the physico-chemical characteristics of the wines produced to a variable extent. The higher rate of fermentation was shown by MTCC 178, compared to MTCC 180 and MTCC 786. Out of various strains, MTCC 178 gave the highest efficiency along with higher ethanol yield. The MTCC 786 strain produced lowest ethanol in wine. The wine made with MTCC 178 had higher titratable acidity. TSS, acidity, pH, total sugar, ethanol and colour did not contribute to separation of yeasts. Sensory evaluation scores of different wines revealed that the must fermented with the yeast strain MTCC 178 had higher scores for colour, taste, flavour, consistency and overall acceptability. Antioxidant potential of different yeast strain wine was evaluated. The antioxidant activity was assessed by estimation of total phenolic & tannins contents, DPPH free radical scavenging activity and FRAP assey. amongest the three yeast strains screened for alcoholic fermentation of reconstituted *Hibiscus* petals extract, *Saccharomyces cerevisiae* MTCC 178 was found most potent strain. The optimal alcoholic fermentation of reconstituted *Hibiscus* extract by *Saccharomyces cerevisiae* MTCC 178 was recorded at 25^0c, initial pH of must 5.0 and total soluble solid 24^0 brix with an inoculam level of 10% (v/v). wine is very important along with other vinification practices as it influenced the chemical and sensorial quality of wine.

Keywords: *Hibiscus rosa-sinensis*, wine, fermentation, Ethanol, Principal Component Analysis, Sensory evaluation**,** Yeast, *Saccharomyces cerevisiae. Bacillus subtilis, Salmonella typhimurium, Staphylococcus aureus*, *Escherichia coli*, antibacterial activity.

1. Introduction
The consumption of a variety of local herbs and vegetables by man is believed to contribute significantly to the improvement of human health, in terms of prevention or cure of diseases because plants have long served as a useful and rational source of therapeutic agents.
Alcoholic beverages have been consumed by humans since the Neolithic era; the earliest evidence of alcohol was discovered in Jiahu, dating from 7000–6600 BC. The production and consumption of alcohol occurs in most cultures of the world, from hunter-gatherer peoples to nation-states.
Till recently, it was perceived that regular consumption of alcoholic drinks is the main reason for several health problems and reduced life-span. However, it has been proved now through various studies that moderate consumption of alcoholic drinks especially wines have better effect on health. Wine, an alcoholic drink is widely accepted, consumed, and preferred due to its nutritive and healthful properties. To enhance the basic qualities of wine, it can be fortified with certain additives that are potentially beneficial to health. Making use of such additives, the wine was fortified, with an aim to further increase the therapeutic applications of wine. For this fortification, extracts from some herbs having high medicinal values were used. Herbs, such as Holy-basil, *Hibiscus* petals, Lemon-grass, Peppermint and Ginger were chosen for fortification of wine which are known for therapeutic and medicinal applications. These Herbal extracts impart the similar properties to the fortified wines and hence, consumption of such fortified wines would give tremendous health benefits than regular wines.

Wine represents one of the examples of functional fermented foods and are a source of food energy. Each gram of alcohol provides 7.1 kcal, and each milliliter provides 5.6 kcal. Alcoholic beverages are worldwide produced and they are most popular around the world for several centuries. The most famous wines are produced in France and made from grape juice. However, several kinds of fruit such as pineapple, Kiwi include honey and rice are used for wine making. Various kinds of herbs and spices play an important role in alcoholic beverage production. They are used as enhancer, preservative and an antioxidant sources. Antioxidants can be found in all part of plants such as fruits, flowers, leaf, stem and root (Rodrigo, 2011). Therefore wines making from herbs are enrich with natural antioxidants. Numerous studies indicated that red wine consumption is associated with a protective effect against disease and ageing.

To improve the quality of life, extensive investments have occurred for the therapeutic applications of herbal plant-resources. Herbal preparations have been known to treat various infectious diseases throughout the history of mankind. It provides relaxation necessary for proper digestion and absorption of food and hence serves as a vital adjunct to the human diet. Strong antibacterial activity of wine is its essential biological function which has been verified under various experimental conditions. Wine serves as a base for medicinal preparations compounded with a range of herbs adapted to treat various disorders. Functional botanical ingredients are more admired than ever in the beverage market. Many wines are made from herbs with perceived medicinal value and such wines have many additional health benefits. Wine have many health benefits including the anti-aging effects in red grape skins, improvement of lung function from antioxidants in white wine, reduction in coronary heart disease, development of healthier blood vessels in elderly people, reduction in ulcer-causing bacteria, destruction of cancer cells by protein in red grape skins, prevention of stroke by keeping the arteries clean by polyphenols in red grape skins, decreasing ovarian cancer risk in women and making the bones stronger.

Previous research has found wines made from herbs to have a bactericidal effect on a variety of pathogens in vitro, including *E. coli* O157:H7 and other virulent strains (Møretrø and Daeschel 2004; Boban *et al.* 2010) [29, 10], *Listeria monocytogenes*, *Salmonella typhimurium*, *Salmonella enteritidis* (Boban *et al.* 2010) [10], *Staphylococcus aureus*, *Campylobacter jejuni*, *Helicobacter pylori*, *Streptococcus pyogenes*, *Shigella sonnei*, and *Vibrio parahaemolyticus*. Although the effect of herbal wines has been studied on a variety of pathogenic bacteria, information about the bactericidal effect of *Hibiscus* wines on pathogens is lacking. The goal of this thesis was to expand upon previous findings from herbal wines, and investigate the bactericidal properties.

The *Hibiscus rosa-sinensis* belongs to the family Malvaceae. Traditionally flower can be used as anti asthamatic agents. many chemical constitute such as cyanidin, quercitin, hentriacontane, calcium oxalate, thiamin, riboflavin, niacin, and antimicrobial substances are present in this flower. The petal have some protective mechanism against microbial attack in most of the parts. The *H. rosa-sinensis* flower petal of large number of plant species growing in the vicinity of our environment were screened for their antimicrobial activity. the antioxidant properties of flavonoids, polyphenolic Compounds and anthocyanins contained in the flower can prevent the oxidation of Low-Density Lipoproteins (LDL), which is associated with the disease. So several studies has shown the antibacterial activity of *H. rosa-sinensis* against human pathogens viz., *Staphylococcus aureus, Bacillus subtillis, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella* spp. Recently, the antimicrobial effects of various plant extracts against certain pathogens have been reported by a number of researchers. Particularly, polyphenols of plant origin have been reported to have a variety of biological effects, including anti-oxidant, anti-carcinogenic, anti-inflammatory and anti-microbial activities. Specifically some phenolic compounds such as resveratrol, hydroxytyrosol, quercetin and a number of phenolic acids have been reported to inhibit various pathogenic microorganisms. Also, there are recent studies reporting the antimicrobial activities of wines and wine extracts against various pathogens. The sweetness of the wine alters fermentation of the *Hibiscus rosa-sinensis* and other ingredients, the wine can be made having great taste and at the same time good for our health. Therefore, the incorporation of the dry *Hibiscus* flower-petals into wine by fermenting remains healthy since it is used all over the world for different ailments.

There is great demand of herbal wine in the market, so this study is planned with a view to manufacture herbal wine from *Hibiscus rosa-sinensis* flower and to evaluate its antimicrobial effect against common food borne pathogens with following objectives-

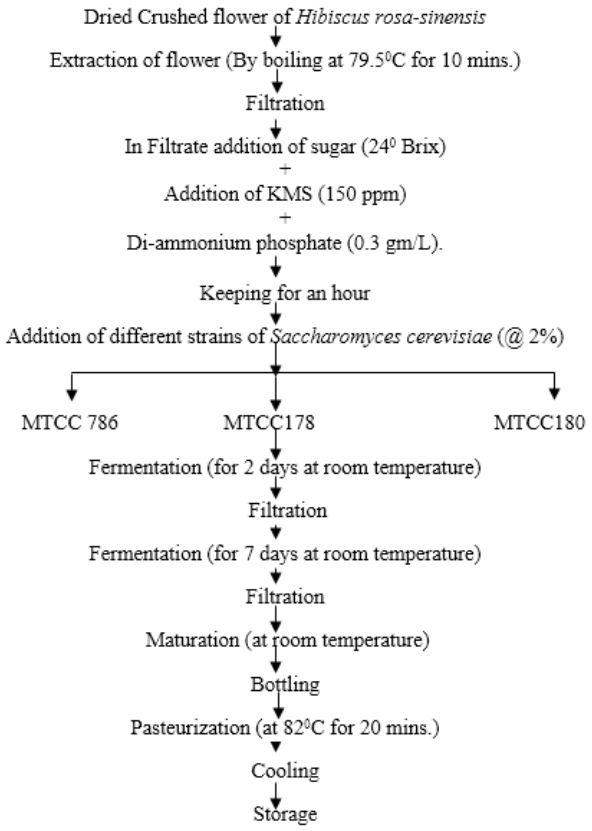
1. To prepare herbal wine from *Hibiscus rosa-sinensis* flower.
2. To study the sensory and physico-chemical properties of prepared wine.
3. To assess the rheological properties of prepared wine.
4. To evaluate the effect of prepared herbal wine against common food borne pathogens (viz. *Bacillus subtilis*, *Salmonella typhimurium, Staphylococcus aureus, Escherichia coli*).

Material and Methods

The present study entitled "Production, Optimization, Characterization and Evaluation of Antimicrobial Activities in *Hibiscus rosa-sinensis* wine" was conducted in the research laboratory of Warner College of Dairy Technology, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad.

The material and methods were adopted during this investigation are given below -

- *Hibiscus rosa-sinensis* flowers were collected from Agronomy department of SHUATS, Allahabad.
- Sugar, KMS, Di-ammonium phosphate were purchased from local market of Allahabad.
- Different types of *Saccharomyces cerevisiae* strains MTCC 786, MTCC 178, MTCC 180 were collected from IMTECH, Chandigarh, India.



Flow Chart for making Herbal wine

Analyses

Physico-chemical Analysis

The wines prepared were analysed for TSS (^{o}B), total solids, pH, titratable acidity, Protein content, reducing sugars and ethanol. TSS was measured using Erma hand refractometer (0-32^{o}B) and corrected for the temperature variation. total solids was done by gravimetric method The pH was measured with ELTOP 3030 pH meter, Titratable acidity (as per malic acid) was estimated by titrating a known aliquot of sample against N/10 NaOH solution. Protein content was estimated by kjeldhal method whereas The reducing sugars were estimated by Lane and Eynon's volumetric method. Ethyl alcohol in the finished wines was determined by the colorimetric appropriately. DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The color changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition. For FRAP assay, a modified method described by was adapted to measure the ability of extracts to reduce ferric ions. Ferrous sulfate solution (concentration ranging from 0.1 to 1 µm) was used for preparing the standard calibration curve. FRAP activity was expressed as micromoles of Fe (II)/100 g of dry weight of samples. Total flavonoid contents of M. borneensis were determined by using the aluminium chloride colorimetric method as described by. The Total Phenolic content of the flower extracts were determined based on the Folin–Ciocalteu (FC) method. For total tannins, the vanillin–HCl method was employed.

Rheological Analysis

Samples of herbal wine was subjected to rheological tests also. These tests consisted in determination of viscosity, colour and brightness. viscosity was estimated with the help of viscometer while colour and brightness was determined were based on measurements of absorbance at 420 nm and 520 nm respectively by spectrophotometric method by using a 1 mm cuvette. The standard curve was plotted considering the concentration against absorbance. it was determined in triplicate.

Sensory evaluation

The sensory evaluation of wines of different treatments was conducted by a trained panel of 10 judges. Each judge was given a set of wines separately in isolated booths and provided with a glass of fresh water to rinse their mouth before tasting the next sample. Each sample was evaluated for various quality attributes, viz. colour, consistency, aroma, overall acceptability, etc. as per the prescribed performa. Judges were asked to rate the samples on a prescribed sensory evaluation Performa with earlier stated attributes. samples were assessed organoleptically using a 9-point hedonic scale, where 9 correspond to "like extremely" and 1 corresponds to "dislike extremely"

Microbiological Analysis

Preparation of media

All the cultures were grown at 30^0 c under agitation(150 rpm) for 24h in yeast extract peptone dextrose broth (YEPD) medium and adjusted to pH 5.0.All the strains were sub-cultured on YEPD agar slants at fortnight intervals and stored in refrigerator at 4±^{0} C, until further use. for muller hinton broth and agar, Beef Extract Peptone, Hydrolysate Acid of Casein, Starch were heated in one litre of distilled water. Then boiled, agar was added to the above mixture and stirred constantly. pH was adjusted to 7.2 by using 1.0 N Hcl or NaOH drop wise. The media in the conical flask was plugged with cotton and sterilized in autoclave at 15 lbs pressure for 15-20 minutes. When the media was cooled, it was stored in the refrigerator and heated whenever required. The turbidity was measured by adjusting to 0.5 Mac Far land standards.

Maintenance of yeast culture

Three yeast strains namely *Saccharomyces cerevisiae* MTCC178, *S. cerevisiae* MTCC 180, *S. cerevisiae* MTCC 786, were procured from institute of Microbial Type Culture Collection, IMTECH, Chandigarh, India.

Bacterial strains and growth conditions

Bacterial strains used in the study were *Escherichia coli*, *Salmonella typhimurium*, *Bacillus subtilis* and *Staphylococcus aureus* obtained from the institute of Microbial Type Culture Collection, IMTECH, Chandigarh, India. All cultures were propagated in Muller Hinton broth. The culture isolates were all aerobically incubated at 37 °C for 24 hrs.

Determination of Minimum inhibitory concentrations (MIC)

MIC was determined according to agar dilution method. Various concentrations of extract were prepared in 10 cm experimental tubes containing Mueller Hinton Broth for fungi and 10 cm experimental tubes containing Mueller Hinton Broth for bacteria. Each tube contains 9 ml of Mueller Hinton for bacteria were sterilized by autoclaving. On cooling, 1 ml of each extract concentration were added to each tube, to make the final concentrations of 2, 4, 8, 16, 32, 64, mg/ml. The mixture of Muller Hilton and extract was poured into

plates aseptically in a laminar flow cabinet. On solidification of the agar medium, 2 µl of adjusted spore suspension were added to each plate by micropipette and incubated at 37 C° for bacteria. The MIC was regarded as the lowest concentration of the extract that did not show any visible growth after 24 hours of incubation. After incubation time, the diameter of free zone was measured exactly by using a ruler in millimeters. All experiments were performed with 3 replicates

Zone of inhibition by Agar well diffusion assay

An agar well diffusion assay was performed to determine if the organisms were susceptible to *Hibiscus* Wine. Isolates of *Escherichia coli, Salmonella, Bacillus subtilis, Staphylococcus aureus* were from the culture collection of the MTCC, Chandigarh. The Wells were aseptically cut out of each agar plate with a sterile metal cork borer of 6 mm diameter. The three wells in each plate were filled with 100 µl of different percentages of each *Hibiscus* wine to be tested. The plates were accordingly incubated at 37°C and observed after 24 h. The diameter of inhibition zones surrounding each well was measured. The experiment was repeated. Concentrations of the selected wines that gave the most inhibitory effects on the tested pathogens were analyzed as described in Result & Discussion.

Statistical analysis

All the assays were carried out in triplicates. The experimental results were expressed as mean ± standard deviation. The data were analyzed using one way analysis of variance (ANOVA). Differences were determined using Fisher's Protected Least Significant Difference (LSD) with P ≤ 0.05. The results of sampling cases and species were plotted simultaneously in a graph to interpret the results. In the data, physico-chemical parameters were used as sampling units and the yeast as species.

Results and Discussion

Table 1: Effect of different wine yeast strains on the Physico-chemical & Rheological characteristics of Herbal wine

Parameters	MTCC 178	MTCC 180	MTCC 786
Physico-chemical analysis			
Ethanol (%)	11.50	10.51	9.51
TSS (^{0} Brix)	16.38	15.46	14.40
Total Solids (%)	11.00	9.27	8.64
PH	4.62	4.34	3.36
Titrable Acidity (%)	0.50	0.61	0.78
Protein (%)	1.26	1.19	0.87
Reducing Sugar (%)	0.050	0.070	0.090
Rheological Analysis			
Viscosity (cp)	3.13	4.31	4.52
Colour (nm)	1.410	1.550	1.930
Brightness(nm)	1.489	2.833	3.470

The physico-chemical characteristics (Table1) of the *Hibiscus* wines prepared with different yeast strains clearly show that All the yeast strains recorded rate of fermentation higher than 1.6. strain MTCC 178 had highest rate of fermentation. The yeast strain suitable for wine production should have high fermentabiltiy, tolerance to ethanol, sedimentation property and no effect on titratable acidity. Further, The Fig. 1 reveals that the strain MTCC 178 produced higher Alcohol (11.50 %) than other strains, which is of great interest to produce high quality wine. The amount of ethanol produced by the yeast is desirable character and from this point of view MTCC 178 proved to be the best. Further, all the three strains MTCC 180 and MTCC 786.

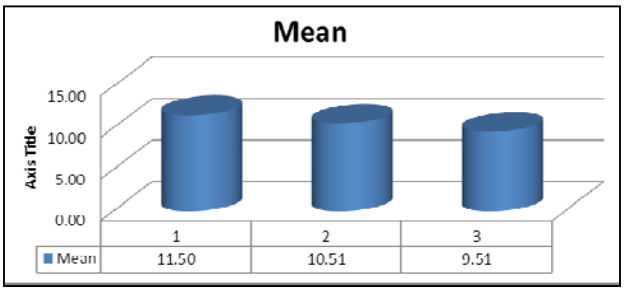


Fig 1: Fermentation efficiency of different yeast strains employed in *Hibiscus* wine preparation

TSS (Table 1) of all the wines ranged from 14.40 to 16.38 and all the wines were fermented to dryness as the residual concentrations of total sugars of all the wines was less than 0.5%. Although the The Fig. 2 reveals that the TSS of the wines (MTCC 178) was quite high, the residual sugar content was very low. It is because of the fact that TSS includes all soluble components including sugar. total solids of all the wines ranged from 8.64 to 11.00 The pH (Table 1) of wines was according to their respective acidities. here exists a correlation between PH and acidity of the sample. The higher the acidity, the lower the PH of the wine. A similar study conducted by revealed that there is a corresponding reduction in pH as the acidity increased in sour wine. The pH of the wine was approx 4.00.

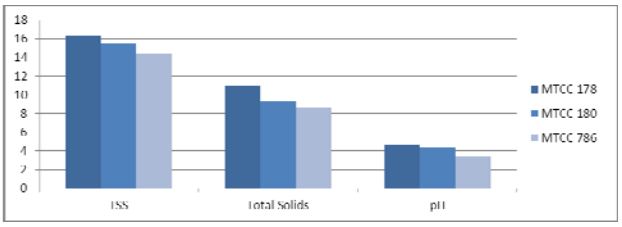


Fig 2: Effect of different yeast strains on TSS, Total solids and pH

The titratable acidity is an important characteristic varying between 0.50 to 0.78% (Table1) which was virtually comparable in all the wines. Thus, yeasts did not influence the acid production in the wines and is desirable. The protein content of the beverage ranged between 0.05-1.00%. The low protein content may be attributed also to the effect of heat process involved in its extraction which might have destroyed some amino acids with resulting beverage. However, the protein contents of the formulated wine (1.70-1.90)%.The reducing sugar was higher in wine MTCC 786 (0.090) as compare to MTCC 178 (0.050).Which shows that yeast strain MTCC 178 consumed more sugar comparable in all the wines. Thus, yeasts did not influence.

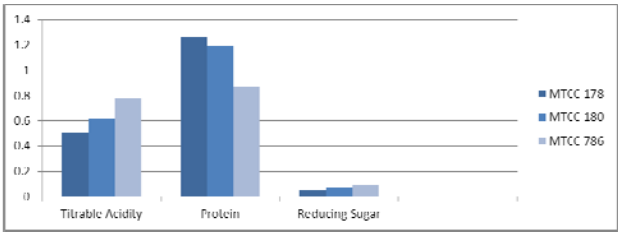


Fig 3: Effect of different yeast strains on titrable acidity Protein and Rrducing sugar

Viscosity of wine MTCC 178 is mainly influenced by colloids. Colloids perform thermal motion (Brownian motion), gradually diffuse and settle, give rise to osmotic

pressure, and participate in formation of gels. Wine (MTCC 786) contain relatively big amounts of thermolabile proteins. When the temperature increases above critical temperature, these proteins coagulate, hence viscosity increases, too Fig 4 reveals that Color and brightness of the wine decreased significantly with difference in the inoculum level. The highest intensity of color and Brightness (Table 1) was (1.930) (3.470) respectively for MTCC 786.

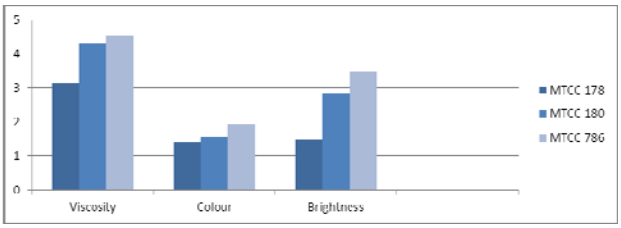


Fig 4: Effect of different yeast strains on viscosity Color and Brightness

Table 2 total antioxidant activity in wines and Content of phenolic compound,total tannins and flavonoids content

Parameteres	Control	MTCC 178	MTCC 180	MTCC 786
DPPH (%)	90.22±0.03	97.43±0.03	96.42±0.03	94.25±0.03
FRAP (%)	32.10±0.06	35.34±0.06	32.30±0.06	30.18±0.06
Total Phenolics (%)	12.22±0.09	16.64±0.09	15.78±0.09	15.73±0.09
Total Tannins (%)	80.00±0.08	83.54±0.08	81.41±0.08	80.55±0.08
Total flavonoids (%)	8.90±1.02	10.48 ±1.02	9.42±1.02	9.15±1.02

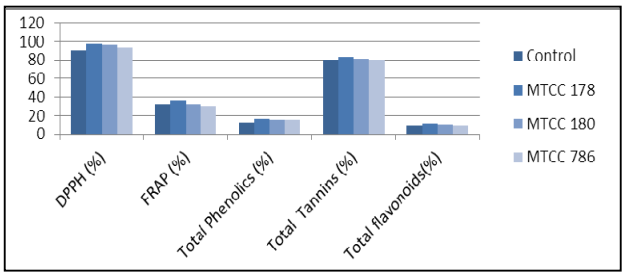


Fig 5: Total antioxidant activity determined by DPPH, FRAP & determination of total phenolic, tannin & flavonoid content

DPPH assay is being used widely as a preliminary test which provides information on the reactivity of test compound with a stable free radical since odd electron of DPPH gives strong absorption band at 517 nm. wine prepared from strain MTCC 178 showed a very good anti-radical activity in scavenging DPPH radical comparable to the control with a maximum inhibition of about 97.43. The Ferric reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. For the estimation of the reductive ability we investigated the Fe3+ to Fe2+ transformation. wine prepared from yeast strain MTCC 178 showed good reducing capacity comparable to the control with a maximum inhibition of about 35.34. gives strong absorption band at 700 nm. The average values of Phenolic compounds content in herbal wines determined by folin–Ciocalteu method were in the interval of 12.22–16.64 mg/l GAE. the differences between our values and the published results could be primarily affected by the nature of the analysed wines, i.e. by their actual contents of phenolic compounds. Among the three wine of different strains, MTCC 178 Containing the highest (16.64%) amount of phenolic compound followed by MTCC 180 (15.78) whereas MTCC 786 containing (15.73%) lowest amount of phenolic compound at absorbance of 765 nm. The content of Tannins determined by vanillin–HCl method at absorbance of 500 nm was approximately the same in MTCC 180 and MTCC 786 wines. although highest percentage of tannin content was 83.54 in wine prepared from strain MTCC 178. The tannin content change significantly. The result of Total Flavonoid contents of the three wines of different strains are given in Table 1. The total flavonoid contents varied from 9.15 to 10.48%. Chloroform extract has been found to be rich in flavonoids.

Sensory Analysis

Herbal Wine production sounds common but the novelty of this work is the use of *Hibiscus* flower and juice which is fermented using a different yeast strains of *Saccharomyces cerevisiae* purchased from IMTECH chandigarh for wine production. Below are the findings.

Table 3: Average mean values analyzed for various quality attributes of Herbal wine

Parameters	Treatments		
	MTCC 178	MTCC 180	MTCC786
Colour & Appearance	8.30	7.90	8.24
Flavour & Aroma	8.70	8.06	7.76
Consistency	8.84	8.36	7.74
Overall Acceptability	8.32	7.73	7.13

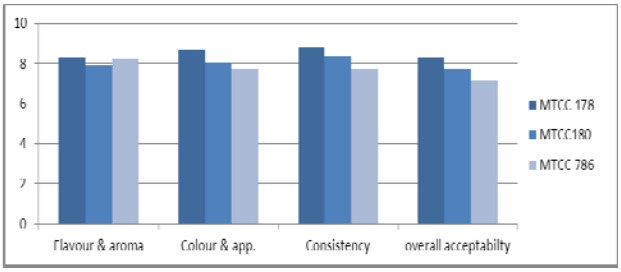


Fig 6: Average scores in various quality attributes of Herbal wine

More recently, wine makers and wine researchers have come to realize that *Saccharomyces* yeasts contribute in significant measure to the flavour and quality of wine than previously thought for determining their potential effects on the organoleptic qualities of the final product. The differences in the composition of wines made from different yeast species appear to be quantitative rather than qualitative. The products of fermentation are usually identical, but the relative amounts of the various compounds differ between different yeast species. From the sensory analysis result shown in Table-1 that there was significant difference ($P > 0.05$) among those three wines in terms of colour, flavor taste and overall acceptability. MTCC 178 wine is rated higher than MTCC 180 & MTCC 786 wine in all attributes.

Microbiological Analysis

Almost all the samples showed antimicrobial activity. It may be because of the presence of secondary metabolites like polyphenol, flavonoids etc, by the yeast from *Hibiscus* petals after. Wine MTCC 786 shows a good antimicrobial activity against *Salmonella typhimurium Staphylococcus aureus*,

Bacillus subtilis and *Escherichia coli.*

The results of the antimicrobial effects of *Hibiscus* Wine MTCC 178, MTCC180 and MTCC 786 by the agar diffusion method are presented in following tables. The results show that *Hibiscus* Wine MTCC 178 at concentrations (2,4 and 8 mg/ml) in table-1 had less significant antimicrobial effect on *Salmonella typhimurium Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli.* and it is not able to prevent the growth of bacteria on culture ($p<0.05$). However, (16, 32 and 64 mg/ml) *Hibiscus* Wine MTCC 178 concentrations, have significant antimicrobial effect on *Salmonella typhimurium, Staphylococcus aureus*, *Bacillus subtilis,* and *Escherichia coli*. The results show that *Hibiscus* Wine MTCC 180, at concentrations (2,4 and 8 mg/ml) in table-2 had less significant antimicrobial effect on *Salmonella typhimurium Staphylococcus aureus*, *Bacillus subtilis,* and *Escherichia coli.* and it is not able to prevent the growth of bacteria on culture ($p<0.05$). However, (16, 32, and 64 mg/ml) *Hibiscus* Wine MTCC 180 concentrations, have significant antimicrobial effect on *Salmonella typhimurium Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. The results show that *Hibiscus* Wine MTCC 786, at concentrations (2,4 and 8 mg/ml) in table-3 had less significant antimicrobial effect on *Salmonella typhimurium Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli.* and it is not able to prevent the growth of bacteria on culture ($p<0.05$). However, (16, 32 and 64 mg/ml) *Hibiscus* Wine MTCC 786 concentrations, have significant antimicrobial effect on *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus subtilis,* and *Escherichia coli*.

All the samples showed zones of inhibition around 11-16 mm against food borne pathogens which has proved the antimicrobial activity of *Hibiscus* wine.

Table 4: Minimum Inhibitory Concentration (MIC) of *Hibiscus* wine prepared from *Saccharomyces cerevisiae* MTCC 178 on *Bacillus subtilis, Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*

	***Hibiscus* wine concentrations (mg/ml)**					
Microorganisms	**2**	**4**	**8**	**16**	**32**	**64**
Bacillus subtilis	-	-	-	+	+	+
Escherichia coli	-	-	-	+	+	+
Salmonella typhimurium	-	-	-	+	+	+
Staphylococcus aureus	-	-	-	+	+	+

Table 5: Minimum Inhibitory Concentration (MIC) of *Hibiscus* wine prepared from *Saccharomyces cerevisiae* MTCC 180 on *Bacillus subtilis, Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*

	***Hibiscus* wine concentrations (mg/ml)**					
Microorganisms	**2**	**4**	**8**	**16**	**32**	**64**
Bacillus subtilis	-	-	-	+	+	+
Escherichia coli	-	-	-	+	+	+
Salmonella typhimurium	-	-	-	+	+	+
Staphylococcus aureus	-	-	-	+	+	+

Table 6: Minimum Inhibitory Concentration (MIC) of *Hibiscus* wine prepared from *Saccharomyces cerevisiae* MTCC 786 on *Bacillus subtilis, Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*

	***Hibiscus* wine concentrations (mg/ml)**					
Microorganisms	**2**	**4**	**8**	**16**	**32**	**64**
Bacillus subtilis	-	-	-	+	+	+
Escherichia coli	-	-	-	+	+	+
Salmonella. typhimurium	-	-	-	+	+	+
Staphylococus aureus	-	-	-	+	+	+

\+ Positive inhibition
\- Negative inhibition

Table 7: Average diameter (mm) of microbial free zone area of *Hibiscus* wine (at different concentrations) prepared from *Saccharomyces cerevisiae* MTCC 178 on *Bacillus subtilis, Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*

Samples	**Zone of inhibition(mm)**			
***Hibiscus* wine MTCC 178**	***Bacillus subtilis***	***Escherichia Coli***	***Salmonella typhimurium***	***Staphylococcus aureus***
2	2.10	2.00	2.11	2.29
4	2.80	2.10	2.14	2.50
8	2.44	2.90	2.10	2.00
16	13.10	13.00	13.10	13.10
32	15.00	14.90	14.15	15.11
64	15.00	15.90	15.00	15.20

Table 8: Average diameter (mm) of microbial free zone area of *Hibiscus* wine (at different concentrations) prepared from *Saccharomyces cerevisiae* MTCC 180 on *Bacillus subtilis, Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*

Samples	**Zone of inhibition(mm)**			
***Hibiscus* wine MTCC 180**	***Bacillus subtilis***	***Escherichia coli***	***Salmonella typhimurium***	***Staphylococcus aureus***
2	1.22	1.10	1.55	1.39
4	1.82	1.30	1.27	1.79
8	1.44	1.40	1.19	1.00
16	12.10	11.22	11.69	12.90
32	13.12	13.20	13.11	12.20
64	15.10	15.10	14.22	14.25

Table 9: Average diameter (mm) of microbial free zone area of *Hibiscus* wine (at different concentrations) prepared from *Saccharomyces cerevisiae* MTCC 786 on *Bacillus subtilis, Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*

Samples	**Zone of inhibition(mm)**			
***Hibiscus* wine MTCC 786**	***Bacillus subtilis***	***Escherichia coli***	***Salmonella typhimurium***	***Staphylococcus aureus***
2	1.20	1.00	1.10	1.50
4	1.90	1.10	1.30	1.90
8	1.40	1.00	1.35	1.50
16	12.00	11.12	11.38	11.90
32	11.00	11.30	11.80	11.50
64	12.10	12.30	12.10	12.20

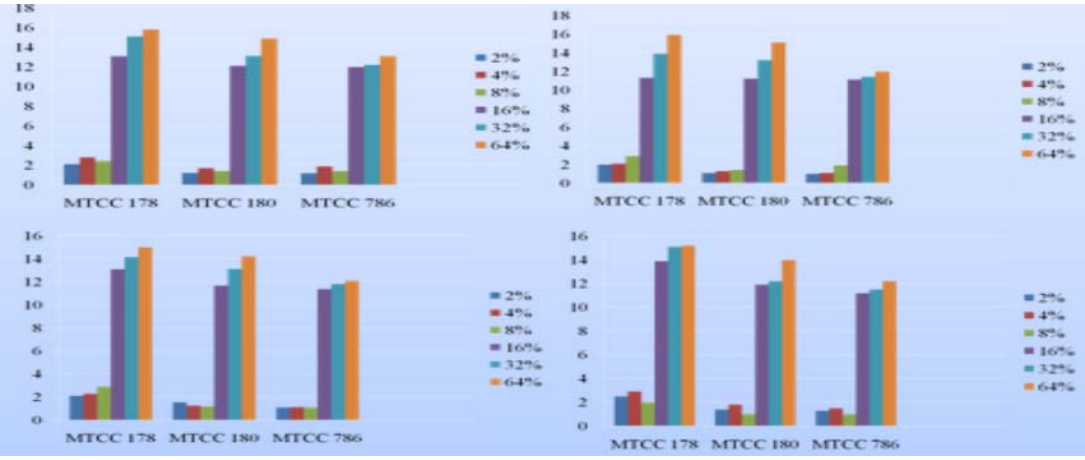


Fig 1: MIC of *Bacillus subtilis, E. coli, Salmonella typhimurium & Staphylococcus aureus,* for different yeast strains of *Saccharomyces cerevisiae* in *Hibiscus rosa-sinensis* wine respectively

The antimicrobial activities of the wine prepared from of *H. rosa-sinensis* flowers were carried out against food borne pathogens such as *Bacillus subtilis, E.coli, Salmonella typhimurium & Staphylococcus aureus* as shown in fig.1. with the help of fig. 1 we can see that *Hibiscus* wine prepared from MTCC 786 was not effective as much as MTCC 178 in respect of all pathogens. Based on the results *Hibiscus* wine prepared from different strains in this study have significant antimicrobial activity on the studied microorganisms. Tables (1,2,3,4,5,6) given above are representative of the MIC of *H. rosa-sinensis wine*. The growth of all pathogens were inhibited at different concentrations of wine, while the bactericidal effect of the herb varies for all organisms. all wines of different yeast strains showed promising zone of inhibition of growth in the range of 11.00±0.17 to 16.00 ±0.10 mm.

Conclusion

The current work was aimed at exploring the vast alternatives of beneficial herbs and botanical ingredients to further enhance the efficacy and functionality of ever popular health beverage i.e. wine. The work was carried out to develop process methodology for the production of *Hibiscus* wine with different strains. Furthermore, the newly developed variants were assessed for the antimicrobial potential. The resultant physico-chemical and rheological characteristics are different for different yeast strains used. The higher acidic conditions, presence of CO_2 and Alcohol content more than 8% ensures good keeping quality of the product. The prepared *Hibiscus* based herbal wines were found to possess bactericidal effect against common food-borne pathogens. Our results are of great practical importance as the prepared herbal wines besides being a tasteful addition to food might also prove to be a health drink with antimicrobial potential against a variety of food-borne pathogens. samples showed zones of inhibition around 11-16 mm against food borne pathogens which has proved the antimicrobial activity of *Hibiscus* wine. It has been suggested that the antioxidant capacity of wine is strongly correlated to the type of phenolic compounds present in wines. This points out the predominant role of phenols in antioxidant activity. The sensory evaluation has indicated that the wine possesses novel characteristics in aroma and taste and good acceptability. amongst the three yeast strain screened for alcoholic fermentation of reconstituted *Hibiscus* petals extract, *Saccharomyces cerevisiae* MTCC 178 was found most potent strain. This study demonstrates that *Hibiscus* Extract can be used successfully in the production of herbal wine. Therefore after analyzing the above findings It can be adjudged that *Saccharomyces cerevisiae* MTCC 178 strain is most suitable for *Hibiscus* wine production.

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