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GINGER VODKA: ETHANOL PRODUCTION USING GINGER AS AN ANTIBACTERIAL.

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ABSTRACT

Ethanol is one of the efficient energy sources that can be produced by using any agricultural product like grains, straws, fruits etc. Ethanol like vodka can be distilled from virtually any fermentable ingredients. Ginger (*Zingiber officinale*) has long been used as naturopathy due to their potential antimicrobial activity against different micro-organisms. Maize is relatively inexpensive compared with other feed stock and grains. However, before ethanol fermentation pretreatment is needed so we liquefied and saccharified the sprouted maize for conversion of starch into fermentable sugars. The fermentation of maize was conducted by *Saccharomyces cerevisiae* under aerobic condition with optimum shaking of broth. The novelty of this experiment is use of ginger extract for its antimicrobial activity which decreases the impurities in product and worked as enhancer for alcohol production. The gas chromatographic estimation has confirmed that the presence of ginger extract increased the quantity as well as quality of ethanol.

KEYWORDS: **Ethanol, Antimicrobial activity, Gas Chromatography, Ginger.**

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INTRODUCTION

Spirits are the drinking beverages include whisky, rum, vodka, gin etc. They are distinct from wine due to distillation, and have an over 35 to 45% alcohol by volume, repeated distillation of vodka will make its level much higher up to 90-96% by volume but for consumption purpose they are diluted before bottling by addition of water. Traditionally, vodka is made by the distillation of fermented cereal grains or potatoes. Presence of unwanted micro-organisms or contaminant like lactic acid bacteria's causes spoilage of fermentation broth, make it undesirable and responsible for less concentrate alcohol production[1,2,3]. This is because the redundant micro-organisms compete for nutrient with alcohol producing yeast and spoil the fermentation broth which terminates in production of other bi-products like lactic acid, acetic acid, propanol, methanol etc. One can ensure the safety and increase the percent alcohol production by using antimicrobials in fermentation process of ethanol from yeast (S*accharomyces cerevisiae*). These ethanol sensitive contaminants like Candida, Hansaniaspora, Kloeckera etc will die automatically as soon as the ethanol concentration starts to increase during the fermentation, but with number is high 10^6 – 10^7 cfu/ml before death, they significantly influence the composition of alcohol [4,5]. Many of the plants used today were known to the people of ancient cultures throughout the world and they valued their preservative and medicinal powers. Scientific experiments on the antimicrobial properties of plants and their components have been documented in the late 19th century [6]. Naturally occurring microbial inhibitors have been recovered from a wide variety of foods including Onions, Garlic, Fruits, Vegetables, Cereals and Spices. Ginger (*Zingiber officinale*) is also one of the medicinal plants, which has been widely used all over the world [7]. Since ancient times ginger was known to cure a wide array of untreated disorders including Arthritis, Cramps, Rheumatism, Sprains, Sore-throats, Muscular aches, Pains, Constipation, Vomiting, Hypertension, Indigestion, Dementia, Fever and Infectious diseases [8]. This information confirms the antimicrobial activity of ginger. In addition, it has been reported that the main ingredients of ginger like Volatile oil, Gingerol, Shogaol and Diarylheptanoids work as Antioxidant, Anti-inflammatory, Anti-lipid, Anti-diabetic, etc. The antimicrobial potency of ginger mainly caused by the presence of Oxygenated mono- and Sesquiterpenes, Phenolic compounds (Shogaol, Gingerol), which are lipid-soluble phenol compounds primarily isolated from the root of ginger. Ginger extract are effective against Gram-positive bacteria compared to the Gram-negative ones [9]. These compounds not only attack cell walls and cell membranes but also affecting their permeability, release of intracellular constituents (e.g. ribose, Na glutamate) and with membrane functions (electron transport, nutrient uptake). Thus, these compounds might have several targets which are lead to the inhibition of bacterial pathogens [10]. The good antimicrobial activity of ginger extract against the food borne pathogens like *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, etc at room temperature and boiling temperature was studied by Pankaj and et al [11]. The objective of this study aimed to increase quality as well as quantity in % bio-ethanol production.

MATERIALS AND METHODS

Collection of sample

Ginger, maize and the activated Baker's yeast (S*accharomyces cerevisiae*) were collected from local market of Jalgaon, Maharashtra, India. The ginger and maize was washed with tap water to clean and removes dirt.

Preparation of crude extracts of ginger

The 350gm of ginger was grinded in mortar and pestle and 150ml juice was extracted. It was filtered by passing it through muslin cloth[12,13].

Antimicrobial activity of ginger against air born contaminants

The ginger extracts were dissolved in distilled water to yield final concentration 5% v/v and 10% v/v and sterilized in autoclave at 121°C for 15min.[14] Antibacterial activities of ginger (*Zingiber officinale*) extracts were evaluated by 5% and 10% ginger extract containing Nutrient agar (NA) plates. The plates were exposed to air for 20 sec and incubated at room temperature 27°C for 48 hrs. [15]

Antimicrobial activity of ginger against Saccharomyces cerevisiae

The antimicrobial activity of ginger extract should not affect the growth of alcohol producing microbes (S*accharomyces cerevisiae*) which was confirmed by agar diffusion technique. 15 ml of molten Potato dextrose agar (PDA) (45°C) was poured in sterile Petri plates. Working cell suspensions of *S. cereviciae* was prepared and 100µl was evenly spread on PDA plates. Once the plates had been aseptically dried, 6 mm wells were punched into the agar. The ginger extracts were dissolved in distilled water to get the final concentration 5% v/v and 10% v/v and sterilized in autoclave at 121°C for 15min. The 100µl ginger extract was pour into wells and the plates were incubated at 31°C for 48 hrs. [15]

Inoculum preparation

Activated Yeast culture (*S. cerevisiae*) was collected from local bakery of Jalgaon. The inoculum was prepared by using Yeast-Malt-Peptone-Dextrose (YMPG) broth media in place of dextrose sugar we have used sprouted maize mash as a carbon source. The commercially available fungal α- amylase enzyme was added externally to convert starch in to simple sugar (degradable carbon source). [16] The inoculum incubated for 48 hours at 31˚C and pH was set to 6.

Preparation of fermentation media

Sprouted maize mash

350 gm of raw maize was soaked in water for overnight. Extra water was drained off, and maize was covered with towel to conserve moisture. Thereafter four days, sprouted maize was grinded in the electric mixer and the maceration of maize was used for further process.

Fermentation process

A fermentation protocols were set for maize one as a control and another test. The fermentation was carried out in 5 L of Erlenmeyer flask for 7 days at 31°c. In fermentation, maize mash (carbon source) was taken in sterilized conical flask and prepared the volume up to 3000 ml by adding sterile distilled water. Thereafter mixtures were kept in boiling water bath at 66°c for 1hr to gelatinize and cooled to room temperature. The fungal α-amylase was prepared with 10 mM $CaCl_2$ buffer and 1 % concentration was added aseptically to the mashed substrate for scarification and sterilized. The flasks were allowed to stand for overnight. On next day, an inoculum was added in the test and control to start fermentation process. In test broth 150 ml sterile ginger extract was added to avoid the growth of other contaminant and control kept as it is. For proper mixing and aeration the fermentation vessel were kept on shaker for 2 days at 31°C. To prevent explosion of carbon dioxide produced by *S. cerevisiae* in fermentation process, 1ml pipette was fixed at the mouth of the flasks. The pH was monitored throughout the course of fermentation using pH meter (Sigma digital pH meter). At the end of the fermentation; filtrate (fermentation broth) was obtained by centrifugation at 5000 rpm for 15min. [17]

Estimation of ethanol

To purify the ethanol from unwanted side products the filtrate was subjected to distillation process at 78°c and alcohol content was determined by Gas chromatography (shimadzu), a non-reactive gas, helium was used to carry the components of the mixture through the column. Using the chromatogram, the percent composition (amount) of each component in the mixture can be determined. The percent composition is directly related to the area of each peak in the chromatogram. 1ul sample was injected in to capillary GC for estimation of ethanol against standard methanol and ethanol run in institute of chemical technology, North Maharashtra University's, Jalgaon. [18]

RESULT AND DISCUSSION

Antimicrobial activity of ginger against air

When Positive control of NA plates without ginger extract exposed to air for 20 seconds the mix microbial colonies were observed as shown in figure 1b while negative control no exposer of NA with air no growth was observed (figure 1a). In the test, 5% v/v and 10%v/v ginger extract containing NA plate exposed to air for 20 seconds have no growth after incubation of 48hr (figure 1c and 1d). This observation confirms the antimicrobial activity of ginger extract against air borne micro-organisms.

Antimicrobial activity of ginger against air

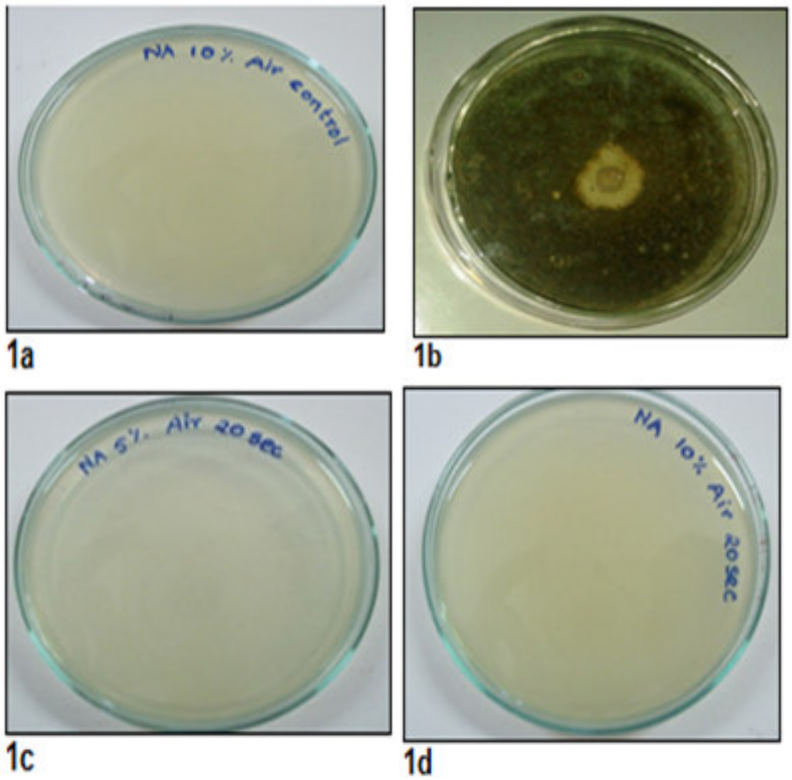


Figure 1

(a) Negative control without exposer of air
(b) Positive control showing the growth of air born micro-organisms,
(c) NA plate with 5% ginger extracts no growth of air born micro-organisms,
(d) NA plate with 10% ginger extracts no growth of air born micro-organisms.

Antimicrobial activity of ginger against S. cerevisiae

PDA plate containing 5% and 10% ginger extract v/v after 48 hr of incubation, growth of *Saccharomyces cerevisiae* was observed. Ginger extract did not affect the growth of alcohol producing yeast. (Figure 2b and 2c).

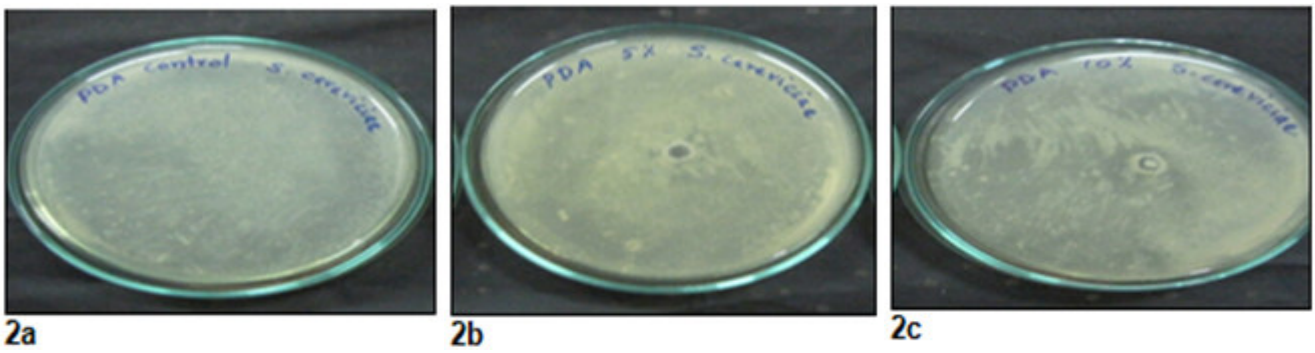


Figure 2

(a)Positive control PDA (Potato dextrose agar)Show growth of S. cerevaciae,
(b) PDA with 5% ginger extract having growth of S. cerevisiae,
(c) PDA with 10% ginger extract having growth of S. cerevisiae.

The results of antimicrobial activity of ginger extract against air contaminants and ethanol producing organism *S. cerevisiae* is given in Table 1.

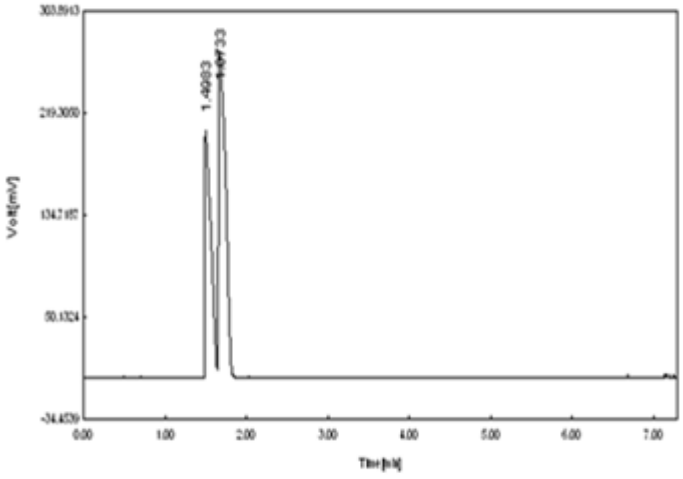
Table 1
Effect of presence of ginger extract on air-born and ethanol producing organisms

Negative control without exposed to air	-
Positive Control exposed to air	++
Test - NA with 5% ginger extract exposed to air	-
Test - NA with 10% ginger extract exposed to air	- -
Positive control growth of *S. cerevaciae on PDA*	+
Test- Growth of 5% ginger extract on *S. cerevaciae*	+
Test- Growth of 10% ginger extract on *S. cerevaciae*	+

Absence of microbial growth: (-), Presence of microbial growth: (+), Lawn of microbial growth: (++)

Estimation of Ethanol by gas chromatography

According to alcohol estimation by GC, Figure 3 shows the standard methanol and ethanol run. The ethanol produced without ginger having presence of unwanted impurities (Figure 3b) whereas, ethanol produced in presence of ginger has almost no impurities (Figure 3a). In GC the percent composition is directly related to the area of each peak in the chromatogram. The peak of ethanol with and without ginger produced from maize having 424.2974 and 48.6616 Area [mV*s] of ethanol, respectively. From above information we can conclude that, ginger has worked as an enhancer. In presence of ginger the other microbes does not interfere with ethanol production so qualitatively and quantitatively ethanol production increases.



Name	RT[min]	Area[mV*s]	Area%
Methanol	1.4983	1110.7492	40.2419
Ethanol	1.6733	1649.4287	59.7581

Figure 3
GC runs of standards Methanol + Ethanol

Estimation of ethanol by GC

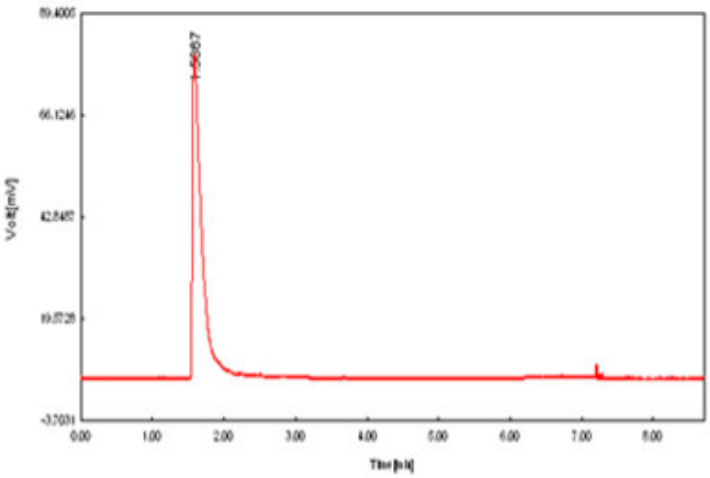


Figure 3A
Ethanol estimation by GC in presence of ginger extract

RT[min]	Area[mV*s]	Width[sec]	Area%
1.5867	424.2974	11.9000	100.0000

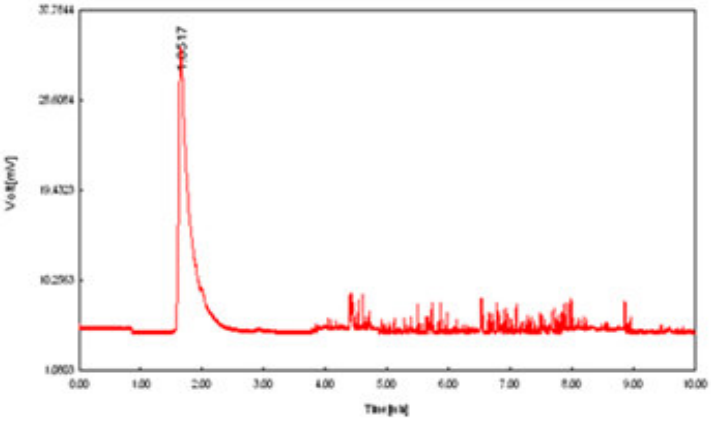


Figure 3B
Ethanol estimation by GC in absence of ginger extract

RT[min]	Area[mV*s]	Width[sec]	Area%
1.6517	48.6616	6.2000	100.0000

DISCUSSION

The maize, sugarcane and sugar beets are major traditional agricultural crops used in bio-ethanol production as well as cellulosic materials such as agro-residues are attractive feedstock for bio-ethanol production.[19] Selection of maize for alcohol production, is cost effective it is abundantly present with low cost. Maize can be used for bio-ethanol production as well as alternative source of energy. Considerable progress has been made over last decades in ethanol production but the problem of spoilage of wine, beer, vodka etc still persist, which directly affect on product concentration and ultimately on human health. The crucial question remains; what should be done to control these contaminants? We all are aware of the antimicrobial property of ginger. So the present study have highlighted the antibacterial property of ginger, which was tested in this experiment and selected as potential antimicrobial agent for alcohol production in fermentation process. This study emphasizes on the synergistic antimicrobial effect of ginger on contamination cause by microbes in fermentation. Presence of contaminant decreases the quality as well as quantity of final product and may cause harm to human health after consumption. In GC estimation no noise was observed so, according to

observations we can say that in presence of ginger the quality of final product increase, while in absence of ginger impurities interferes in quality as well as quantity of product.

CONCLUSION

The result of our experiment showed that the ginger extract having antimicrobial activity against wide range of air born micro-organism while it do not have any lethal activity against *S. cerevisiae*. Today the contamination of ethanol (wine, beer etc) is a main problem for fewer yields and spoilage. To overcome this problem the discovery of novel active compound is a matter of urgency. We found that ginger having biological active compound, in fermentation the presence of ginger at log phase was responsible for increase yield as well purity of product. When studied comparatively we got more concentration of ethanol produced when added the extract of ginger in media while in absence less concentration as well as impure product was obtained.

CONFLICT OF INTEREST

Conflict of interest declared none.

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