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Research Article

Antimicrobial Activity of Sugarcane Vinegar with Eatables Against Selected Food Borne Pathogens

Jyotsana Singh and Amar P Garg*

School of Biological Engineering and Life Sciences, Shobhit Institute of Engineering and Technology, Deemed to-be-University, Meerut, India

***Corresponding Author:** Amar P Garg, School of Biological Engineering and Life Sciences, Shobhit Institute of Engineering and Technology, Deemed to-be-University, Meerut, India.

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Abstract

Natural vinegar is a fermented product of two successive biochemical processes. Primary fermentation of sugar to ethanol is followed by its secondary fermentation to acetic acid. Sugarcane having high content of sugar, is a potential substrate for making vinegar through alcoholic and acetous fermentation. In daily routine, we use sugar cane vinegar (SCV) with different eatables such as ginger, garlic, onion, raw papaya, white radish and green chilli. These eatables have well known beneficial effects. In present study, we used sugarcane vinegar (SCV) with selected eatables such as ginger (*Zingiber officinale*), garlic (*Allium sativum*), onion (*Allium cepa*), raw papaya (*Carica papaya)*, white radish (*Raphanus sativus*) and green chilli (*Capsicum annuum*) for assessment of their antimicrobial activities against food borne pathogens - *Escherichia coli* (ATCC8739), *Bcillus subtilis, Staphylococcus aureus* (ATCC 6539), *Shigella flexneri* (ATCC 12022), *Salmonella typhi* (ATCC 14028), *Cronobacter sakazakii* (ATCC 29544), *Vibrio parahaemolyticus* (ATCC 17802), *V. cholera* (ATCC 3906) *and Pseudomonas aeruginosa.* We also analyzed the phytochemical that are present in eatables and sugarcane vinegar. The study revealed the differences between various phytochemicals that are present in vinegar and different eatables. Alkaloids are not present in sugarcane vinegar but when the eatables are soaked with SCV for 7 days it showed the presence of alkaloid. SCV treated green chili and raw papaya generally enhanced antimicrobial activity against *Vibrio parahaemolyticus*, but only raw papaya did not exhibit any effect against *Pseudomonas aeruginosa* or showed very little antimicrobial activity.

Keywords: *Zingiber officinale*; *Allium sativum*; *Allium cepa*; *Carica papaya*; *Raphanus sativus*; *Capsicum annuum*

Introduction

Fermented vinegar is a food supplement having immense antioxidant potential. However, its use in developing countries is negligible, primarily due to lack of awareness of its benefits and cost difference between fermented and synthetic vinegars. Fruit vinegar is aa popular natural product with multiple uses, most often in daily diet [1-3]. The quality characteristics of local products determination are very important to promote vinegar marketing for its safe and healthy usage [4]. The industries dealing with the production of natural vinegar still use the traditional batch fermentation which generally spans 4-5 weeks [5-7]. Vinegar is known for its antimicrobial properties that makes it useful for a number of applications in food industry. Vinegar is also used for cleaning and treating nail fungus, head lice, warts, ear infections and to remove bad smell from room air and refrigerators [8,9]. Consumers prefer natural preservative methods for inhibiting the growth of foodborne pathogenic microorganisms [10]. The organic acids in vinegar, mainly acetic acid passes into cell membranes of microorganisms leading to bacterial cell death [11-15]. The bacterial strains, temperature, pH, acid concentration, and ionic

strength influence the antimicrobial activity of organic acids [16-18]. Vinegar is used to enhance the good tastes of many foods and accelerates the solubility of calcium, iron and phosphorus in vegetables and restrain the loss of vitamins. It improves the absorption of nutrients for human body. Vinegar is added into salads not only to adjust the tastes but also to eliminate bacteria and toxins in the dishes.

The bioactive compounds in vinegars consist of organic acids, polyphenols, melanoidins, and tetra methyl pyrazine, which exhibit several health benefits, because of their antibacterial and antioxidant activities, aiding in weight loss, blood pressure, glucose control and expansion of blood vessels [19-21]. Ginger (*Zingiber officinale*), garlic (*Allium sativum)*, onion (*Allium cepa*), white radish (*Raphanus sativus*), raw-papaya *(Carica papaya)* and green chilli (*Capsicum annuum*) are normally used in winter season after soaking in sugarcane vinegar. Ginger (*Zingiber officinale*); Family- Zingiberaceae, is widely cultivated for its medicinal uses and as condiment. It is very useful to treat the common cold, headache, muscular and rheumatic disorders [22]. It contains gingerol, which has powerful medicinal properties. White radish (*Raphanus sativus*); Family- Brassicaceae, is commonly referred as radish. Regular use of radish helps in weight loss and increase the efficiency of the metabolism for bodily processes. It helps in treating colon, kidney, intestinal, stomach and oral cancers. Radish improves the immunity, removes mucous from throat and fight cold and cough when used as a part of diet. It is antiviral and also used to treat jaundice.

Onion (*Allium cepa*); Family -Liliaceae, was cultivated 6000 years BC in the nail valley. It contains large number of minerals and vitamins. Raw onion is used as a medicine and after boiling it loose its efficiency [23]. Garlic (*Allium satium); Family* -Liliaceae, is effective against many common pathogenic bacteria [24] and is a strong antimicrobial agent both against Gram-positive and Gram-negative bacteria. Papaya (*Carica papaya*); Family Caricaceae, a rich source of antioxidant and vitamins (A, B, C and E), along with minerals like magnesium and potassium, has medicinal property against dyspepsia, hyperacidity, dysentery and constipation. It is also useful in digestion of proteins because of high proteolytic enzymes [25]. Green chilli (*Capsicum annum*) belongs to the family "Solanaceae", is rich in vitamins; especially vitamin C [26]. There are five cultivated species of the genus *Capsicum* (*C. frutescens, C. chinense, C. pubescens, C. baccatum and C. annuum*) of which *C. annuum* is the most widely cultivated all over India and is known for its characteristics taste as non-pungent and pungent [27].

Sugarcane vinegar, with its own health-promoting properties, is a good alternative to apple cider vinegar. Sugarcane juice contains amino acid, vitamins such as niacin and riboflavin, and minerals like calcium, phosphorus, manganese, zinc, and iron [28]. Sugarcane vinegar is produced from sugarcane juice through the processes of alcoholic fermentation and ethanoic acid fermentation [29].

Materials and Methods

Sample preparation

All eatables were collected from the local market of Meerut (U.P) India and were washed to clean the soil, peeled off and washed again under clean tap water. After washing, all these eatables were cut into small pieces and dried under sunlight for 2 to 4 h. After drying all eatables were put into 25ml of sugarcane vinegar for 7 days. Thereafter, these were crushed with vinegar and filtered through normal filter paper and the extract stored in glass jar at room temperature for further use.

Qualitative phytochemical analysis of vinegar

Qualitative standard chemical tests were carried out for phytochemical screening of untreated and vinegar treated eatables using AOAS protocol (1990) as described by Trease and Evans (1978) and Singh and Garg (30). These were used to detect the presence of alkaloids, saponins, tannins, flavonoids, anthraquinones, terpenoids and glycosides.

Antimicrobial activity by agar diffusion method

The antimicrobial activity of different eatables with sugarcane vinegar (SCV), were studied using agar well diffusion method. The selected bacterial strains were inoculated into 10 mL of nutrient broth and incubated at 37 ± 1°C for 24h.The Mueller Hinton agar plates were inoculated separately with 100 µL test bacterial strain and evenly spread aseptically on entire surface of the plate. The wells were cut aseptically using sterile crock borer (6mm diam.) and 50µLof test extract of each eatable with sugarcane vinegar (SCV) was added. The plates were incubated in an upright position at 37 ± 1°C for 24 to 48 h and the diameter of zone of inhibition (in mm) was measured against *Escherichia coli* (ATCC8739), *Bcillus subtilis, Staphylococcus aureus* (ATCC 6539), *Shigella flexneri* (ATCC

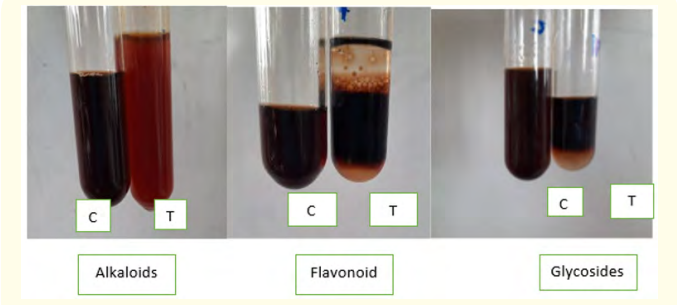
12022), *Salmonella typhi* (ATCC 14028), *Cronobacter sakazakii* (ATCC 29544), *Vibrio parahaemolyticus* (ATCC 17802), *V. cholera (*ATCC 3906) *and Pseudomonas aeruginosa.* the common foodborne pathogens.

Results and Discussions

The eatables like ginger, garlic, onion, raw papaya, green chilli and white radish are commonly used with and without vinegar in daily diet. Phytochemical analysis of extracts of these eatables in water and sugarcane vinegar (SCV) revealed that addition of SCV did not remove/destroy any of the phytochemicals of raw eatables rather the ingredients of SCV were also incorporated into the treated eatable (Table 1). The qualitative analysis of SCV revealed that it contained significant phytochemicals like saponins, tannins, flavonoids, terpenoids, glycosides, carbohydrates and phenolic compounds while alkaloids and athraquinones were not detected (Table 1, Figure 1). Ginger and raw papaya did not possess anthaquionones while garlic, onion, green chillies and white radish after soaking in SCV showed all ingredients of vinegar as well eatables. Phytochemicals also known as phytonutrients, are naturally occurring substances found in plants and play vital role in detoxification of harmful and deleterious chemicals of the body. The beneficial properties of naturally fermented vinegar are anti-oxidative, anti-diabetic, antimicrobial, antitumor, anti-obesity, antihypertensive, and cholesterol-lowering vinegar products [31]. The alkaloids present in plants are used in medicines as anaesthetic agents. Sugarcane juice is highly beneficial to human health as it contains many amino acids such as aspartic acid, alanine, citric acid; vitamins such as vitamin A, B1, B2, B3 and C, niacin, riboflavin; and essential nutrients like calcium, phosphorus, manganese, zinc, especially iron [32,33] and is commonly consumed in India as anti-thirst drink summer.

Phytochemicals	Sugarcane vinegar	Eatables											
		Ginger		Garlic		Onion		Raw Papaya		Green Chilli		White radish	
		WE	SCE	WE	SCE	WE	SCE	WE	SCE	WE	SCE	WE	SCE
Alkaloids	–	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	–	+	+	+
Tannins	+	–	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	–	+	+	–	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+	+	+	+	+
Anthoquinones	–	–	–	+	+	+	+	–	–	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenolic compounds	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 1: Qualitative analysis of vegetables treated and untreated with sugarcane vinegar.



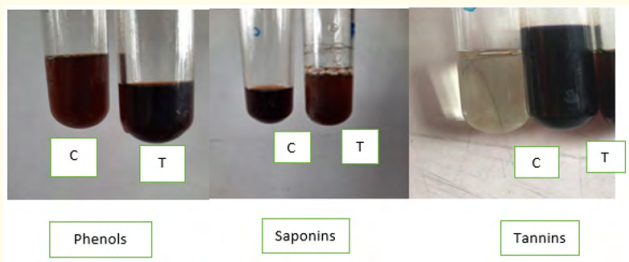


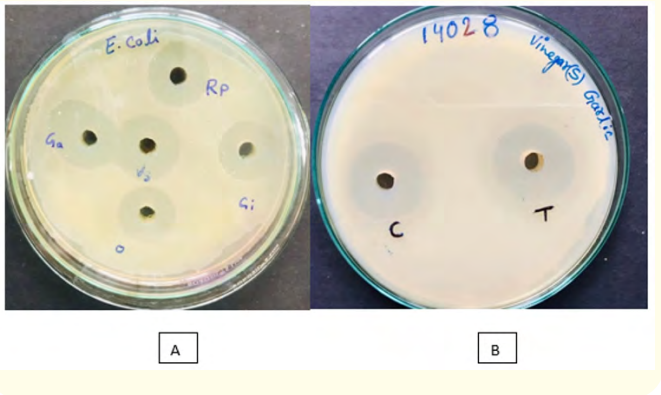
Figure 1: Qualitative phytochemical tests for analysis of Sugarcane vinegar.

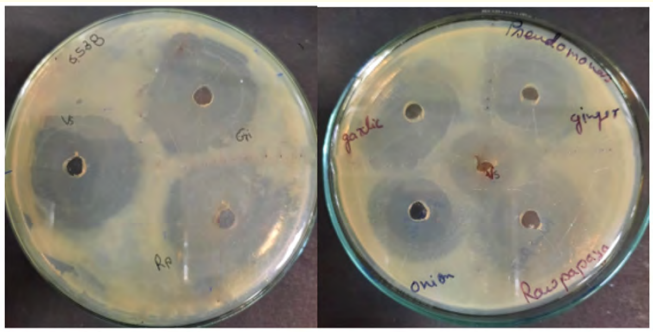
The antimicrobial studies using agar well diffusion method with and without vinegar of selected eatables revealed that antimicrobial activities of garlic, ginger, onion, raw papaya, white radish and green chili are higher against all test food borne pathogens - *E. coli (*ATCC8739), *Bcillus subtilis, Staphylococcus aureus* (ATCC 6539), *Shigella flexneri* (ATCC 12022), *Salmonella typhi (*ATCC 14028), *Cronobacter sakazakii* (ATCC 29544), *Vibrio parahaemolyticus* (ATCC 17802), *V. cholera* (ATCC 3906) *and Pseudomonas aeruginosa* when soaked for 7 days in sugarcane vinegar (Table 2). This study shows that the extract of eatables with vinegar possesses antimicrobial compounds which could be substitutes for mild antibiotics. The findings of this present study support and improve the knowledge on health-promoting and food safety related potentials of vinegar.

Bacterial strains	Control (mm)	Garlic (mm)		Ginger (mm)		Onion (mm)		Rawpapaya (mm)		White radish (mm)		Greenchilli (mm)	
	SCV	WE	SCE	WE	SCE	WE	SCE	WE	SCE	WE	SCE	WE	SCE
Pseudomonas aeruginosa	7.0	7.0	12.0	7.75	11.25	9.5	13.0	9.0	8.5	8.25	12.5	6.0	12.25
Staphylococcus aureus	10.0	6.5	12.75	8.25	12.25	7.75	10.25	7.0	10.75	9.0	13.75	8.0	12.75
Shigella flexneri	9.0	9.0	11.75	9.25	10.25	10.25	12.5	8.5	11.75	7.0	12.0	7.94	10.0
Salmonella typhi	10.0	9.5	11.25	7.57	12.25	7.0	13.75	8.5	10.75	8.0	11.25	7.68	10.25
Cronobacter sakazoii	10.0	10.25	12.25	7.5	11.25	8.751	3.25	9.0	11.0	10.65	12.5	7.0	10.5
Vibrio parahaemolyticus	9.5	8.5	12.25	9.0	9.75	7.5	13.25	9.0	16.25	8.8	12.75	8.23	10.75
Bacillus subtilis	9.0	8.5	12.0	9.0	11.25	8.25	14.0	9.0	11.5	7.89	13.25	7.5	11.75
E. coli	10.25	9.5	11.5	7.5	12.25	7.7	12.75	9.0	13.25	10.0	12.75	9 .0	11.25
Vibrio cholera	7.85	7.0	11.5	6.5	12.5	6.0	12.5	8.0	10.25	8.25	12.75	6.0	10.0

Table 2: Zone of inhibition of Sugarcane vinegar extract with eatables after 24 h (SCV = Sugarcane Vinegar; WE = Water Extract; SCE = Sugarcane Vinegar Extract).

The major objective of this research was the comparison of antimicrobial activities of bioactive compounds of selected eatables with and without sugarcane vinegar. Hence, we analysed the phytochemical bioactive compounds of eatables with or without sugarcane vinegar like carbohydrates, proteins, flavonoids, alkaloids, saponins, glycosides, terpenoids, anthoquinones (Table 1 and Figure 1). This study showed that alkaloids are absent in sugarcane vinegar but when treated different eatables, it contained alkaloids also as bioactive compound. It suggests that some bioactive compounds are active when eatables are soaked with sugarcane vinegar for 7 days. The bacteriostatic and bactericidal actions of vinegar on food borne pathogenic bacteria including *E. coli, Pseudomonas aeruginosa, S. aureus, Shigella flexneri, Salmonella typhi, Cronobacter sakazakii, Vibrio perahaemolyticsa, Bacillus subtilis and Vibrio cholera,* showed that SCV with eatables has both antagonistic and synergistic activity. All eatables showed higher antimicrobial activities with sugar cane vinegar (Table 2 and Figure 2).





C D

Figure 2: Zone of inhibition of sugar cane vinegar extract with eatables ginger(Gi), garlic(Ga), onion (O), raw papaya (Rp) and sugar cane vinegar control denoted by Vs against on E. coli on plate A and on plate B, C is control and T is test against strain 14028 (*Salmonella typhi*); on plate C raw papaya (Rp) and ginger (Gi), vinegar control denoted by Vs show zone of inhibition against strain 6538 (*Staphylococcus aureus*) and on plate D show zone of inhibition against P*seudomonas aeruginosa*.

The Onion is well known for its antimicrobial activity but when it is treated with sugarcane vinegar it shows more antimicrobial activity against *Shegella flexneri* in comparison to untreated SCV. Hence, it is suggested that onion treated with sugarcane vinegar is more beneficial for human consumption. The presence of medicinally active constitutes like flavonoids, alkaloids, saponins, tannins, anthraquinones, terpenoids and glycosides in untreated and sugarcane treated eatables determines their nutritive value and antimicrobial activities [34]. Most eatables are beneficial for our health, and if these are soaked with sugarcane vinegar, these are even more beneficial for us. 35 have evaluated antimicrobial activities of medically relevant green leafy vegetables and found that ethanolic and methanolic extracts of leaves of *Azadiracta indica*, *Coccinia grandis*, *Ipomoea aquatica* and *Paederia foetida* possess antimicrobial activities against *Staphylococcus spp., Klebsilla spp*. and *Pseudomonas spp*., while crude and hot water extract showed almost little or no effects on bacterial growth [35]. Ricci., *et al.* [36] also found that sugarcane vinegar with eatables has a positive effect against food borne pathogens. Rai [37] and Narender., *et al.* [38] also found that fruits and vegetables extracts have antimicrobial potential [37,38].

Conclusion

The findings of the present study concluded that overnight soaking of eatables with SCV exhibit high antimicrobial activities against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Shigella flexneri, Salmonella typhi, Cronobacter sakazokii, Vibrio parahaemolyticus and V. cholera*. Our studies further suggest that soaking of eatables with sugarcane vinegar should be minimum for 7 days for human health.

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Conflict of Interest

The authors declare that there is no conflict of interests.

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