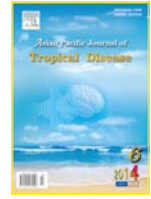




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# Microbiological analysis of common preservatives used in food items and demonstration of their *in vitro* anti-bacterial activity

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## PEER REVIEW

**Peer reviewer**

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**Comments**

This is a good study in which the authors evaluated the distribution of microbial loads in common food preservatives. The results are interesting and suggested that sodium bisulfate and citric acid samples were satisfactory preservatives both in terms of microbiological criteria and their anti-bacterial traits.

Details on Page 455

## ABSTRACT

**Objective:** To quantify the microorganisms contaminating the common preservatives used in food as well as to detect their *in vitro* anti-bacterial traits.

**Methods:** A total of 9 preservatives were subjected to conventional cultural and biochemical methods for microbial enumeration. Anti-bacterial activities were demonstrated through the agar well diffusion method.

**Results:** All samples were found to be contaminated with bacteria up to  $10^5$  CFU/g and with the fungal flora within a range of  $10^1$ – $10^2$  CFU/g. *Escherichia coli*, *Pseudomonas* spp. and *Staphylococcus* spp. were demonstrated in most of the samples. Sodium sulfite and citric acid possessed the strongest anti-bacterial trait against all of the test bacteria. Acetic acid exhibited activity against 6 out of 8 test bacteria while vinegar exhibited the activity against 4 bacteria. Activity of salt was demonstrated only against *Listeria* spp. and *Bacillus* spp., while activity of sugar and honey was found only against *Escherichia coli* and *Klebsiella* spp., respectively.

**Conclusions:** According to the current investigation, sodium sulfite and citric acid samples were found to be satisfactory preservatives both in terms of microbiological criteria and their anti-bacterial traits.

## KEYWORDS

Preservatives, Microorganisms, Anti-bacterial activity, Food safety, Public health

## 1. Introduction

Emergence of food borne infectious diseases is a principal public health concern as well as an imperative economic hitch for many countries[1–4]. Association of harmful microorganisms in cosmetics and pharmaceutical drugs are also not unlikely[5,6]. Preservatives, which are commonly known as natural or synthetic substances, are principally affixed to food items including fruits and fruit juices, vegetables, processed foods, and additionally to the cosmetics and pharmaceutical products to enhance their quality as well as shelf life[7,8]. The mode of action

frequently lies on their anti-oxidative, antimicrobial and anti-enzymatic properties which in turn hinder the chemical decomposition, fermentation, acidification, and microbiological proliferation within the product[7,9–11].

Aside from their advantages, some of the artificial preservatives including nitrates, benzoates, sulfites, sorbates, formaldehyde and several others may possess life-threatening side effects[7,9,12]. Among the earliest preservatives, high concentrations of sugar (mainly used for jams and jellies) and salt (for meat and fish), pickling with salt, vinegar, lemon juice or mustard oil (for vegetables) are well known. Other advancement in preservation efficiency

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arose through canning, pasteurization, irradiation, filtration, addition of natural or synthetic preservatives<sup>[13–16]</sup>.

While the microbiological spoilage in food, pharmaceutical and cosmetics items are globally common, the use of preservatives used in those products against microbial contamination is also expected<sup>[17–20]</sup>. In context of Bangladesh the microbial prevalence in food and consumer items is too frequent, resulting in disease outbreaks<sup>[21–27]</sup>. However, the knowledge on the extent of microbiological contamination of the associated preservatives have not been provided. Moreover, the microbial content in the preservatives as well as the demonstration of antimicrobial activity would imply the efficiency of the preservatives. Based on these facts, current study attempted to isolate and enumerate the microorganisms accessing the preservatives and to detect their anti-bacterial traits.

## 2. Materials and methods

### 2.1. Sampling, sample processing and microbiological analysis

A total of 9 samples of different categories of natural and synthetic preservatives (with appropriate dates of manufacturing and expiry on the packs) were collected from different super shops in Dhaka city during September 2013 to December 2013. Samples included salts, sugars, sodium sulfite, sodium benzoate, acetic acid, citric acid, vinegar, honey and turmeric preservatives. All samples were transported to the Microbiology Laboratory in order to assess their microbiological quality. A total of 10 g of samples were homogeneously mixed with 90 mL of buffer peptone water, and serial dilutions were prepared up to  $10^{-4}$  following the standard protocols<sup>[5–6]</sup>. An aliquot of 0.1 mL of each suspension from the dilution  $10^{-2}$  and  $10^{-4}$  was spread onto nutrient agar plate to enumerate the total bacteria and on Sabouraud dextrose agar plate for the estimation of fungal load. Then the nutrient agar plate and Sabouraud dextrose agar plates were incubated at 37 °C for 18 to 24 h and at 25 °C for 48 to 72 h, respectively.

For the enumeration of specific pathogens, 0.1 mL from the dilution of  $10^{-2}$  and  $10^{-4}$  of each sample was spread onto membrane fecal coliform, MacConkey agar, mannitol salt agar, and cetrimide agar for the enumeration of total fecal coliform, *Escherichia coli* (*E. coli*), *Staphylococcus* spp., and *Pseudomonas* spp., consecutively. All the plates were incubated at 37 °C for 24 h except membrane fecal coliform agar which was incubated at 44.5 °C for 18–24 h. Presence of *E. coli* was further confirmed by the appearance of bluish–black colonies with the production of green metallic sheen on the eosin–methylene blue agar<sup>[6]</sup>. Confirmative biochemical tests revealed the identity of the specific pathogens<sup>[28]</sup>.

### 2.2. Determination of anti-bacterial activity of the preservatives

The anti-bacterial activity of the preservative samples

was performed by using agar well diffusion method as described previously<sup>[21]</sup>. Lawns of bacterial pathogens (*E. coli*, *Klebsiella* spp., *Pseudomonas* spp., *Salmonella* spp., *Staphylococcus* spp., *Vibrio* spp., *Listeria* spp. and *Bacillus* spp.) were prepared over the Mueller Hinton agar plates and holes were made in the Mueller Hinton agar by cork borer. Each of the homogenized preservative blends (around 10 µg/mL) was then introduced separately in the specified hole with a positive control (streptomycin, 10 µg/mL) and negative control (normal saline). Presence of clear zone around the sample suspension indicated the presence of anti-bacterial activity.

## 3. Results

All samples studied were found to be populated with bacteria within a range of  $10^2$ – $10^5$  CFU/g with the presence of specific pathogenic microorganisms, i.e., *E. coli*, *Pseudomonas* spp. and *Staphylococcus* spp. in most of the samples (Tables 1 and 2). Sodium benzoate, vinegar and honey samples were found to harbor the highest number of spoiling bacteria ( $\sim 10^5$  CFU/g). Next prevalence was noticed in case of sugar and turmeric samples ( $10^4$  CFU/g) while salt and acetic acid samples were populated by a lesser extent of bacteria ( $10^3$  CFU/g). The least microbial spoilage was in the citric acid and sodium sulfite samples ( $10^2$  CFU/g). Except sugar and vinegar, the other 7 samples exhibited the proliferation of fungal population.

**Table 1**

Microbial analysis of different preservative used in food.

Sample	Total aerobic bacteria (CFU/g)	Fungi (CFU/g)	<i>E. coli</i> (CFU/g)	<i>Pseudomonas</i> spp. (CFU/g)	<i>Staphylococcus</i> spp. (CFU/g)
Sugar (n=5)	$4.1 \times 10^4$	0	$7.0 \times 10^1$	0	$1.9 \times 10^2$
Salt (n=5)	$3.2 \times 10^3$	$3.7 \times 10^2$	0	$8.0 \times 10^1$	0
Acetic acid (n=5)	$3.6 \times 10^3$	$4.9 \times 10^2$	0	$1.2 \times 10^1$	0
Citric acid (n=5)	$2.9 \times 10^2$	$2.0 \times 10^2$	0	0	0
Sodium benzoate (n=5)	$5.8 \times 10^5$	$4.5 \times 10^2$	$1.6 \times 10^1$	0	$3.8 \times 10^2$
Vinegar (n=5)	$6.4 \times 10^5$	0	$2.2 \times 10^1$	$3.6 \times 10^2$	$4.6 \times 10^2$
Sodium sulfite (n=5)	$2.8 \times 10^2$	$2.1 \times 10^2$	0	0	0
Honey (n=5)	$3.9 \times 10^5$	$3.3 \times 10^2$	$1 \times 10^1$	$1.8 \times 10^2$	$3.3 \times 10^2$
Turmeric (n=5)	$4.3 \times 10^4$	$3.9 \times 10^1$	0	$1.6 \times 10^1$	$5.9 \times 10^2$

Fecal coliforms were absent in all cases.

Acceptable microbial limits<sup>[48]</sup>: Total aerobic bacteria:  $10^3$  CFU/g; total fungal load:  $10^2$  CFU/g; absence of fecal coliforms, *E. coli*, *S. aureus* and *Pseudomonas* spp. per 1 g of the preservative; absence of *Salmonella* spp. per 10 g of the preservative.

**Table 2**

Confirmative biochemical tests for the different isolates.

Assumed organism	TSI			H <sub>2</sub> S reaction	Indole test	MR test	VP test	Citrate Test	Motility	Oxidase test
	Slant	Butt	Gas							
<i>E. coli</i>	Y	Y	+	–	+	+	–	+	+	–
<i>Klebsiella</i> spp.	Y	Y	–	–	–	–	–	–	–	+
<i>Pseudomonas</i> spp.	Y	R	–	–	–	–	–	–	–	–
<i>S. aureus</i>	Y	R	+	+	–	+	–	+	+	–

TSI: Triple sugar iron test; Y: yellow (acid); R: red (alkaline); MR: methyl red; VP: Voges–Proskauer.

Sodium sulfite and citric acid were found to be free from any contaminating specific pathogenic bacteria while

vinegar and honey samples were found to be contaminated with *E. coli*, *Pseudomonas* spp. and *Staphylococcus* spp. Salt and acetic acid samples harbored only *Pseudomonas* spp. ( $\sim 10^2$  CFU/g), sodium benzoate was found to be contaminated with *E. coli* and *Staphylococcus* spp., and the turmeric samples were contaminated with *Pseudomonas* and *Staphylococcus* spp.

### 3.2. Anti-bacterial traits of the preservative samples

Among the samples studied in our study, sodium sulfite showed the highest activity against all of the test bacteria (Table 3). Acetic acid was found to exhibit the anti-bacterial activity against 6 out of 8 test bacteria while vinegar exhibited the activity against 4 bacteria. Anti-bacterial activity of both of these preservatives was most prominent against *Pseudomonas* spp. (Table 3). Another organic acid, the citric acid in our study also exhibited the activity against all bacteria; however, to a lesser extent compared to that of sodium sulfite. Compared to sodium sulfite, acetic acid, citric acid and vinegar, other samples in our study were found to pose the anti-bacterial activity to a lesser extent.

**Table 3**

Anti-bacterial activity of different preservatives (mm).

Sample	<i>E. coli</i>	<i>Klebsiella</i> spp.	<i>Pseudomonas</i> spp.	<i>Salmonella</i> spp.	<i>Staphylococcus</i> spp.	<i>Vibrio</i> spp.	<i>Listeria</i> spp.	<i>Bacillus</i> spp.
Sugar	18	0	0	0	0	0	0	0
Salt	0	0	0	0	0	0	10	20
Acetic acid	8	0	25	0	12	12	15	15
Citric acid	20	0	25	20	10	15	10	20
Sodium benzoate	0	0	0	0	0	0	7	0
Vinegar	6	0	15	0	0	0	6	8
Sodium sulfite	30	32	32	30	34	30	36	33
Honey	0	7	0	0	0	0	0	0
Turmeric	0	0	0	0	0	0	0	0

Each of the preservative was tested with a concentration of approximately 10 µg/mL.

Activity of salt was scored only against *Listeria* spp. and *Bacillus* spp., while activity of sugar and honey was found only against *E. coli* and *Klebsiella* spp., respectively. No activity was found in case of turmeric samples. Since this preservative was found to be populated with the ubiquitous *Pseudomonas* spp. and the easily transmittable staphylococcal species, absence of anti-bacterial trait was not unlikely.

## 4. Discussion

Food preparations or pharmaceutical/cosmetics solutions often tend to provide suitable media for the growth of microorganisms and hence require the incorporation of a preservative[5,6,22,24]. While bacteria and fungi spoiled food are well known, reports on the food associated preservative spoiling microorganisms are rare. According to our study, the huge proliferation of microorganisms within the samples tested may reveal a possibility of food and pharmaceutical contamination apart from exogenous sources. A limited report on the preservative spoilage by microorganisms exists so far, and hence the reasoning of the microbial prevalence appears a bit difficult; however, considering the pharmaceutical bio-burden cases, we assume that the microbial access into the preservatives

tested in our study might be due to the relatively low concentrations of these food grade products as well as due to the common cause of unhygienic preparation of the preservatives[7,9,11,14,18]. Nevertheless, to our knowledge, the abundance of microorganisms in common preservatives used in Bangladesh has been first time reported in our study. Additionally, the microbial load among the preservatives tested further poses the extended spoilage of the intended food or pharmaceutical products to be used and hence raises the application risk.

It is indeed well reported that the natural substances including salt, sugar and vinegar are also used as traditional preservatives[29]. Besides non-toxicity with acceptability in taste and odour, a preservative should be effective against a wide spectrum of microorganisms. Almost all of the preservatives, either natural or synthetic, act as either antimicrobials or antioxidants or both and hence are known to prevent the growth of molds, yeasts and bacteria[13,29–33]. The history of sodium sulfite's anti-bacterial trait has long been known and this preservative has been shown to pose the therapeutic efficacy[7,34–36]. Consistently, our study also demonstrated the highest anti-bacterial activity of this preservative, which is interestingly in consistent to the least microbial prevalence as observed through microbiological enumeration assay.

Organic acids are popular preservatives with marked anti-bacterial traits[37–40]. In our study, both acetic acid and vinegar exhibited the anti-bacterial activity against *Pseudomonas* spp.; however, both were initially found to harbor *Pseudomonas* spp., with a relatively higher bacterial load especially in the vinegar samples. The reason behind this discrepancy might be the higher concentration of the preservatives used for the study of the anti-bacterial activity. Such a baffling result is suggestive of the further determination of the minimum inhibitory concentration of acetic acid and vinegar. The notable anti-bacterial activity of citric acid in our study is totally consistent with the microbial prevalence data since no pathogen was found to prevail within this preservative.

A recent report has shown sodium benzoate to be effective to extend the shelf life of fruit juice; however, in our study this preservative has been found to exhibit the anti-bacterial activity only against *Listeria* spp. to a minor extent[41]. Besides the vinegar and honey samples, sodium benzoate was also found to be largely propagated with microbial population which in turn could be explanatory behind the anti-bacterial inefficiency of this preservative.

Salting, salt curing, corning or sugar curing of foods by sodium chloride, brine (for bacon, salt pork, etc.) and sucrose (sugar-cured ham, fruit preserves, jams and jellies, etc.) has long been employed to protect food from microbiological spoilage. Salts have long been used for food preservation and have been found to be effective for bacterial killing[31,42–46]. Nevertheless, in the present investigation, the activity was limited. The bacterial prevalence of  $10^3$ – $10^4$  CFU/g in salt and sugar samples in our study was also in line with such a weak anti-bacterial activity of these preservatives. Several reports proved honey to possess significant anti-bacterial activity; however, according to the present study, the activity was not notable probably due to the difference in source, or processing deficiency, or might be due to different experimental conditions[10,12]. Furthermore, presence of all

pathogenic bacteria in honey samples was also suggestive of the weak anti-bacterial activity of this preservative.

While the aspects of anti-bacterial traits of the preservatives could be well discussed in cohort with an array of reports, the scarcity of microbial bio-burden indeed limits the focus on acceptable criteria of the preservatives. However, considering the preservatives used in the current investigation as non-sterile pharmaceutical products, the acceptance criteria of microbial limits should be set for the total aerobic bacteria, total fungal load, and the complete absence of specific pathogens including fecal coliforms, *Staphylococcus aureus* (*S. aureus*), *E. coli*, *Pseudomonas* spp. and *Salmonella* spp., as specified by the pharmacopoeia standards<sup>[47,48]</sup>. In this context, the overall microbiological quality of the preservatives citric acid and sodium sulfite was found to be within the limit which is also in consistent to their anti-bacterial activity against the test bacteria.

Overall, according to our study, sodium sulfite and citric acid samples were found to be satisfactory preservatives both in terms of microbiological criteria and their anti-bacterial traits. The results of anti-bacterial activity of the preservatives presented in this study are in line with their microbiological load, which indeed mark a complete bacteriological profile of the samples tested. Thus, in relation to the microbial proliferation in food samples, the study of anti-bacterial activity of all the common preservatives may further raise the public health concern regarding food safety.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgements

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### Comments

#### Background

Food borne infectious diseases is a principal public health concern, and it has negatively effect on economic growth in many countries. Food preservatives help to protect health by decreasing the risk of food-borne illness caused by microorganisms in food, and by lowering oxidation in the body, which may occur as a result of ingredients in foods that become oxidized. Food spoiling bacteria and fungi are well known, but the knowledge on the extent of microbiological contamination of the associated preservatives has not been reported extensively.

#### Research frontiers

In this article authors performed to quantify the microorganisms in some common food preservatives as well as to detect their *in vitro* anti-bacterial traits.

#### Related reports

It is indeed well reported that the natural substances including salt, sugar and vinegar are also used as traditional preservatives. In addition, a recent report has shown sodium benzoate to be effective to extend the shelf life of fruit juice.

#### Innovations & breakthroughs

The present research shown that sodium sulfite and citric acid samples were satisfactory preservatives both in terms of microbiological criteria and their anti-bacterial traits.

#### Applications

This study significantly contributes to understand microbial load in some common food preservatives as well as anti-bacterial activity of the same food preservatives.

#### Peer review

This is a good study in which the authors evaluated the distribution of microbial loads in common food preservatives. The results are interesting and suggested that sodium sulfite and citric acid samples were satisfactory preservatives both in terms of microbiological criteria and their anti-bacterial traits.

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