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Research Article

FERMENTED SAUERKRAUT JUICE AS ANTIMICROBIAL AGENT: AN *IN VITRO* STUDY

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ABSTRACT
In the present study, the antimicrobial activity of fermented sauerkraut juice was evaluated against human pathogenic bacterial and fungal strains by using agar well diffusion assay. Interestingly the pathogenic fungi was found to be most sensitive than the bacterial pathogens. All the three test fungi such as *Aspergillus fumigatus*, *Aspergillus sp.*, and *Candida albicans* were inhibited by fermented sauerkraut juice with zone of inhibition 36 mm, 35 mm and 15 mm respectively. Antibacterial activity of Sauerkraut juice was observed against *Escherichia coli, Salmonella enterica* ser. *typhi, Staphylococcus epidermidis* and *Bacillus amyloliquifaciens.* The maximum zone of inhibition was observed against *Staphylococcus epidermidis* of 21 mm, followed by *B. amyloliquifaciens* (18 mm) and minimum against *E. coli* (14 mm). No activity was observed against *Salmonella enterica* ser. *typhi.* Present finding suggested that, the fermented sauerkraut juice may be used as antifungal and antibacterial agent to cure infectious diseases and also used as functional foods as it has nutritional values and may also be used as biopreservative for the other food products.
Keywords: Acidic, *Aspergillus, Candida,* cabbage, human pathogens, antibacterial and antifungal activity.

INTRODUCTION

Sauerkraut is an acidic cabbage which result from natural fermentation by bacteria mainly lactic acid bacteria (homofermenter and heterofermenter) indigenous to cabbage in the presence of 2 to 3 % non-iodide salt[1]. Sauerkraut undergoes a sequential fermentation that is initiated by heterofermentative lactic acid bacteria and completed by homofermentative bacteria[2]. Lactic acid bacteria are useful in producing fermented foods such as yoghurt, pickles and are also used as probiotics[3]. Fermented sauerkraut has a long history and is generally considered to be a health promoting product[4]. The sole ingredients in sauerkraut, the cabbage, are rich in vitamins (C and K), boost the immune system and have other beneficial effects[3]. Fermentation of the cabbage juice could enhance the bacteriostatic effect of the cabbage juice as acidified sodium chloride may be formed from the ferment[5]. Such juice may have potential for extended use in the bio preservation of other foods and also has been reported to be used traditionally in the treatment of lung diseases[6]. Sauerkraut is not known as food among the masses of India. Although sauerkraut is manufactured occasionally in the small quantity by a few industries in India, they have conducted no research on preparation of sauerkraut and antimicrobial activity of its juice[7]. However, limited information on the antifungal activity of fermented cabbage juice extracts to further gauge its possibility as a novel drug. So the objective of the present study was to determine the antimicrobial activity of fermented sauerkraut juice against human pathogenic bacteria and fungi to replace the commercially available antibiotics and preservatives.

MATERIALS AND METHODS

Preparation of Sauerkraut

Sauerkraut was prepared by obtaining cabbages (Band Gobi, *Brassica oleracea* var. captita) from the local market of Ambala, Haryana, India. The spotted and defective cabbage heads were trimmed off and the cabbages were shredded with sterile knife. The shredded cabbages were weighed 50 g in two layers followed by sprinkling of NaCl (non-iodized). The shredded cabbage and salt were placed in alternating layers in wide mouth jars. A heavy weight was placed over the mixture in the jars and pressed gently to squeeze out the juice. The jars were covered with sterile lids and incubated at room temperature for 28 days for complete fermentation of cabbage[4].

Tested Microorganisms

Human pathogenic microorganisms were obtained from MTCC, Chandigarh, India for testing antimicrobial activity of sauerkraut juice. The three test fungi were *Aspergillus fumigatus* (MTCC 4163), *Aspergillus sp.* (MTCC 1344), and *Candida albicans* (MTCC 143) and four test bacteria were *Escherichia coli* (MTCC 723), *Salmonella enterica* ser. *Typhi* (MTCC 3216), *Staphylococcus epidermidis* (MTCC 435) and *Bacillus amyloliquifaciens* (MTCC 1488).

Standardisation of Tested Microorganisms

The tested microorganisms were standardised by using 0.5 Mc Farland standards. Mc Farland Standard was used as reference to adjust the turbidity of microbial suspensions so that their number will be within a given range. 0.5 Mc Farland gives approximate cell density of 1.5×10^8 CFU/ml, having absorbance of 0.132 at wavelength of 600 nm. The microbial suspensions were prepared in their respective sterile nutrient broth and are compared either visually or by measuring the absorbance with that of the standard[8].

Agar well diffusion method for antimicrobial activity

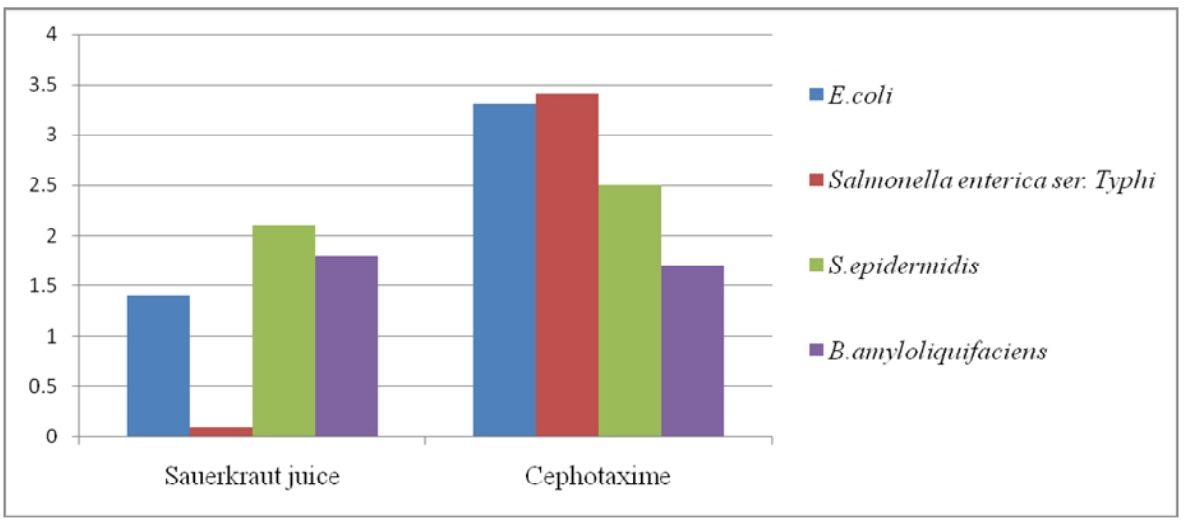
The antimicrobial activity of the fermented sauerkraut was determined by using agar well diffusion method. The protocol of antimicrobial activity is as follows.

Petri plates were prepared by pouring 20 ml of respective sterile molten media for the growth of tested microorganisms and allowed it to solidify
↓
Swabbing of agar plates with 100 µl of each standardized test microorganism
↓
Wells (8 mm in diameter) made into agar plates with sterile borer (8 mm)
↓
Wells loaded with 100 µl of fermented sauerkraut juice
↓
Plates were incubated at 37^{0}C for 24 to 48 h
↓
Measurement of diameter of zone of inhibition (in mm)

Table 1: Antibacterial activity of fermented sauerkraut juice and antibacterial drug against human pathogenic bacterial strains

Sample ↓	Tested microorganisms			
	Escherichia coli **ZOI (in mm)**	***Salmonella enterica ser. Typhi*** **ZOI (in mm)**	***Staphylococcus epidermidis*** **ZOI (in mm)**	***Bacillus amyloliquifaciens*** **ZOI (in mm)**
Sauerkraut juice	14	-	21	18
Cephotaxime	33	34	25	17

ZOI- Zone of Inhibition; - No activity



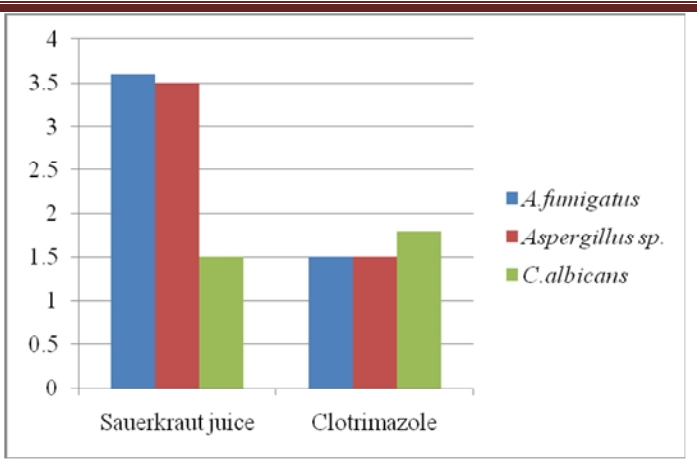
Y axis - Zone of inhibition in cm

Figure 1: Comparison of antibacterial activity of sauerkraut juice with commercially available antibacterial drug (Cephotaxime)

Table 2: Antifungal activity of sauerkraut juice and antifungal drug against pathogenic fungal strains

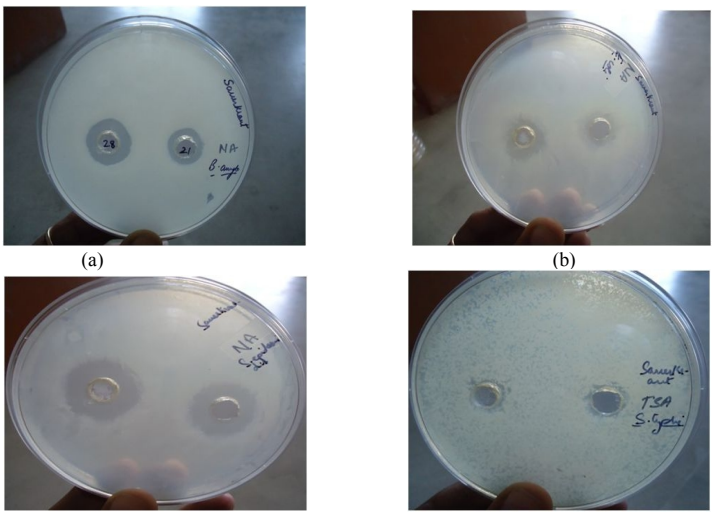
Sample ↓	Tested microorganisms		
	Aspergillus fumigates **ZOI (in mm)**	***Aspergillus sp.*** **ZOI (in mm)**	***Candida albicans*** **ZOI (in mm)**
Sauerkraut juice	36	35	15
Clotrimazole	15	15	18

ZOI- Zone of Inhibition



Y axis - Zone of inhibition in cm

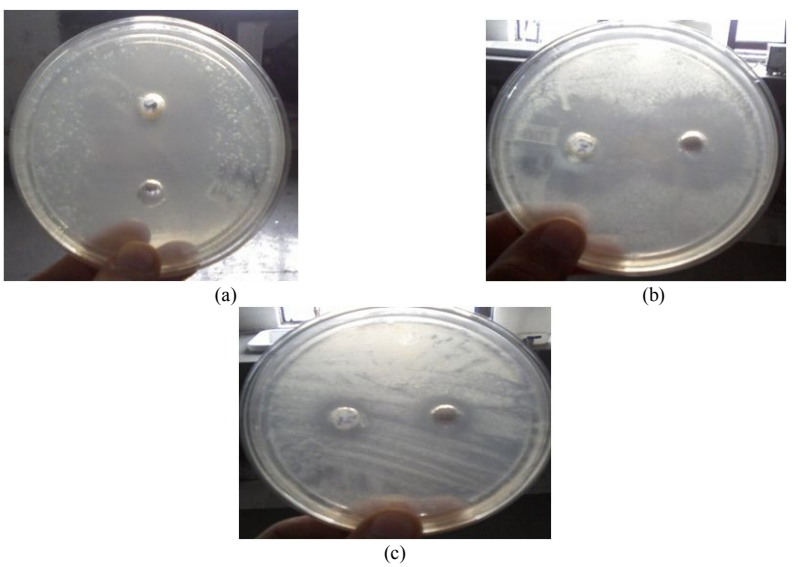
Figure 2: Comparison of antifungal activity of sauerkraut juice with commercially available antifungal drug (Clotrimazole)



(a) (b)

(c) (d)

Figure 3: Antibacterial activity of sauerkraut juice against pathogenic bacterial strains a) *Bacillus amyloliquifaciens,* b) *Escherichia coli,* c) *Staphylococcus epidermidis,* d) *Salmonella enterica ser. typhi.*



(a) (b)

(c)

Figure 4: Antifungal activity of sauerkraut juice against pathogenic fungal strains a) *Aspergillus fumigatus* b) *Aspergillus sp.* c) *Candida albicans*

RESULTS AND DISCUSSION

Antibacterial activity of fermented sauerkraut juice was observed against *Escherichia coli, Salmonella enterica* ser. *typhi, Staphylococcus epidermidis* and *Bacillus amyloliquifaciens* using agar well diffusion method. The maximum zone of inhibition was observed against *S. epidermidis* of 21 mm, followed by *B. amyloliquifaciens* (18 mm) and minimum against *E. coli* (14 mm). No activity was observed against test *Salmonella enterica* ser. *Typhi.* The Gram positive bacterial strains were found to be more sensitive than that of Gram negative bacterial strains. It may be due to the presence of outer layer around the peptidoglycan that acts as a barrier to external molecules. Antibacterial activity of broad spectrum Cephotaxime (30 µg) against tested bacterial strains was also determined by agar disc diffusion method. Cephotaxime showed excellent activity against Gram negative bacteria (33 mm – 34 mm) followed by Gram positive bacteria (17 mm – 25 mm) (Table 1, Figure 1, 3). Antimicrobial activity of fermented sauerkraut juice against food borne bacterial pathogens has also been recorded by Gogo *et al*[8]. Our results negate the finding of Gogo *et al*[8]. They reported that the *E. coli* was the most resistant pathogen whereas our results showed the highest growth inhibition on *Staphylococcus epidermidis* and lowest on *E. coli*. The variation in the diameter of the zone of inhibition against *E. coli* may be due to the type of strain used. The antibacterial activity of cabbage juice has been reported to be due to the glucosinolates degradation by-products found in the juice[9]. Antifungal activity of juice was observed against *Aspergillus fumigatus*, *Aspergillus sp.*, and *Candida albicans.* Maximum zone of inhibition of sauerkraut juice was observed against *Aspergillus fumigatus* with zone 36 mm, followed by *Aspergillus sp.* (35 mm) and minimum against *C. albicans* (15 mm). Antifungal activity of Clotrimazole drug was evaluated against tested fungal strains by agar disc diffusion method. Clotrimazole showed maximum inhibition to *C. albicans* (18 mm) followed by *A. niger* and *A. fumigatus* with zone of inhibition 15 mm (Table 2 and Figure 2, 4). This inferred that the naturally fermented sauerkraut juice showed better antifungal activity than commercially available antifungal drug. The diameter of zone of inhibition was found more in sauerkraut juice than commercially available drug due to the different mechanism of action of the components present in the sauerkraut juice. Cabbage juice has been shown to have antibiotic and antifungal activity against a wide range of bacteria and fungi, and has been used traditionally in the treatment of lung diseases[6]. The antimicrobial activity of sauerkraut juice may be due to low pH and high acidity[7]. The growth inhibitory substance was suggested to be carbohydrate in nature and of low molecular weight.[10] Sulphur compounds from cabbage have been found to show strong antimicrobial activity. The organosulfur components methyl methanethiosulfinate and s-methyl -L- cysteine sulfoxide and sulphides have been found in unfermented cabbage to have antimicrobial effects on some strains[11]. These compounds were however not determined in this study but they might be able to persist during the process of fermentation. It has been shown that organic acids found in fermented foods can inhibit a wide range of microorganisms[12,13].

CONCLUSION

It can be concluded from the present findings that naturally fermented sauerkraut juice showed good antifungal activity followed by antibacterial activity against test human pathogenic fungi and bacteria respectively. The antifungal activity of sauerkraut juice is comparatively better than commercially available antibiotic. It may be suggested that sauerkraut juice is good for consumption upto 28 days of fermentation and also used to control fungal infections. Further research is required for the identification of bioactive molecules present in the sauerkraut juice and *in vivo* efficacy against pathogenic microorganisms before it is used for commercialization in the form of drugs/preservatives as alternative to commercially available antibiotics and preservatives.

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