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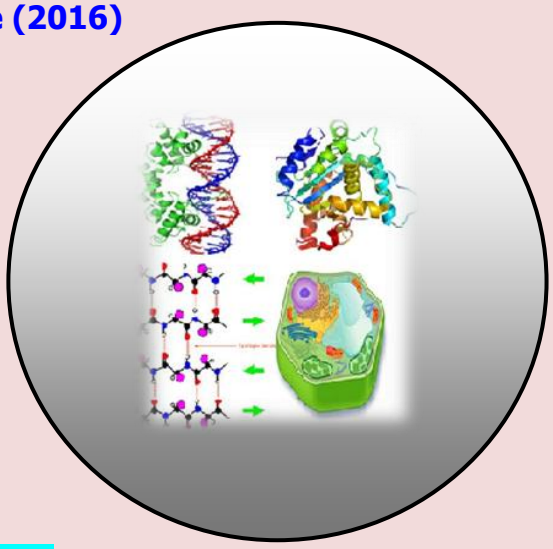

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# *In vitro* Evaluation of Antimicrobial Activity of Sauerkraut against *Burkholderia cepacia* and *Candida glabrata*

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**ABSTRACT**

***Antibacterial and antifungal activities of lactic acid bacteria (LAB) from sauerkraut were studied in vitro. Homofermentative Pediococcus pentosaceus and Lactobacillus plantarum strains were used to conduct a trial and determine their feasibility to eliminate Burkholderia cepacia species of Gram negative bacteria which is an important pathogen in immunocompromised patients associated with long term hospitalization during recovery. Also the antagonistic activities of both LAB isolates were studied against clinically isolated Candida glabrata fungal strains predominantly present in immunocompromised patients. There is limited information about antagonistic activity of fermented sauerkraut extracts to further gauge its possibility as a bio-preservative. Methanol-water extract (MWE), crude protein extracts (CPE) and water-soluble polysaccharide extracts (WSPE) were prepared and tested for their ability to inhibit target pathogens. Agar well diffusion and disk diffusion methods by using different media were performed for screening of selected LAB strains. Extracts from fermented products were used for the same. According to the variations in diameter of inhibition zones antagonistic activity exerted by P. pentosaceus was greater against B. cepacia than C. glabrata .The antimicrobial activity of the sauerkraut extract preparations also varied against both pathogens with more activity exerted by CPE, moderate by WPE and slightly lower by MWE. The overall analysis will be useful in selecting fermented probiotic foods and some of these extracts as natural antimicrobials in healthy and/immunocompromised consumers. Application of both LAB species as a functional starter culture for the safe consumption can contribute to different economically important neutraceutical and technological circumstances in developing countries.***
***Keywords: Sauerkraut, Lactic acid bacteria, Burkholderia cepacia, Candida glabrata and Antimicrobial activity.***

## INTRODUCTION

The antimicrobial effect of fermented sauerkraut as well as evaluation of anti pathogenic potential associated with two homofermentative probiotic strains was done against selected microbial pathogens. Probiotics are well known to possess specific properties such as tolerance towards gastric juice and bile, adhesion to the epithelial cells of the intestine, and improvement of the intestinal microbial balance (Ministry of Food and Drug Safety, 2015a; Isolauri *et al* 2002). The main feature of probiotic is based on their antagonistic or

antimicrobial activities against pathogenic bacteria in the intestine (Quigley, 2010). Some important compounds sulfotransferase, glutathione transferase, and epoxide hydrolase are also found in cruciferous vegetables (Chang 1996; Fahey 1997). Indole and isothiocyanate enzymatic products of myrosinase from glucosinolates found in cauliflower and cabbage lower the incidence of tumor formation and have an antioxidative effect (Nestle 1998). There are also antifungal components such as iprodione (Datta 1999), chitosan (Rhoades 2000; Roller 1999), and a peptide (Lucca 1999) in cruciferous vegetables. *Burkholderia cepacia* complex (BCC) is an established pathogen in two patient populations with genetic diseases *viz*. cystic fibrosis (CF) and chronic granulomatous disease. Infection with BCC leads to a rapid decline in lung function and is often a contra indication for lung transplant, significantly influencing morbidity and mortality associated with CF disease (Narayana swamy 2017) .Moreover, BCC has become an increasingly common nosocomial pathogen due to its high intrinsic and acquired antimicrobial resistance, lack of effective antibiotics, and survival ability in the environment for prolonged periods of time. The increasing incidence of infections by these organisms along with the rising drug resistance warrants a close monitoring of the antimicrobial susceptibility of these organisms (Vikas 2012). Several studies showed that "*B. cepacia*" strains can spread between CF patients via simultaneous hospital admissions or social contact outside the hospital. (Li Puma, 1998).*Candida glabrata* is a growing challenge in clinical settings where it causes mucosal infections and is associated with approximately 15% of all Candida related systemic bloodstream infections (Pfaller 2004; 2007). Infections due to *C. glabrata* are characterized by high mortality rates and they are difficult to treat due to reduced susceptibility of these species to azoles, especially fluconazole (Rinaldi 2006; Germain 2001; Paul 1999; Karen 2002).

Sauerkraut LAB activity is likely to be because of production of antibacterial & antifungal peptides & exopolysaccharide during fermentation period. The trial conducted showed that the sauerkraut LAB cultures and extracts have the ability to prevent growth of the *Burkholderia* and *Candida spp*. The direct addition of selected starter cultures of LAB to raw materials in sauerkraut production has been a break through resulting in a high degree of control over the fermentation process and standardization of the end products.

## MATERIALS AND METHODS

### *Experimental Design and Treatments*

The aim of this research was to study the antimicrobial activities of sauerkraut as well as the antagonistic activity studies of some selected LAB strains isolated at different time intervals during fermentation process. The treatments consisted of selection of LAB strains, preparation of sauerkraut extract components, antibiotic sensitivity testing of target pathogens and antimicrobial activity assays of both LAB strains as well as sauerkraut extract fractions. The experiment was designed as a complete randomized design and replicated three times.

### *LAB Strains Isolation and Selection from Sauerkraut*

All isolated pure LAB cultures used in this study were recovered from traditional raw cabbage fermentation product known as sauerkraut. A number of bacterial strains were isolated from 10 grams of samples collected aseptically from the fermentation container during different time intervals. Each sample was homogenized in 90 ml sterile quarter strength Ringer Salt Solution (HiMedia Laboratories) using the vortex and $10^{-1}$ to $10^{-8}$ serial dilutions were made. From each dilution 0.1 ml of suspension was spread on sterile Lactobacillus MRS Agar (De Man, Rogosa and Sharpe agar. Lactobacillus MRS Agar, M641- HiMedia Laboratories) plates with bromocrysol purple dye (0.02%) and incubation was done in $C0_2$ incubator [ESCO CelCulture $C0_2$ Incubator(CCL)] at 35°C in presence of 5% $C0_2$ concentration. 60 Colonies were purified twice by streaking on MRS Agar plates at 35°C for 48 hrs in $CO_2$ incubator. Gram positive, Catalase negative rods or cocci in tetrads, acid producing colonies were cultivated in MRS broth supplemented with 10 % (v/v) glycerol and maintained at -20°C for further studies. On the basis of probiotic potential studies in previous work 10 best strains of *Lactobacillus* and *Pediococcus* (data not included) were selected for further studies (Bhosale 2018).

### *Preparation of Cell Free Supernatants*

Selected probiotic LAB strains were inoculated at a cell concentration of $10^5$ cells/ ml in 200 ml of sterile MRS broth (M369- HiMedia Laboratories) and incubated as still cultures at 35°C for 24 hrs and 48 hrs in $CO_2$ incubator. Cell free supernatant was obtained by centrifugation at 10000 rpm for 15 min and sterile filtration (Sterlitech stainless steel syringe Filter holder KS 13) (Jesper 2003).

***Methanol Water Extract (MWE)***

At different time intervals of sauerkraut preparation, 500 gram samples were freeze-dried and macerated in methanol (ideal) for 12 hours. (Not real liquid nitrogen, this is poor man's "LIQUID NITROGEN" W. Beaty 2008). The methanol extract was filtered and the residue was extracted with boiling distilled water (1:10 w/ v) for 15 minutes and then centrifuged (10000rpm) for 10 minutes. The supernatant obtained was freeze dried to provide a methanol-water extract and was studied for antimicrobial activity capacity against both targeted pathogens.

***Water Extracts and Crude Protein Extracts (CPE)***

Water extracts and crude protein extracts were obtained by blending freeze-dried (500 gram) sauerkraut with sterile distilled water (1:10 w/v) in a blender (Hamilton Beach Hbh550 Series Fury Electric Blender). Slight modification was done in a method by Shu-Hui Hu, Jinn-Chyi Wang, 2004 .The homogenate was maintained at 20°C for 10 hours and filtered through filter paper (Whatman™ 1001-090 Grade 1Whatman/GE Healthcare). Part of the filtrate was freeze dried to give the water extract. The remainder was subjected to ammonium sulfate precipitation. Ammonium sulfate was added to the filtrate to a final concentration of 50% and the precipitate collected by centrifugation at 12400 rpm at 4°C for 20 minutes. The precipitate was dissolved in 0.02 M (20mM) Tris-HCl (pH 8.2) buffer solutions containing 0.1, 0.3, and 0.8 M NaCl, respectively. Protein content was determined by the Bradford assay (Copeland 1994). The sample was designated as the crude protein extracts (Roe 2001).

***Water Soluble Polysaccharides Extract (WSPE)***

Water-soluble polysaccharide extract was prepared by adding sterile distilled water (1:10 w/v) to the sauerkraut. Mixture was heated at 60°C for 20 minutes, and then filtered through Whatman No. 1 filter paper. Ethyl alcohol (95%) was added to the filtrate, followed by 75% alcohol to precipitate polysaccharides (Wang1998). The precipitate was collected by centrifugation and dissolved in distilled water and filtered to collect the components with molecular weight below $10^5$ Da. These components were concentrated by freeze drying and then redissolved in deionized water (pH 7.0). The content of the water-soluble polysaccharides were determined using the phenol-sulfuric acid method (Chaplin 1994).

***Targeted Test Strains***

*Burkholderia cepacia* and *Candida glabrata* isolated from patient's samples were used as pathogenic bacteria and fungi target test strains respectively. Strain identification was done by VITEK 2 automated microbial identification system. (Sahyadri Speciality Labs Pune) .Bacteria was cultured in sterile nutrient broth (NA- MM 244 HiMedia Laboratories) at 37°C for 18 hours. Yeasts were cultured in sterile Sabouraud Dextrose Broth (SDA-M 033HiMedia Laboratories) at 30°C for 48 hours. Both cultures were appropriately diluted in sterile normal saline solution to obtain the cell concentration at $10^6$ CFU/ml. 10 % (v/v) glycerol stocks were maintained at -20$^{o}$C for further studies.

***Antibiotic Resistance of Burkholderia cepacia***

Antibiotic sensitivity of *Burkholderia cepacia* was determined on Vitek 2 and interpretation done according to CLSI guidelines 28$^{th}$ Edition–Jan 2018. Following antibiotics were tested - Ticarcillin/Clavulanic acid, Ceftazidime, Meropenem, Minocycline, Levofloxacin and Trimethoprim- sulfamethoxazole. *Escherichia coli* ATCC 25922 was used as quality control strain and results were within the recommended ranges.

***Antibiotic Resistance of Candida glabrata***

Antibiotic sensitivity test of *Candida glabrata* was performed on Vitek 2 and interpretation was done by the system as per breakpoints according to CLSI and EUCAST guidelines [(document M27$^2$ 2018 & document M44-A2. Second edition Vol.29 No.17.2009); (Epidemiological Cut off Values for Antifungal Susceptibility Testing M59, 2nd ed. January 2018)]. Four antifungal drugs Caspofungin, Amphotericin- B, Micafungin and Flucytosine were reported. *Candida tropicalis* ATCC750 was used as quality control strain and results were within the recommended ranges.

**ANTIMICROBIAL ASSAY**

***Antibacterial Activity by Agar Well Diffusion Method***

*Burkholderia cepacia* fresh broth culture was activated into sterile nutrient broth (10 ml) followed by incubation at 37$^{0}$C for 24 hrs. The optical density of each active culture was adjusted to 0.1 at 625 nm to give standard inoculums of $10^6$ cfu / ml. Bacterial counts were confirmed by plating out on sterile nutrient agar (NA- MM 012-HiMedia Laboratories) plates incubated at 37$^{0}$C for 48 hrs. After that 100 µl ($10^5$cfu / ml) of prepared culture was spread on surfaces of sterile Mueller–Hinton agar and Brain Heart Infusion Agar (BHI Agar-SM211D-Hi-media Laboratories) plates with sterile cotton swabs.

Then, a well with a diameter of 6 mm was punched aseptically with a sterile cork borer and a volume 50µl of the extract as well as cell-free supernatants from 24 hrs and 48 hrs old LAB cultures were introduced into the well. 50 µl sterile nutrient broth processed same as above was used as control. These plates were then kept at ambient temperature for 30 min to allow diffusion of extracts prior to incubation at $37^0$C for 24 hrs. Inhibition zones (including the diameter of well) were measured, and values < 12 mm were considered as non active extracts against bacteria. Inhibition zone size were recorded as 13 -18 mm (+), 19 - 24mm (++) and 25-35mm (+++) respectively.

***Antibacterial Activity by Disk Diffusion Method***

The disk diffusion method of Bhunia *et al*. (1988) was followed with modifications. Fresh cells of indicator bacteria, at concentrations of $10^5$cells/ml, were spread on sterile MH and BHI agar plate surfaces. Sauerkraut extract preparations and cell-free supernatants from 24 hrs and 48 hrs old LAB cultures were added to sterile paper discs (disc diameter 6mm) and placed on the agar surface and incubated at $37^0$ C for 24 hrs. Sterile BHI broth was absorbed to a disc and placed as a control. After incubation, a clear zone around a disc was evidence of antimicrobial activity. Antibiotic discs meropenem, chloramphenicol and levofloxacin were used as standard drugs.

***Antifungal Activity by Agar Well Diffusion Method***

The antifungal activity of the sauerkraut and both LAB isolates was checked against *Candida glabrata* by agar well diffusion technique. In short supernatants of both LAB isolates as well as sauerkraut extract preparations were monitored for antifungal activity against indicator yeast. Inoculation of 0.1 ml ($10^5$ CFU/ ml) targeted fungal strain was done on Sabouraud Dextrose Agar (SDA agar M 063-HiMedia Laboratories) and Antifungal Assay Agar (AFA agar M164-HiMedia Laboratories) plates. A volume of 50µl of cell free supernatants of LAB preparations as well as extract fractions were filled in 6 mm diameter wells cut in the SDA and AFA agar plates seeded with test organism. The diameter of the inhibition zone was measured after 48 hrs of incubation at $30^0$C. Control experiments were carried out under similar conditions by using nystatin and griseofulvin as standard drugs. The results were confirmed by repeating the experiments thrice. The antifungal activity was classified according to the zone of inhibition such as strong +++ (25-30mm), moderate ++ (15-24mm) and mild + (11-14mm) and no inhibition(less than 10mm) on respective media agar plates.

***Antifungal Activity by Disk Diffusion Method***

Method described by Oh *et al.* 2000 with some modification. Briefly, target yeast was transferred onto a 9 cm diameter Petri dish containing sterile SDA and AFA agar using a sterile cotton swab and spread over the whole surface of the medium as a thin film. Sterilized 9 mm diameter antibacterial susceptibility blank disc (Sterile Discs SD067 HiMedia Laboratories) was loaded with 50 µl of each LAB supernatant after the cells were removed from the filtered medium centrifuged at 10000 rpm for 10 min and left to dry in an open sterile petri dish in a incubator at $37^0$C for 10 min. Same method was applied for sauerkraut extract preparations .After that, the discs were placed on the agar plates seeded with test organism. The inhibition of fungal growth was evaluated by measuring the diameter of the transparent inhibition zone around each disc.

**RESULTS**

Total 60 lactic acid producing bacterial strains were isolated from fermented sauerkraut; of which16 were rod shaped and 18 were cocci in tetrads. Ten LAB strains were identified using the MALDI TOF analysis (National Centre for Microbial Resource NCCS, Pune) as homofermentative *Lactobacillus plantarum* & *Pediococcus pentosaceus* species. All were screened for *in vitro* antibacterial and antifungal activities towards acquired emerging pathogens. *B. cepacia* was reported susceptible towards only three drugs Ceftazidime, Meropenem and Trimethoprim/Sulfamethoxazole .Intermediate type of sensitivity pattern was observed by Levofloxacin and Minocycline against target bacterial pathogen. *C.glabrata* was reported resistant against Caspofungin & Micafungin but sensitive to Amphotericin B and intermediate sensitive to Flucytosine. The antimicrobial activity of the CFS & sauerkraut extracts preparations at different time intervals of fermentation was quantitatively assessed by the presence or absence of inhibition zone and zone diameter, respectively (Table 1 and 2). Cell free supernatants of all LAB strains exhibited the effective inhibition activity against pathogenic bacteria & fungi by agar well diffusion & disk diffusion methods. The diameter of inhibition zones varied from 9 mm to35 mm against *Burkholderia cepacia* & the diameter of inhibition zones varied from 9 mm to 30 mm against *Candida glabrata*. The antagonistic activity exerted by *Pediococcus pentosaceus* was greater against Gram negative bacteria than the fungal strain.

**Table 1. Antimicrobial activity of MWE, CPE & WSPE against *B. cepacia* and *C. glabrata.***

| Sauerkraut Extract Type | Days of Fermentation | Agar Well Diffusion *(B. cepacia)* | | Disk Diffusion *(B. cepacia)* | | Agar Well Diffusion *(C. glabrata)* | | Disk Diffusion *(C. glabrata)* | |
|---|---|---|---|---|---|---|---|---|---|
| | | MHA | BHIA | MHA | BHIA | SDA | AFA | SDA | AFA |
| Methanol Water | Day 05 | - | - | - | - | - | - | - | - |
| | Day 10 | + | + | + | + | + | - | + | - |
| | Day 15 | +++ | ++ | ++ | ++ | ++ | + | + | + |
| | Day 20 | ++ | + | + | + | - | - | - | - |
| Crude Protein | Day 05 | + | + | + | - | - | - | - | - |
| | Day 10 | +++ | +++ | ++ | + | ++ | + | + | - |
| | Day 15 | +++ | +++ | ++ | ++ | +++ | ++ | ++ | ++ |
| | Day 20 | ++ | ++ | + | + | ++ | + | + | + |
| Water Soluble PS | Day 05 | - | - | - | - | - | - | - | - |
| | Day 10 | + | + | + | + | + | + | - | - |
| | Day 15 | ++ | + | - | - | + | - | + | - |
| | Day 20 | + | + | - | - | - | - | - | - |

**PS: Polysaccharides, MHA: Mueller Hinton Agar, BHIA: Brain Heart Infusion Agar, SDA: Sabouraud Dextrose Agar and AFA: Antifungal Assay Agar**

**Table 2. Antimicrobial activity of CFS against *Burkholderia cepacia* and *Candida glabrata.***

| Assay Method | Agar Well Diffusion *(Burkholderia cepacia)* | | | | Disk Diffusion *(Burkholderia cepacia)* | | | | Agar Well Diffusion *(Candida glabrata)* | | | | Disk Diffusion *(Candida glabrata)* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Type of Media | BHIA | | MHA | | BHIA | | MHA | | SDA | | AFA | | SDA | | AFA | |
| Time hrs / CFS | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 48 |
| SSLb03 | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ | + | ++ | - | - | + | ++ | - | - |
| SSLb07 | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ | + | ++ | - | - | + | ++ | - | - |
| SSLb10 | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ | + | ++ | - | - | + | ++ | - | - |
| SSLb15 | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ | + | ++ | - | - | + | ++ | - | - |
| SSLb18 | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ | + | ++ | - | - | + | ++ | - | - |
| SSP01 | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ | - | - | ++ | +++ | - | + |
| SSP04 | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ | - | - | ++ | +++ | - | + |
| SSP09 | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ | - | + | ++ | +++ | - | + |
| SSP11 | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ | - | + | ++ | +++ | - | + |
| SSP14 | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ | ++ | +++ | - | + | ++ | +++ | - | + |

**CFS: Cell Free Supernatant, BHIA: Brain Heart Infusion Agar, MHA: Mueller Hinton Agar, SDA: Sabouraud Dextrose Agar and AFA: Antifungal Assay Agar**

Crude protein extract preparations showed better activity than methanol water extract against both pathogens. Water soluble polysaccharide extract preparations showed minimal activities against *Burkholderia cepacia* and almost no inhibition zone were observed against *Candida glabrata* by disk diffusion method. Zone of inhibition measured in mm were greater on SDA agar plates than AFA agar plates. Also the diameter of zone of inhibition was different on BHI & MH agar plates for *Burkholderia cepacia*; zone size was greater on MH agar plates than BHI agar plates. Variable results were observed for the antagonistic activities by using different sauerkraut extract preparations. The extraction products prepared at 10 to 15 days of fermentations exhibited the highest antimicrobial activities by agar well diffusion assay than the disk diffusion methods.

The actual size of zone of inhibition by CPE was greater than MWE. WSPE preparations showed lowest activity against bacterial pathogen and no activity was observed against fungal target strain by disk diffusion method. On prolonged incubation the antimicrobial activities of sauerkraut extract preparations were reduced slowly (after 18-20 days of fermentation period) at room temperature. Within 16-18 days of fermentation the final product was stored in refrigerator temperature (2 to 4°C).

**DISCUSSION**

*Burkholderia cepacia* is multidrug resistant organism. The multidrug resistance appears to result from ability of formation of bio films and action of various efflux pumps that efficiently remove antibiotics from the cell and decrease contact of antibiotics with the bacterial cell surface. *B. cepacia* is resistant to many disinfectants, cleansers and is unaffected by many preservatives including betadine. Drugs contaminated with *B. cepacia* pose a serious threat to susceptible patients, particularly those with cystic fibrosis or who are otherwise immunocompromised (Lynn 2011). In general, *Candida glabrata* is transmitted nosocomially in immunocompromised debilitated patients through the hands of healthcare workers (Rodrigues 2014). The agent may also be acquired from colonized mucosa after chemotherapy. In patients with malignancies *C. glabrata* is one of the most frequent non- albicans *Candida* clinical isolates (Farmakiotis 2015). Consequently, *C. glabrata* infections have a high mortality rate in compromised, at-risk hospitalized patients. Primary metabolites are produced during natural fermentation of sauerkraut along with the growth of LAB at successive stages of fermentation. The role of antimicrobial compounds produced by probiotic strains as prophylactic agents against enteric infections is crucial and well documented (Zhao 2008; Chiu 2013; Wen 2013). Some of LAB produces bacteriocins, antibacterial proteinaceous substances with bactericidal activity against related species (narrow spectrum) or across genera (broad spectrum of activity) (Rogelj 1994; Cotter 2005). The mechanisms behind the prevention of gastrointestinal and urinary tract infections by probiotic bacteria have been elucidated in animal, but also in human studies, confirming enhancement of immune responses and production of antimicrobial substances (Vanderbergh1993; Antikainen 2009; Gill 2003; Reid 2006).However, there is increasing clinical evidence that probiotics are effective not only in the treatment and prevention of gastrointestinal diseases, but also in chronic liver disease, multiple organ dysfunction syndrome and autoimmune diseases (Demeria 2009, Loguercio 2005; Matsuzaki2007). Antifungal peptides are potential candidates due to their membrane-disrupting mechanism of action. By specifically targeting fungal membranes and being rapidly fungicidal, they can reduce the chances of resistance development and treatment duration. (Ng Sm 2017).Numerous strains of lactobacillus genera have a potential to produce exopolysaccharide under specific growth conditions with a wide range and diversity of structure and have a potential to be used as neutraceutical (Badel 2011). In recent years, some bioactive polysaccharides extracted and separated from plants, especially from medicinal plants, have attracted significant attention in the fields of pharmacology and biochemistry because of their potential biological activities, such as antioxidant (Hoang, 2015; Boual, 2015), glycosidase inhibitory (Cui 2015), anticoagulant (Li 2015; Cai 2016), prebiotic (Nadour 2015), antitumor (Zhu2016) and immunobiological effects (Heiss 2015).There are comparatively few reports on antifungal effects of LAB and overall vegetable fermentation products. Antifungal activities of LAB isolated from Tarhana against *Alternaria alternata, Aspergillus parasiticus, Aspergillus oryzae, Penicillium griseofulvum, Penicillium chrysogenum, Penicillium notatum, Penicillium citrinum, Penicillium roqueforti, Aspergillus fumigatus* were studied in 2014. El-Gendy and Marth (1981) reported that *Lactobacillus casei* inhibited the growth and the aflatoxin production of *Aspergillus parasiticus*. Ström *et al* (2002) and Magnusson *et al* (2003) purified three antifungal substances, cyclo(*L*-Phe-*L*-Pro),cyclo(*L*-Phe-*trans*-4-OH-*L*-Pro) and phenyl acetic acid, from *L. plantarum*, *L. pentosaceus*, and *L. sakei*, respectively. This indicates that other antifungal compounds are widely distributed among different species of lactic acid bacteria. In this study, the ten LAB strains isolated from sauerkraut exhibited antifungal activities against *C.glabrata* which was measured by diameter of zone of inhibitions on SDA and AFA. Although there was difference in result parameters by two different methods , agar well method can be accepted as preferable method and more perfect studies can be carried out with more precision .Further investigations on the nature of inhibiting compounds and their mechanisms will be able to provide a great potential for the control of other pathogenic fungal strains. More than one compound is responsible for the antimicrobial activities from mixed culture of lactic acid bacteria. *Pediococcus pentosaceus* & *Lactobacillus plantarum* to produce antimicrobial peptides and polysaccharides can be used as either the starter cultures or their products as protective cultures or biopreservatives in sauerkraut – probiotic vegetable based fermentation product.

The overuse and misuse of antibiotics causes the spread of community and hospital acquired infections by drug resistant bacteria throughout the world .These bacterial and fungal strains are resistant to single and multiple antimicrobial drugs .The application of probiotic cells and competitive exclusion preparations of lactic acid bacteria in human host may be prophylactic and curing therapy as one of the alternatives in future. During sauerkraut fermentation application of standard LAB preparations with optimal growth parameters for production of antimicrobial bacteriocins is more cost effective approach than direct application of pure antibiotics in clinical infections. Sauerkraut is considered as food supplement, not drugs with new generation of live probiotics. It will be a positive futuristic step made for different gastrointestinal and urogenital disease therapies or immunoglobulin and other protein based therapies. Further work on sauerkraut can be carried out based on genomic & proteomic research for evaluation of sauerkraut specific probiotics as drugs for prevention and treatment of critical infections and diseases.

## CONCLUSION

These results suggest that Pediococcus and Lactobacillus from sauerkraut have different characteristics and functional properties related to antimicrobial mechanism against pathogenic bacteria and fungi. Combined effects of two probiotic sub-organisms definitely generate the multi-functions of commercial probiotic product sauerkraut. Ethanol extraction is a simple procedure that can be applied to industrial production. CPE and WSPE preparations on the industrial production level should be of great importance in utilizing the unique functions of CPE more efficiently and satisfactorily.

**Conflicts of interest**

All authors declare no conflicts of interest.

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