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Screening of Antimicrobial and Synergistic Activity of *Withania somnifera, Aloe vera* Plant Extracts and Certain Lactic Acid Bacterial Strains Against Food Borne Pathogens

B. Srinu*[1], K. Kasturi Devi[2], J. S. Bedi[1#]. T. Madhava Rao[1] and K. Kondal Reddy[3]

Department of [1]Veterinary Public Health, [2]Pharmacology and Toxicology, [3]Livestock Products Technology, [#]School of Public Health and Zoonosis, GADVASU, [1,2,3]College of Veterinary Science, Rajendranagar, Hyderabad, Telangana
*beesamsrinu@gmail.com

Abstract

The methanolic extract of plants *Withania somnifera, Aloe vera* and certain lactic acid bacterial (LAB) strains were evaluated for their antibacterial and synergistic effect against food borne pathogens. Antagonistic/synergistic activity of plant extracts and LAB were tested alone and combinely against selected food borne pathogens by using well diffusion method. The results exhibited that the extracts of *Withania somnifera, Aloe vera* and the lactic acid bacterial strainswere showed excellentantimicrobial activity when they are tested alone against the test organisms.But the results obtained from the combined testing of methanolic extracts of plants and Lactic acid bacterial strains significantly lesser antibacterial activityagainst all the tested organisms except with *Pediococciacidolactici 252* which showed synergistic activity with methonalic extract of *Withania somnifera* against *E.coli*. The present study indicated that the methanolic extracts of *Withania somnifera, Aloe vera* and LAB showed good antibacterial activity when they are used individually then the combined and these can be used as a natural drug for the treatment of several infectious diseases.

Keywords: Antimicrobial activity, Food borne, *Withanea somnifera, Aloe Vera,* LAB

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Introduction

Antibiotics play a major role in clinical medicine in eradicating pathogens. Worldwide excessive usage of antibiotics is increasing from last few decades, due to this there is development of multidrug resistance among the pathogenic bacteria (7). Due to the emergence of multiple drug resistance in human pathogens has necessitated a search for new antimicrobial substances from other sources. To generate suitable alternatives, we first need to understand better why antibiotics are being overused and to define the unintended consequences. Antibiotics are used for disease treatment and prevention in both humans and animals. Historically, antibiotics have also been used for improving growth promotion in food-producing animals.

There are various alternatives to the antibiotics, which are developed for treating the specific diseases, like bacteriophage therapy (2), predatory bacteria (5), bacteriocins (3), and competitive exclusion of pathogens (13). According to World Health Organization (4) medicinal plants would be the best source to obtain a variety of drugs. Bacteriocins are the compounds which are produced by the lactic acid bacteria that have a biologically active protein moiety and bactericidal action (6). The use of plant extracts, Lactic acid bacterial strains (LAB) with

* *Author for correspondence*

known antimicrobial properties, can be of great significance in the treatment of various microbial infections.

In the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulteration and side effects (14). Antibiotics primarily that target bacterial infection, there are numerous approaches to either reduce the selection for antibiotic resistance or to replace the antibiotic altogether. Each approach has its own benefits and costs. To treat antibiotic-resistance problem should be a multifaceted and it includes educating the people for reduced use of antibiotics by replacing with other available alternative products. But no alternative method will replace the all uses of antibiotics, because a variety of specific and general methods are needed to both prevent and treat disease. Therefore, there is the need to search for efficient economical alternative to the antibiotics. In the present study the methanolic extracts of *Withanea somnifera, Aloe vera* and LAB were evaluated for their antagonistic/ synergistic activity against common food borne pathogens.

Material and methods

Collection of plant material

The plant materials were collected from different localities in Tirupati. The samples were washed thoroughly to remove dirt particles present on the surface, disinfected. The samples were then dried in oven. The dried samples were crushed into powdered form by mortar and pestle.

Preparation of extracts

The extracts obtained by soaking of twenty five grams plant material powder sample in 100 ml of methanol and allowed to stand for 72 hrs followed by filtration. The filtered extracts were kept in water bath at 40°C (methanolic extract) till evaporation of the solvents. The crude extracts left after water bath were scratched against the walls of the beaker and were weighed. The crude extracts were dissolved in DMSO (Dimethyl Sulphoxide) according to the standard of 1ml DMSO/mg of extracts. The extracts were then collected in centrifuge tubes and were stored at 4°C in a refrigerator (12).

Biological and Chemicals

The lactic acid bacterial strains *Bifidobacterium bifidum 229, Lactobacillus helviticus 194,* and *Pediococcus acidilactici 252* were obtained from National Dairy Research Institute (NDRI, Karnal) and National Institute of Nutrition (NIN, Hyderabad). Media and chemicals were procured from Himedia Laboratories, India and prepared according to standard procedures. The lactic acid bacterial strains were sub-cultured three times before use in sterile de Mann Mclean Rogosa Sharpe broth (MRS) using 1% inoculums and incubated at 37°C for 48 h.

Procurement and maintenance of test pathogens

Various food borne pathogenic microorganisms are used in the study. The food borne pathogenic microorganisms are sub cultured 3 times at 37^{0}C for 24 h before the usage and 1ml of culture inoculated into nutrient broth and incubated at 37^{0}C for 24 h. Overnight cultures were used to test the antibacterial activity of plant extracts and LAB by using well diffusion method.

Antibacterial activity by lactic acid bacteria

The antibacterial activity of different extracts of the various parts of the plant and Lactic acid bacterial strains was studied by agar well diffusion method described by (7) was followed. An overnight culture of food borne pathogenic microorganisms namely *Escherichia coli*, *Klebsialla pneumoniae, Salmonella typhimurium, and Staphylococci aureus* were grown in nutrient broth. A lawn of an indicator strain i.e. pathogenic organisms were made by spreading the cell suspension over the surface of MRS agar plates with a sterile cotton swab. The plates were allowed to dry and a sterile cork borer of diameter 7.0 mm was used to cut uniform wells in the MRS agar plates. Each well was filled with 50 µl of lactic acid bacterial inoculums from MRS broth and incubated at 37°C for 36-48 h. After incubation, the diameter (mm) of the inhibition zone around the well was measured.

Antibacterial activity by plant extracts

Antimicrobial activity of methonolic extracts of plants were tested against food borne pathogenic bacteria using agar well diffusion method (8). Bacteria (200 µl) were aseptically introduced and spread using cotton swabs on surface of nutrient agar plates. A well of about 7 mm diameter with sterile cork borer was aseptically punched on each agar plate. 50 µl of the plant extracts were introduced into the wells in the plates. Plates were kept in laminar flow for 30 min for pre diffusion of extract to occur and were then incubated at 37°C for 24 h. The presence of zone of inhibition was regarded as the indicator of antimicrobial activity and

was expressed in terms of average diameter of the zone of inhibition in millimeter. To evaluate the efficiency of the methodology and to compare the potentiality of the antibacterial effect of the crude extracts tested, a negative control well was made with 50 µl of the extracting solvent and a positive control was made by placing standard antibiotic disc. The standard antibiotic used in this assay are as follows: Tetracycline (T 30 µg, Oxoid), Vancomycin (V 30 µg, Oxoid) and Sulphamethoxazole trimetoprine (SXT 25 µg, Oxoid).

Antibacterial activity of plant extracts against LAB

Overnight cultures of LAB were grown in MRS broth. A lawn of an indicator strain i.e. LAB were made by spreading the cell suspension over the surface of MRS agar plates with a sterile cotton swab. The plates were allowed to dry and a sterile cork borer of diameter 7.0 mm was used to cut uniform wells in the MRS agar plates. Each well was filled with 50 µl plant extract and incubated at 37°C for 36-48 h. After incubation, the diameter (mm) of the inhibition zone around the well was measured.

Antagonistic/synergistic activity of plant extracts, LAB combinely against food borne pathogens

An overnight culture of food borne pathogenic microorganisms namely *Escherichia coli*, *Klebsialla pneumoniae, Salmonella typhimurium, and Staphylococci aureus* were grown in nutrient broth. A lawn of an indicator strain i.e. pathogenic organisms were made by spreading the cell suspension over the surface of nutrient agar plates with a sterile cotton swab. The plates were allowed to dry and a sterile cork borer of diameter 7.0 mm was used to cut uniform wells in the nutrient agar plates. Each well was filled with 25 µl of lactic acid bacterial inoculums from MRS broth, 25 µl of plant extract (1:1) and incubated at 37°C for 36-48 h. After incubation, the diameter (mm) of the inhibition zone around the well was measured.

Results

Antimicrobial activityof methanolic extracts of *Withanea somnifera* and *Aloe Vera*, were tested against food borne pathogens by using Well diffusion method and the results obtained are presented in Table 2.The present study revealed that the methonolic extract of *Withanea somnifera* showed higher zone of inhibition against *Staphylococcus aureus* (24mm) (gram positive) when comparative to other test organisms of *E.coli* (16mm), *S.typhimureum* (18mm) and *K. pneumonea* (13mm) (gram negative) when tested alone. Whereas, methonolic extract of Aloe vera showed higher zone of inhibitionin *K.pneumonea*(14mm) then *E.coli* (10mm) and *S. typhimureum* (4mm) (gram negative). *Staphylococcus aureus* showed resistance to methonolic extract of *Aloe vera.*

The three LAB strains used in the study have shown good antibacterial activity (Table 3, fig 1.) against different food borne pathogens with varying degree of zone of inhibition when they are used alone. *Bifidobacterium bifidum*-229 showed good antibacterial activity against the tested pathogens with the range of zone of inhibition 13 mm to 17 mm. *Lactobacillus helviticus 194* inhibited the growth of all pathogens tested with the range of zone of inhibition from 11mm to 18mm. Among three LAB strains used in the study *Pediococci acidolactici 252* showed higher antibacterial activities with a range of zone of inhibition from 19mm to 22mm.

The study exhibited that all the LAB strains tested were showed resistance (absence of zone of inhibition) to the methonolic extracts of *Withanea somnifera* and *Aloe vera* by using well diffusion method. Antibacterial and synergistic activity of methonolic extracts of *Withanea somnifera, Aloe vera* and LAB combine against food borne pathogens showed resistance (no zone of inhibition) except *Pediococci acidolactici-252* and *Withanea somnifera* were showed good antibacterial activity against *E. coli* with a zone of inhibition about 22mm which is higher when they are tested alone.

Discussion

Methanol extracts of *Withanea somnifera* exhibited more inhibitory activity on gram positive bacteria than gram negative bacteria and these results were similar to the findings of (16). The extracts of *Aloe vera* showed good antibacterial activity against the all the tested pathogenic micro organisms except on *Staphylococcus aureus* which were differing with the findings of (9). The zone of inhibition more on *Klebsialla pneumonia* which findings are in accordance with (17). The present study identified that the plant extracts from the *Withanea somnifera* showed good antibacterial activity when compared with the plant extracts of *Aloe vera* on all the tested pathogenic micro organisms.

The lactic acid bacterial strains used in the study have exhibited good antibacterial activity against the food borne pathogens tested and the results are in agreement with the findings of the (1). Antibacterial activity of LAB on enteric pathogenic bacteria viz, *S. aureus* and *E. coli* are in accordance with (11). According to (10) slight differences in antagonistic activity of LAB's on tested pathogenic organisms may due to production of antimicrobial compounds to varying degree. The antimicrobial effect of lactic acid is due to undissociated form of acid, which penetrates the membrane and liberates hydrogen ion in the neutral cytoplasm thus leading to inhibition of vital cell functions.

The present study revealed that when combine testing of methonolic extracts of *Withanea somnifera, Aloe vera* and LAB were showed no antibacterial activity against test pathogens except methonolic extract of *Withanea somnifera* and *Pediococci acidolacitici* 252 which showed synergistic activity against *E. coli* with a zone of inhibition about 22mm which is significantly higher than testing alone for their antibiotic activity.

Table 1: Antibacterial activity of different solvent extracts from ***Withanea somnifera*** and ***Aloe vera*** against food borne pathogens (zone of inhibition in mm)

Name of the organisms	***Withanea somnifera*(-root)**	***Aloe Vera* (leaf)**
Escheria coli	**16**	10
Salmonella typhimureum	**18**	**4**
Staphylococcus aureus	**24**	**R**
Klebsialla pneumonea	13	14

Note :R =Resistant

Table 2. Antibacterial activity of LAB strains against food borne pathogenic bacteria (Zone of inhibition in mm)

Name of the organisms	*Bifidobacterium bifidum 229*	*Lactobacillus Helviticus-194*	*Pediococci acidolactici-252*
E.coli	15	15	19
S.typhimureum	17	11	22
S. aureus	13	15	21
K.pneumonea	15	18	19

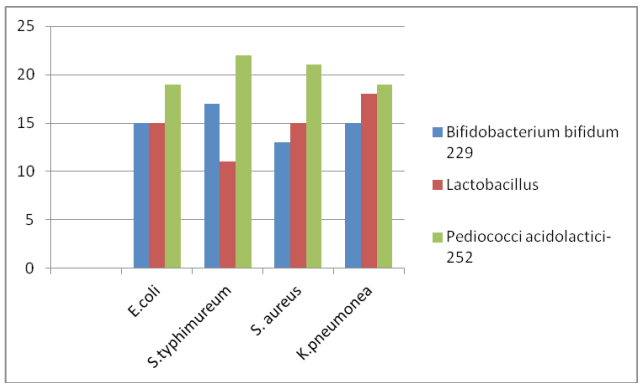


Fig1. Antibacterial activity of LAB strains against food borne pathogenic bacteria

Conclusion

The present study indicated that the methanolic extracts of *Withania somnifera, Aloe vera* and LAB showed good antibacterial activity when they are used individually then the combined and these can be used as a natural drug for the treatment of several infectious diseases caused by these organisms and could be useful in understanding the relations between traditional cures and current medications.

Table 3. Antagonistic /synergistic activity of methonolic extracts of ***Withanea somnifera, Aloe vera*** and certain Lactic acid bacterial strains combine against food borne pathogens (zone of inhibition in mm) R = Resistant

Name of the organisms	*Withanea + B.bifidum 229*	*Withanea+ L.helviticus 194*	*Withanea+ P.acidolactici 252*	*AloeVera+ B.bifidum 229*	*AloeVera+ L.helviticus 194*	*AloeVera+ P.acidolactici 252*
E.coli	R	R	R	R	R	R
S.typhimureum	R	R	R	R	R	R
S. aureus	R	R	R	R	R	R
K.pneumonea	R	R	22mm	R	R	R

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Conflict of Interest

None Declared

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