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Antibacterial Activity of Processed *Tamarindus Indica*. L Seeds against Clinically Important Microorganisms

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ABSTRACT

Plants are the most common source of antimicrobial agents. Their usage as traditional health remedies is most popular and it is reported to have minimal side effects. In the present communication, antibacterial activity of *Tamarindus indica L* have been reported that may be an alternative source to develop an alternative treatment of bacterial infection. *Tamarindus indica L* seeds were processed and tested for their antibacterial activity against clinically important Gram positive and Gram negative bacteria. Different processing methods were employed to the seeds of *Tamarindus indica* L such as soaking, dehulling, cooking, autoclaving and germination. Extraction of *Tamarindus indica* L seeds were carried out at 37˚C with methanol. The extracted materials was then determined for antibacterial activity by disk diffusion and growth inhibition studies method. The results showed that the extracts possessed the highest antibacterial activity against the bacteria, *Enterobacter Sp.* and *Morexella Sp.* Further the sensitivity of two pathogenic bacteria, *Staphylococcus aureus* and *Escherichia coli* to the tannins of the processed tamarind seeds was tested. The highest activity was exhibited by the autoclaved seeds with 70.70% against *Staphylococcus aureus* and 46.21% against *E. coli*. The growth inhibition studies indicated that extracts of control, cooked and autoclaved seeds inhibit *Staphylococcus aureus* and *Escherichia coli* more than soaked, dehulled and germinated seeds.. Similar results were obtained with the agar disc diffusion test. The potent components of *Tamarindus indica* L is suggested for further purification and determination of phytochemicals and other biological properties.

Keywords: Tamarindus indica L, Antibacterial activity, Agar disc diffusion assay, Growth inhibition studies.

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INTRODUCTION

Tamarind (*Tamarindus indica*) is an economically important tree, found in many countries of Asia, Africa and South America. *Tamarindus* belongs to the family Leguminosae. *Tamarindus* itself is a monotypic genus, containing the sole species *T. indica*. The seeds of wild plants including the tribal pulses have received more attention. They are highly resistant to disease and pests and exhibit good nutritional qualities[1].The underutilized legumes / wild tribal pulses have tremendous potential for commercial exploitation, but largely remain ignored. They offer a good scope to meet the ever-increasing demands for vegetable protein. Although they have high protein content and possess good nutritional value, their utilization is limited by the presence of some antinutritional / antiphysiological / toxic substances, such as, saponins, tannins, polyphenols, phytic acid, protease and α-amylase inhibitors, haemagglutinins, lectins, etc. Methods for reducing antinutrients in food are carried out according to their physical (dehulling / cooking, autoclaving / pressure cooking, dry roasting, soaking, milling, selective extraction, irradiation) or biochemical (enzyme processing, germination and fermentation) character[2]. Plants are the most common source of antimicrobial agents. Their usage as traditional health remedies is most popular for 80 % of the world population in Asia, Latin America and Africa and it is reported to have minimal side effects[3,4]. In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies. The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources. Flavonoids and other polyphenols are recorded as antimicrobial agents in many plants. Due to their antimicrobial, antifungal and antiseptic effect, they have an extensive ethnobotanical use in many areas[5]. Catechin and pyrogallol, found in vegetables tannins, are phenols toxic to microorganisms. There are two hydroxyl groups in catechin and three in pyrogallol. The number and location of hydroxyl groups in the phenolic compounds are the key factors in their toxicity to microorganisms. As the number of the hydroxyl groups increase, so does the toxicity[6]. Antibacterial properties of vegetable tannins have been exploited in various fields, particularly in medicine. Tannic acid exhibits antioxidant, antimutagenic and anticarcinogenic properties. Tannins and tannic acid are toxic to fungi, bacteria and viruses and inhibit their growth while digallic acid inactivate influenza viruses[7]. Gallic acid in carob seed has antibacterial, antifungal and antioxidant properties. Several reports are available on the use of tannins in treating various ailments in humans, including diarrhoea, gastric ulcers, snake bites and wounds[8]. However, reports on antimicrobial activity of tannins are scarce. The present study was carried out to determine the effect of various processing treatments on tannin and phenolic content present in the seeds of tamarind. Further, studies were carried out to determine the antibacterial activity of tannins from the seeds of *Tamarindus indica* L.

MATERIALS AND METHOD

Plant Material

The seeds of *Tamarindus indica* were collected using random sampling technique (RST) from local areas of Bangalore district, Karnataka State, India. After dehulling the fruits, equal samples of seeds were combined to give one bulk population sample from which sub samples were taken. The seed samples were dried in the sunlight for 24 hrs. After removing immature and damaged seeds, the matured seeds were washed under tap water, dried and stored in refrigerator until further use.

Processing treatments:

The seeds were subjected to five different types of processing.

1. **Soaking:** The seeds were soaked in water for 5 days, dried at 60° C and ground to a fine powder using a blender.
2. **Dehulling:** The seeds were soaked in water for 5 days and then hand pounded to separate the hull. The dehulled seeds were then dried at 60° C and ground to a fine powder.
3. **Cooking:** The seeds were cooked for 30 minutes, mucus was removed from seed coat and washed. The cooked seeds were then dried at 60° C and ground to a fine powder.
4. **Autoclaving:** The seeds were autoclaved, cooled and then dried at 60° C and ground to a fine powder.
5. **Germination:** The seeds were treated with 50% H_2SO_4 for 30 minutes. After 30 minutes, it was washed and sowed onto a medium containing coco pith and sand in the ratio 1:1. After 10 days, the seeds were cleaned, dried overnight at 60° C and ground to a fine powder.

Antimicrobial activity

Antimicrobial activity for all the five processed samples were analyzed.

Test Microorganisms

The test microorganisms used in this study consisted of 6 bacterial species *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Enterobacter aerogens, Morexella sps and Serratia mercescens* were obtained from Dept. of Microbiology, G. K. V. K. Campus, Bangalore. The bacterial strains were grown and maintained on nutrient agar (NA) slants. The inoculated agar slants were incubated at 37° C for bacteria.

Antibacterial activity of tannins:

Disc Diffusion

Susceptibilities were determined using the disc diffusion method[9]. Filter paper discs of 6 mm diameter saturated with tannin extracts were aseptically placed on agar media that had been spread with test bacteria. Negative control discs were separately prepared by saturating with sterile water.

Growth Inhibition Studies

The growth inhibition studies were carried out as described by Subbalakshmi et al[10]. 0.9 ml of the above culture with 0.1 O.D at 600 nm was taken in sterilized test tubes and 0.1 ml of methanolic tannin extract was added. For control, 0.9 ml of culture and 0.1 ml of buffer was added in a different test tube. The tubes were incubated at 37° C for 1 hour. After 1 hour, 10 ml of nutrient broth was added. As the tannin extracts were colored, separate blanks were also maintained. The test tubes were incubated for 24 hours at 37° C and the absorbance was measured at 600 nm against blank (nutrient broth). The OD of blank with only extract was subtracted from the OD of experimental tubes and compared with that of control. The percentage inhibition of growth was calculated by using the following formula.

$$\%\ \text{inhibition of growth} = \frac{\text{OD of control} - (\text{OD of test} - \text{OD of blank})}{\text{OD of control}} \times 100$$

RESULTS AND DISCUSSION

Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities and important ecological roles. They can be chemical defenses against insects, herbivores and microorganisms. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. In many cases these substances serve as plant defense mechanism against predation by micro- and macroorganisms. In fact, certain substances such as terpenoids, give plant their smells while others, such as quinones and tannins, are responsible for plant pigmentation. Plant seeds are easily available and are the richest source of antioxidants, phenolics and proteins. Legumes have to be processed prior to consumption due to their high content of antinutritional compounds, such as tannins, phytic acid, galactosides and trypsin inhibitors[11]. Processing techniques such as soaking, cooking, germination and fermentation have been found to reduce significantly the levels of phytates and tannins by exogenous and endogenous enzymes formed during processing[12,13,14]. The plants possess significant cytotoxic and antibacterial activities, but the chemical compositions of the extracts were not elucidated. Only a limited number of papers have described the various flavonoid, amino-containing, triterpene, steroid, megastigmane, saccharide, organic and fatty acid compounds found therein[15]. Due to these findings, we have focused on establishing a relationship between the total phenolic content, tannins and antibacterial activity of seeds of *T.indica*. Various processing treatments such as soaking, dehulling, cooking, autoclaving and germination have been carried out on the tamarind seed and the content of tannins, phenolics and its antimicrobial activity determined.

Anti-nutritional Factors

Figure 1 shows the effect of processing methods on tannins. The results indicate that soaking followed by dehulling of seeds and germinated seeds have reduced tannin content in comparison to the control seeds. Tannin content increased with autoclaving. Several plants which are rich in tannins have been shown to possess antimicrobial activities against a number of microorganisms. For example the antibacterial activity of leaf extract Dichrostachys cinerea were investigated and reported the presence of tannins, alkaloids and glycosides[16].

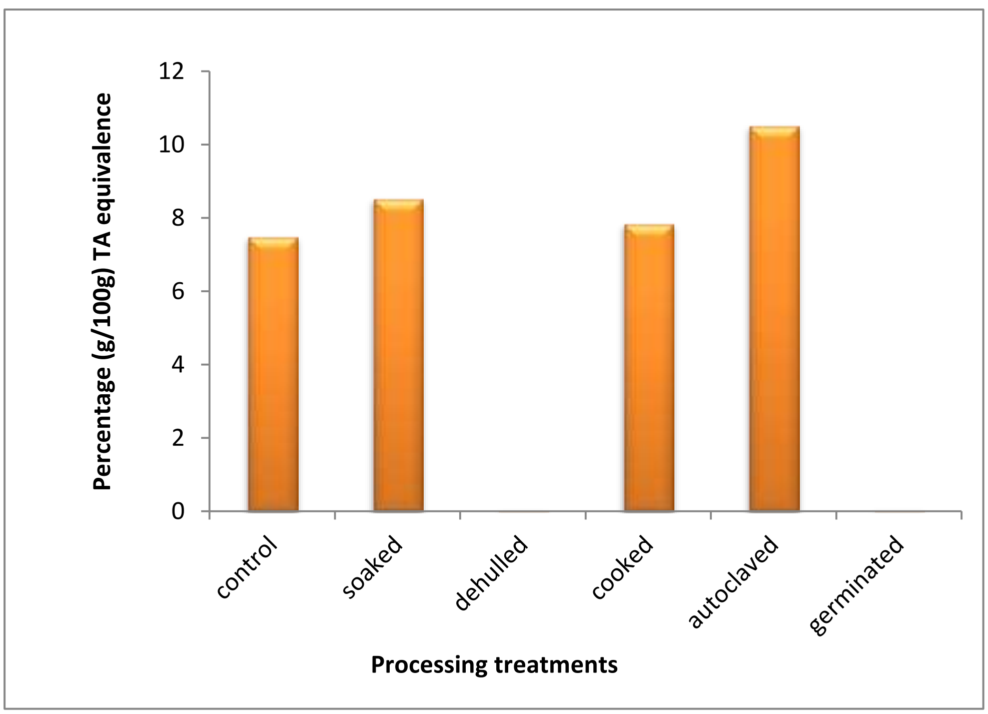

Figure 1: Effect of Various Processing Treatments on the Levels of Tannins in *Tamarindus Indica* Seeds

Figure 2 shows the effect of processing treatments on total phenolics of tamarind seeds. The results indicate that the autoclaved seeds showed an increase in phenolics while a reduction was noticed in dehulled and germinated seeds when compared to that of control seeds. The results of chemical analysis of mango seed kernel (MSK) showed no adverse effect of soaking, boiling, HCl treatment and $Ca(OH)_2$ treatment on the crude protein, ether extract and nitrogen free extract. Treatment of soaked and boiled MSK with $Ca(OH)_2$ was found to effectively enhance the reduction of anti-nutritional factors to barest minimum, if not complete removal thereby enhancing better utilization of the MSK in livestock and poultry nutrition.

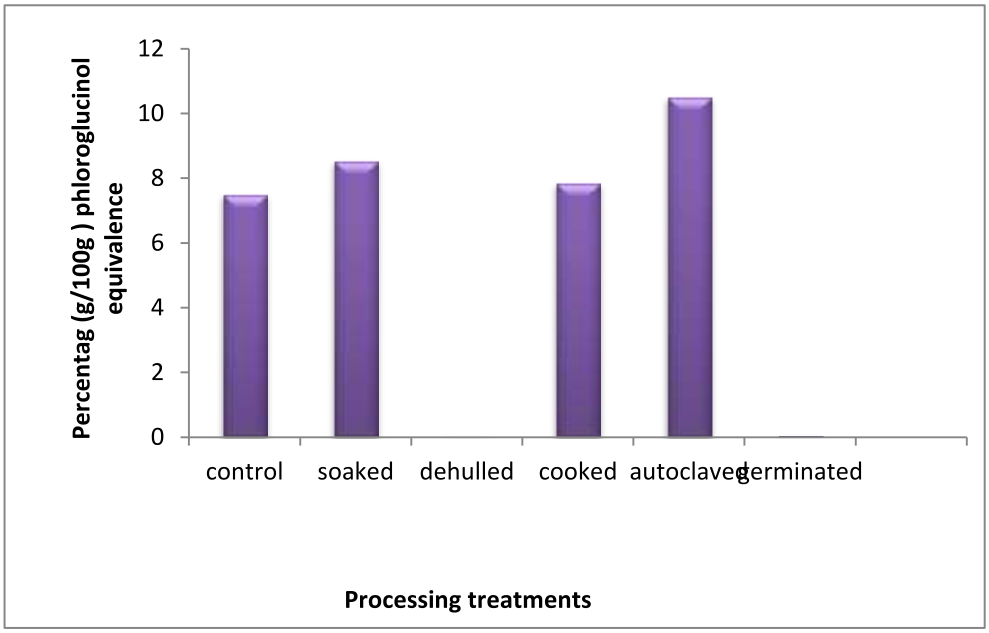

Figure 2: Effect of processing treatments on the content of total phenolics in *Tamarindus indica* seeds

Antimicrobial activity

The presence of antibacterial substances in the higher plants is well established[17]. Results of the antimicrobial activity of the processed seed extracts are shown in Table 1, 2 and 3. The results obtained shows that the extracts are effective against microorganisms. Table 1 and Table 2 deals with the percentage of inhibition studies both with the control group and the processed seeds. The percentage of inhibition with both gram positive and gram negative bacteria with respect to the control group is shown in table 1. The results showed that tannins possessed the highest antibacterial activity against the bacteria, *Enterobacter Sp.* and *Morexella Sp.* Further the sensitivity of two pathogenic bacteria Staphyococcus aureus and *Escherichia coli* to the tannins of the processed tamarind seeds was tested. The highest activity was exhibited by the autoclaved seed with 70.70% against *S. aureus* and 46.21% against *E. coli* (table 2). The ability of tannin compounds to cause the bacterial colonies to disintegrate probably results from their interference with the bacterial cell wall; thereby inhibiting the microbial growth[18].

Table 1: Effect of tamarind seed extract (control) on the growth of Gram positive and Gram negative bacteria by Growth Inhibition Studies

No.	Microorganism	% inhibition of growth
1.	Bacillus cereus	35.71
2.	Escherichia coli	28.57
3.	Enterobacter sps	58.20
4.	Morexella sps	76.92
5.	Serratia sps	32.70
6.	Staphylococcus aureus	45.16

Table 2: Percentage of Growth Inhibition of Control and Processed Tamarind Seed Extract

Sample / Micro organism	Control Seeds	Soaked seeds	Dehulled seeds	Cooked seeds	Autoclaved seeds	Germinated Seeds
S. aureus	45.16	7.9	3.8	15.1	70.7	3.2
E. coli	28.57	14.3	0.8	26.8	46.21	2.9

Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug[19]. The growth inhibition studies indicated that extracts of control, cooked and autoclaved seeds inhibit *Staphylococcus aureus* and *Escherichia coli* more than soaked, dehulled and germinated seeds. Similar results were obtained with the agar disc diffusion test (Table 3).

Table 3: Antibacterial Properties of Processed Seed Extracts Using the Agar Diffusion Technique (mm)

Sample / Micro Organism	Zone of inhibition (mm)					
	Control Seeds	Soaked seeds	Dehulled seeds	Cooked seeds	Autoclaved seeds	Germinated seeds
B. cereus	8	17	R	R	R	R
E. coli	9	6	R	10	11	R
Enterobacter sps	14	12	R	9	12	R
Morexella sps	R	R	R	R	R	R
Serratia sps	9	8	R	12	10	R
S. aureus	12	10	R	11	22	R

Note: number indicates mm and R indicates resistances.

Autoclaved seeds showed highest activity with zone of inhibition of 22 mm against *S. aureus*. Less activity was obtained with the dehulled and germinated seed samples against all the different microorganisms. Soaked seeds showed highest activity against *B. cereus* (17 mm). All the test microbes were resistant with 0.0mm zone of inhibition against dehulled and germinated seed extracts.

CONCLUSION

Tannins are naturally occurring plant polyphenols which combine with protein and other polymers to form stable complexes. They are fairly large molecules having molecular weights of 500-3000 kD. Several phenolic hydroxyl groups located on the surface of tannin molecules are believed to participate strongly in the properties and biological activities of the tannins. There are several reports on the use of tannins in treating various ailments in humans, including diarrhea, gastric ulcers, snake bites and wounds. Amongst the gram-positive and gram-negative bacteria, gram-positive bacterial strains were more susceptible to the extracts when compared to gram negative bacteria. This may be attributed to the fact that these two groups differ by its cell wall component and its thickness. The ability of tannin compounds to cause the bacterial colonies to disintegrate probably results from their interference with the bacterial cell wall; thereby inhibiting the microbial growth (Erasto et al., 2004).

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