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Phytochemical Detection and *in vitro* Evaluation of Tamarind Fruit Pulp for Potential Antimicrobial Activity

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Abstract: The Tamarind (*Tamarindus indica*) pulp extracts were screened for their anti-microbial activities using the agar well diffusion method and detection of phytochemicals was carried out by Gas-Chromatography-Mass Spectrometry (GC-MS). The extracts were tested against seven bacteria and three fungal strains. Among all tested microorganisms the tamarind pulp extracts exhibited higher antimicrobial activities against *Salmonella typhimurium* (NCIM 2501) and *Staphylococcus aureus* (NCIM 5021) and lower activity against *Aspergillus niger* (NCIM 545). Tamarind pulp extracts were more potent than the tartaric acid solution. The major phytochemicals detected in tamarind pulp extracts were 2-Furancarboxaldehyde, 2, 3-Butanediol, 2-Furancarboxaldehyde, 5-methyl. This investigation throws light on possible applications of the tamarind fruit pulp in natural preservation of food and food products.

Key words: Antimicrobial activity, phytochemicals, *Tamarindus indica*, tamarind pulp, tartaric acid, preservation

INTRODUCTION

The plant, *Tamarindus indica* L. commonly known as tamarind belongs to the family Ceasalpiniaceae (Fabaceae) indigenous to India and south East Asia. The pulp of the fruit is widely used for food and beverage products like syrup, juice, concentrates and exotic food speciality products like chutneys, curries, pickles and meat sources (Ishola *et al*., 1990). Fruit pulp is used to alley thirst which is nutritive and forms useful drinks given to persons recovering from sickness (Morton, 1987). It is rich in tartaric acid, citric acid, vitamin C and sugars (Nyadoi and Abdullah, 2004).

In India, Tamarind fruit pulp is used to make Tamarind Fish which is a sea food pickle. The juice is used to preserve fish up to 6 months when mixed with acetic acid. Juice is also used in barbecue sauces (El-Siddig *et al*., 2006). The most outstanding characteristic of tamarind is like taste, the acid is due to blend of tartaric acid and sugars which is uncommon in other fruits (Ulrich, 1970).

It is added to the other foods to give a sour taste and used as antioxidants. The frequent use of tamarind fruit as a food (Bhattacharya *et al*., 1994) and the traditional application of their crude extracts for medicinal purposes have stimulated diverse studies concerning to it's chemical composition (Lanhers *et al*., 1996). Tamarind pulp is popularly employed in the general traditional medicine practice as a drug conveyor and in the treatment of various diseases and skin disorders. Morever, diverse medicinal liquor made of the tamarind pulp are recommended in developing countries for their laxative, antiseptic, diuretic and anti-inflammatory effects (Rimbau *et al*., 1999). They have also been showed to be beneficial in controlling fever (Khurana and Ho, 1989). Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Iwu *et al*., 1999). Making antimicrobial drug therapy effective, safe and affordable has been the focus of interest during recent years (Sharma *et al*., 2002). In the present study attempts have been made to screen mature unripe tamarind fruit pulp extracts for possible antimicrobial activity and finding the expected reasons for antimicrobial activities by determining the phytochemicals present there in.

MATERIALS AND METHODS

Tamarind fruits: The mature unripe and ripe pods of tamarind of Pratishthan cultivar were collected from local area of Shivaji University, Kolhapur (MS) India.

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Preparation of extracts: Crude extract and autoclaved crude extract. About 100 g of clean and infestation free tamarindpods were taken and seeds were removed manually. The flesh was macerated using an electric blender and the juice was extracted by squeezing the homogenized mass through a muslin cloth. The obtained juice was treated as crude extract and stored in pre-sterilized glass bottle at 10°C. The crude extract was autoclaved at 121°C for 15 min (Daniyan and Muhammad, 2008) cooled to room temperature, stored at 10°C and treated as an autoclaved crude extract.

Aqueous extract: The aqueous extracts of mature unripe and ripe pods of tamarind were prepared as per the procedure suggested by Daniyan and Muhammad (2008) with slight modification. In brief the fruit pulps were mixed with warm distilled water (45-50°C) in the ratio 1:1 and blended in electric blender. These mixtures were shaken for 10 min, filtered through muslin cloth and stored at 10°C until further use.

Solvent extracts: The solvent extracts were prepared with acetone, methanol and ethanol as per the procedure given by Doughari (2006) with slight modification. About 10 g of crushed flesh was extracted with 10 mL of acetone, methanol and ethanol respectively and kept on a rotary shaker for 12 h. The extracts were filtered, centrifuged at 5000 rpm for 10 min and supernatants were collected. The supernatants were filtrated and used for phytochemical analysis.

Tartaric acid and autoclaved tartaric acid solution: About 10% tartaric acid solution was prepared in sterile demineralized water. Tartaric acid solution was autoclaved at 121°C for 15 min. Both autoclaved and non autoclaved tartaric acid solutions were screened for antimicrobial activities.

Detection of phytochemicals by GC-MS: Possible presence of active components in the solvent extracts of tamarind pulp prepared with acetone, methanol and ethanol were detected by GC-MS (Gas Chromatography-Mass Spectroscopy). The GC-MS analysis of the samples was performed on a QP-2010 (Shimadzu) gas chromatography coupled with mass spectroscopy. The samples were injected into Rtx-5 MS capillary column (60 m length ×0.25 mm internal diameter ×0.25 μm film thickness). The carrier gas was helium at a flow rate 1.0 mL min^{-1}, linear-velocity 25.6 cm sec^{-1}. The initial column temperature was 80°C, then increased linearly at 10°C min^{-1} -280°C and held for 11 min. The total run time was of 36.14 min. The temperature of the injection port was 280°C and interface temperature was 290°C. The injection volume was 1 μL. The mass spectra were recorded in Electron Ionization (EI) mode at 70 eV. Compound identification was accomplished by comparing the retention times with those of authentic compounds and fragmentation pattern, as well as with mass spectra in the NIST spectral library stored in the computer software (version 1.10 beta, Shimadzu) of the GC-MS.

Microorganism tested: The test organisms included 3 g positive bacteria; *Bacillus cereus* (NCIM 2156), *Staphylococcus aureus* (NCIM 5021), *Micrococcus luteus* (NCIM 2103) and 5 g negative bacteria; *Salmonella typhimurium* (NCIM 2501), *Pseudomonas aeruginosa* (NCIM 2036), *Escherichia coli* (NCIM 2089), *Proteus vulgaris* (NCIM 2027) and three fungal strains; *Aspergillus niger* (NCIM 545), *Fusarium moniliformae* (NCIM 1099) and *Rhizopus stolonifer* (NCIM 880). All the strains were obtained from National Collection of Industrial Microorganisms (NCIM), Pune, India.

***In vitro* screening of extracts for antimicrobial activities:** Each of the above test organisms was sub-cultured on nutrient broth to test viability, subsequently on nutrient agar slants and after 48 h incubation, these slants were kept at 10°C for future use. The direct colony suspension method was used for inoculums (Mathew *et al*., 2006). Agar well diffusion method was used to screen the antimicrobial activities of the extracts (Perez *et al*., 1990). Nutrient agar and nutrient soft agar were used as culture medium for bacterial cultures while potato dextrose agar and potato dextrose soft agar were used as culture medium for the fungal cultures.

Nutrient agar plates were swabbed with the respective cultures (0.2 mL) of the organism and kept for 15 min for absorption to take place. Wells were made in molten agar plates using a sterile cork borer (7 mm dia) and 100 μL of each extracts were added into wells using micro-pipette and allowed for diffusion at room temperature for 2 h. The plates were incubated at 37°C for 24 h. Similar methods as for bacteria were adopted for fungi but instead of nutrient agar potato dextrose agar was used and the inoculated plates were incubated at 2°C for 72 h. The zone of inhibition and stimulation were measured and express in mm.

Effect of temperature and pH on antimicrobial activities: About 5 mL of aqueous extracts were taken in test tubes and treated at 4, 30, 60, 100 and 120°C in a water bath for

30 min and tested for antimicrobial activity against S*almonella typhimurium* (NCIM 2501). To determine the effect of pH, aqueous extracts were treated at pH ranges of 2.5-10 (Monitored by 1N HCl or 1N NaOH solution) in series of test tubes for 30 min and tested for antimicrobial activity against *Salmonella typhimurium* (NCIM 2501) (Doughari, 2006).

RESULTS AND DISCUSSION

Effects of different extracts on growth inhibition of microorganisms: The aqueous extracts of mature unripe and ripe fruit pulp were screened for antimicrobial activities and obtained results are shown in Table 1. The data showed that the extract of mature unripe fruit pulp got higher antimicrobial activities for the entire tested microorganism as compared to extract of ripe fruit pulp. These higher antimicrobial activities might be due to higher amount of free tartaric acid supported by phytochemicals present in mature unripe tamarind fruits (Lewis and Neelakantan, 1964; Salunkhe and Kadam, 2005).

The crude, autoclaved crude extract, 10% tartaric acid solution and autoclaved 10% tartaric acid solution have been tested for their antimicrobial activities (Table 1). Antibacterial activities of extracts were observed against all bacterial strains where as these extracts did not exhibited antifungal activities against few fungi (Table 1). The tamarind pulp extracts exhibited remarkable anti-microbial activities against the tested micro-organisms in the order of sensitivity as *Salmonella typhimurium* (NCIM 2501)>*Staphylococcus aureus* (NCIM 5021)>*Bacillus cereus* (NCIM 2156)>Pseudomonas aeruginosa (NCIM 2036)>*Micrococcus luteus* (NCIM 2103)>*Escherichia coli* (NCIM 2089)>*Proteus vulgaris* (NCIM 2027)>*Aspergillus niger* (NCIM 545).

All the extracts of tamarind pulp and tartaric acid solutions were inactive against *Fusarium moniliformae* (NCIM 1099) and *Rhizopus stolonifer* (NCIM 880). Earlier research on antimicrobial properties of diluted aqueous extract (1:6) of tamarind pulp showed low activity against *Salmonella typhimurium*, *Staphylococcus aureus* and *Escherichia coli* (Abukakar *et al*., 2008; Daniyan and Muhammad, 2008).

About 10% tartaric acid solution with and without autoclaving showed lower activities against all tested bacteria and fungal strains as compared to extracts of tamarind pulp, however it did not show any activity against *Proteus vulgaris* (NCIM 2027) (Table 1). It is reported that tartaric acid is used as an acidulant which shows preservative action against food spoilage causing organisms (Furia, 1980). The autoclaved crude extract of tamarind pulp showed zone of stimulation against *Staphylococcus aureus* (NCIM 5021), *Salmonella typhymurium* (NCIM 2501) and *Bacillus cereus* (NCIM 2156).

The results of stimulation zone are given inTable 1 and the stimulation zone of *Staphylococcus aureus* (NCIM 5021) as a sample presentation is shown in Fig. 1. This zone of stimulation may be due to the changes in phytochemicals, occurred during autoclaving. Another possible reason for this zone of stimulation may be decreased concentration of phytochemicals from the end of growth inhibition (Lorian and Strauss, 1966).

Detection of phytochemicals: Tamarind pulp extracts (ehanolic, methanolic and acetonic) were subjected to GC -MS for presence of various phytochemicals and detected compounds are shown in Table 2. About 17 different compounds were identified from tamarind pulp extracts. The ethanolic extract of tamarind pulp showed presence of more number of compounds as compared to other extracts.

Table 1: Effects of different extracts of mature unripe tamarind pulp on the growth of microorganisms*

	Zone of growth inhibition (mm)						
				Autoclaved crude extract			
Name of Microorganism	Aqueous extract of mature ripe Unripe pods	Aqueous extract of mature ripe pods	Crude extract	Inhibition	Stimulation	10% Tartaric acid	Autoclaved 10% tartaric acid
Bacillus cereus (NCIM 2156)	10	09	16	15	07	11	12
Proteus vulgaris (NCIM 2027)	09	07	14	13	-	-	-
Salmonella typhimurium (NCIM 2501)	12	10	18	16	08	08	09
Staphylococcus aureus (NCIM 5021)	12	11	18	17	07	09	08
Pseudomonas aeruginosa (NCIM 2036)	11	08	15	14	-	07	09
Escherichia coli (NCIM 2089)	09	08	14	13	-	08	10
Micrococcus luteus (NCIM 2103)	08	07	15	14	-	10	10
Aspergillus niger (NCIM 545)	03	---	05	03	-	02	02
Fusarium moniliformae (NCIM 1099)	---	---	-	-	-	-	-
Rhizopus stolonifer (NCIM 880)	---	---	-	-	-	-	-

*Each value is average of three determinations

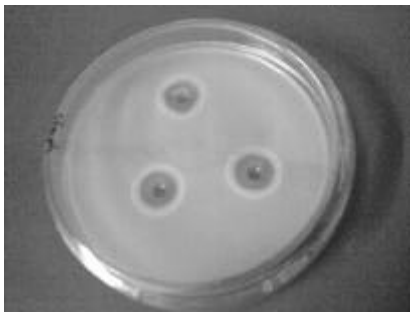

Fig. 1: Plate showing inhibition zone surrounded with stimulation zone of *Staphylococcus aureus* (NCIM 5021)

Table 2: Phytochemicals detected in the acetonic, ethanolic and methanolic extracts of tamarind pulp by GC-MS

Compounds	RT	CAS #	Percentage
Acetinic extract			
2-Furancarboxaldehyde	6.327	98-1-1	6.21
2, 3-Butanediol	6.750	513-85-9	7.13
2-Furancarboxaldehyde, 5-methyl	8.113	620-2-0	2.90
Pyran-4one, 2,3-dihydro-3, 5-dihydroxy-6-methyl	8.428	28564-83-02	1.70
2-Ethyl-5-propylcyclopentanone	8.759	0-0-0	1.81
Methyl 2-furoate	10.387	611-13-2	1.46
1, 3-Dioxolane, 2, 4, 5-trimethyl	11.513	3299-32-99	2.66
Ethanolic extract			
2-Furancarboxaldehyde	6.330	98-1-1	6.21
2-Furancarboxaldehyde, 5-methyl	8.112	620-2-0	2.38
Pyran-4one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl	8.445	28564-83-02	0.85
5, 5-Bi-1-pyrroline, 2, 2-diethoxy	8.781	93042-3-6	0.72
Furanmethanol	10.949	4412-91-3	0.86
2-Propyl-tetrahydropyran-3-ol	11.520	0-0-0	1.86
2-Furancarboxaldehyde, 5-(hydroxy methyl)	13.07	64-47-0	18.92
Cyclohexasiloxane, dodecamethyl	14.011	540-97-6	1.78
D-Allose	18.293	0-0-0	5.13
-o-Methyl-d-glucose	22.147	0-0-0	61.66
Methanolic extract			
2, 3-Butanediol	6.700	513-85-9	6.47
Pyran-4one, 2,3-dihydro-3, 5-dihydroxy-6-methyl	11.501	28564-83-2	2.54
Hydroxymethylfurfurole	13.000	67-47-0	14.28
-o-Methyl-d-glucose	21.776	0-0-0	76.71

RT-Retention Time

It is apparent from the data that, 2-Furancarboxaldehyde, 2, 3-Butanediol, 2-Furancarb oxaldehyde, 5-methyl were dominating compounds in all the extracts. 2-phenylacetaldehyde, 2-furfural, 2-acetylfuran and hexadecanoicacid are the major contributors to the overall aroma of tamarind (Soursop, 1980; Pino *et al*., 2004). Earlier findings report that the phytochemicals i.e., 2-Furancarboxaldehyde, 2,3-butanediol, methyl 2-furoate and hydroxymethylfurfurole (Hegazi and Abd-El-Hady, 2002; Guo *et al*., 2008) and 2,2'-diethoxy-5,5'-bi-1-pyrroline (Yin *et al*., 2009) show antimicrobial activities.

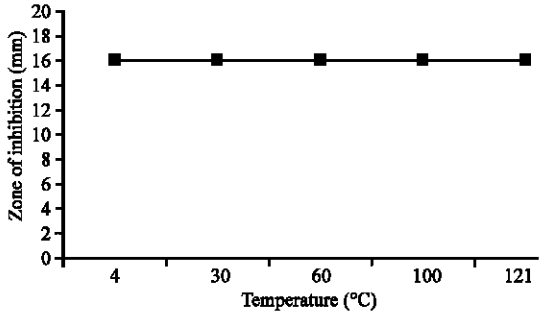

Fig. 2: Effect of temperature on the antimicrobial activity of aqueous extract

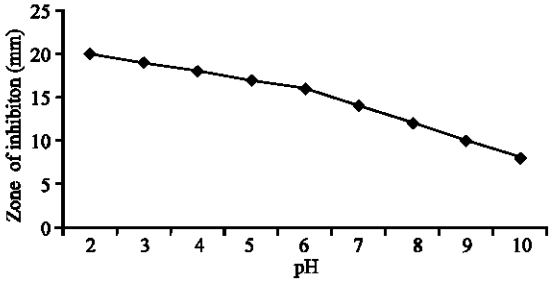

Fig. 3: Effect of pH on the antimicrobial activity of aqueous extract

Effect of temperature and pH on antimicrobial activity: Antimicrobial activity of aqueous extract of tamarind pulp subjected to various temperatures and pH was studied against *Salmonella typhimurium* (NCIM 2501). The obtained results are depicted in Fig. 2 and 3, respectively. It was observed from the plot that temperature did not have any effect on the antimicrobial activity.

This temperature resistance may be an indication that the phyotoconstituents can withstand to higher temperatures (Doughari, 2006). The results of effect of pH revealed that the antimicrobial activity of the extract was higher at lower pH and vice versa. Antimicrobial activity of phyotoconstituents increases in the presence of acidic medium (Molan, 1992).

CONCLUSION

Plant extracts have great potential as antimicrobial compounds against disease and food spoilage causing micro-organisms. Especially extracts of tamarind fruit pulp are effective against many pathogenic bacteria and fungi. The findings revealed that, the antimicrobial activities showed by the tamarind pulp extracts are due to the combine action of the phytochemicals and tartaric acid. This investigation has opened up the possibility of the use of this fruit pulp in natural preservation of food and

food products. The phytochemicals detected need to be further studied individually for the antimicrobial action. The stimulating effect of autoclaved crude extract of tamarind fruit pulp needs to be thoroughly studied for possible mechanism of action contributed by the phytochemicals.

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