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Evaluation of *Tamarindus Indica* Seed Coat for its Antimicrobial Activity and Acute Oral Toxicity

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Abstract

Tamarind (Tamarindus indica, Fabaceae), a tropical fruit found in Africa and Asia is highly valued for its pulp. The in-vitro antimicrobial and in- vivo acute oral toxicity of different extracts of Tamarind seed coat was assessed. The seed coat was extracted using methanol and water and the methanolic extract was then fractionated using different solvents based on the increasing strength of polarity. The phytochemical analysis of the extracts was done qualitatively using standard techniques. Agar gel dilution technique was used to find out the antibacterial and antifungal activities against the various pathogens of veterinary and human importance. The Minimum Inhibitory Concentration was determined by broth dilution and serial dilution plate technique for bacteria and fungi respectively. The extracts were given orally at the limit dose of 2000 mg/kg to adult Wistar rats to find the acute oral toxicity. Well defined margins of inhibition were obtained with MIC of 3.125 -12.5 for the crude alcoholic extract and its fractions. The fractions of the methanolic extract showed inhibition of P. multocida and C. neoformans at dose rate of 3.125 mg/ml. The aqueous extract showed less activity when compared to the alcoholic extract. None of the animals showed any clinical signs of toxicity. Hence the seed coat can be further exploited for its use as a good antimicrobial agent.

I INTRODUCTION

Nature has been a source of medicinal agents for thousands of years. Plants remain as an important source of antimicrobial substances to combat serious diseases of the world. The medicinal values of these plants lie in some chemical active substances that produce a definite physiological action on human body.These traditionally used plants contain many phytochemicals having great therapeutic value. They can either inhibit the growth or kill many pathogens that are infective to animals and human beings and those that have no toxicity can form lead to the development of antimicrobial agents [1]. A phytochemical that inhibits the growth of bacteria through a different mechanism will be of immense use clinically as it can be used for the treatment of resistant infections [2].

Tamarind or *Tamarindus indica* L. of the Fabaceae, subfamily Caesalpinioideae, is an important food in the tropics. It is a multipurpose tree of which almost every part finds at least some use either nutritional or medicinal. Anti-oxidant, anti-inflammatory, anti-microbial and anti-fungal activity has been documented from several plant parts. Traditional antimicrobial therapy is going through a crisis with development of antimicrobial resistance to almost all the present available drugs, necessitating the development of agents with target sites that are unique [1, 3].The phytochemical constituents as well as extensive studies on the antimicrobial and antifungal properties need to be undertaken. Hence in the

present study we report the antibacterial, antifungal and toxicity of the extracts and the fractions of *Tamarindus indica* seed coat.

II MATERIALS AND METHODS

2.1. Preparation of plant material

The Tamarind seed was bought from a local vegetable market. It was roasted in low flame; seed coat separated pulverized and extracted using methanol and water. The extraction with methanol was done in a soxhlet extraction apparatus whereas the aqueous extraction was done as a decoction. The extracts were dried using a rotary vacuum evaporator and stored under refrigeration.

2.2 Fractionation of the methanolic extract

The methanolic extract was fractionated using various solvents based on their increasing strength of polarity. The extract was first mixed with 10 volumes of n-hexane, followed by the mixing of the insoluble part with chloroform. The left over residue was then mixed with 1:1 n-butanol and water and the fractions separated using separating funnel. The fractions were dried using a rotary vacuum evaporator and stored under refrigeration.

2.3. Phytochemical analysis

The extract as well as the fractions was analyzed qualitatively for various phytochemical constituents.

2.4. Antibacterial assay

The extracts as well as the fractions were diluted using 10% DMSO/ tween 80 solutions to get serial dilutions of 500, 250, 100, 50, 25, 12.5 and 100, 50, 25, 12.5, 6.25 and 3.125 µg/ml of the solution respectively. In all the tests 10% DMSO/tween 80 solutions were kept as negative control. Octadisc (Himedia) containing Amoxicillin 10 mcg, Tetracycline 30 mcg, Co- Trimoxazole 25 mcg, Ciprofloxacin 5 mcg, Gentamycin 10 mcg, Erythromycin 15 mcg, Chloramphenicol 30 mcg and Cefalexin 30 mcg were used as positive control.

Antimicrobial assay was done in Muller Hinton agar plates. Microbial cultures with 0.5 McFarland standard turbidity equivalents were prepared as suspensions in nutrient broth. The suspensions were further diluted to 1:10 to get a concentration of 10^6 CFU/ml. About 0.2 ml of the diluted inoculums was applied directly to the plate and spread using the sterile L shaped spreader. Wells were bored into the agar using a sterile 6 mm well borer. The wells were then filled with 25 µl of the DMSO/tween 80 diluted extracts in different concentrations and incubated at 37^0C for 24 hrs. Inhibition zones were measured and recorded as the mean diameter (mm) of complete growth inhibition. The tests were done in triplicate [4].

2.5 Assessment of minimum inhibitory concentration for antibacterial activity

The test was performed in microtitre plates. 100 µl of MH broth was mixed with equal volume of the extract. The dose range between the last concentration that showed antibacterial property and the first concentration that did not show inhibition were divided equally into 5 dilutions. These dilutions were used for calculating MIC. 100µl of the inoculums specimens were added to each well and incubated for 24 hrs at 37°C [4].

2.6 Antifungal Assay

The inoculums was prepared as described earlier and the plates were made using SDA. The extracts as well as the negative control were added as described in the antibacterial assay. After incubation for 48 hrs at 28°C, the plates were examined for the presence of growth/ inhibition of growth and the diameter of zone of inhibition was measured in mm. Octadisc containing Amphotericin B 100 units, Clotrimazole 10 mcg, Fluconazole 25 mcg, Itraconazole 10 mcg, Ketoconazole 10 mcg and Nystatin 100 units were used as positive control. The tests were done in triplicates [5].

2.7 Minimum inhibitory concentration for antifungal activity

Minimum inhibitory concentration determination was done by a serial dilution plate technique where solutions of the reconstituted extracts (based on the results of agar dilution technique) were added into the pre-sterilized and pre-cooled SDA; the media poured and allowed to set. The plates were then inoculated with the test fungi and incubated at 28^0C for 2-7 days. Control plates which contained no extract were also prepared along with the extract. The MIC of each plant was determined after incubation, being the lowest concentration with no visible growth [6].

2.8 Acute oral toxicity testing

The acute oral toxicity testing was done for the extract and its fractions in adult wistar rats, 6 animals per group as per OECD guide lines 420.

III RESULTS AND DISCUSSION

3.1 Phytochemical constituents

Table 1: Phytochemical constituents of different extracts and fractions

Extract	Phenolics	Alkaloids	Steroids	Glycosides	Tannins	Terpenes	saponins	Flavonoids
Aqueous	+	+	-	-	+	+	+	-
Methanolic	+	+	-	-	+	-	+	-
Hexane fraction	+	+	-	-	+	-	-	-
Chloroform fraction	+	+	-	-	+	-	-	-
Butanol fraction	+	+	-	-	+	-	-	-
Water fraction	-	+	-	-	+	-	+	-

All the extracts and fractions showed the presence of alkaloids and tannins where as the water fraction of the aqueous extract was shown to be deficient in phenolics. None of the extracts showed the presence of steroids, glycosides and flavonoids. The presence of alkaloids, terpenes or flavonoids provides antimicrobial properties to the plants [7, 8]. Saponins are agents that can enter the bacterial and fungal cell causing damage to the cell wall causing leakage to the cellular contents resulting in cell death

[9, 10, 11]. In the present study, it was seen that alkaloids were present in all the extracts and the antimicrobial activity may be attributed to the presence of this phytochemical.

Table 2: Antibacterial and antifungal activity: MIC of the extract and fractions against various pathogens (mg/ml)

Organism	Methanolic extract	Hexane fraction	Chloroform fraction	Butanol fraction	Water fraction	Aqueous extract
Escherichia coli	-	-	-	-	-	-
Salmonella typhimurium		-	-	-	-	-
Pasteurella maltocida	50	3.125	3.125	3.125	3.125	25
Pseudomonas aeruginosa	-	-	-	-	-	-
Staphylococcus aureus	12.5	6.25	50	25	100	25
Streptococcus pyogenes	12.5	25	12.5	50	50	25
Listeria monocytogenes	12.5	50	25	25	50	-
Enterococcus faecalis	12.5	25	50	25	-	-
Klebsiella pneumonia	-	-	-	-	-	-
Candida albicans	12.5	25	100	25	100	-
Aspergillus fumigatus	-	-	-	-	-	-
Aspergillus parasiticus	-	-	-	-	-	-
Cryptococcus sp.,	12.5	6.25	3.125	3.25	3.125	12.5

From the results it is seen that there was no activity in case of Gram –ve bacteria where as very good activity with a well-defined margin was seen against Gram +ve organisms. The selectivity toward Gram-positive microorganisms would suggest that, the external membrane of Gram-negative bacteria acts as a barrier against this compounds.The same results has been observed many times and has been reported. An extract or fraction having MIC of 3.125 and 6.25 mg/ml can be considered to be having

very good antimicrobial activity with a potential to be presented as an antimicrobial molecule. It is proposed by various workers that extracts being crude fusions containing much number of components show high minimum inhibitory concentrations and those extracts that show MIC's below 50 mg/ml can be considered to be having antifungal property [12, 13, 14, 15, 16]. A very good activity was shown against *Pasturella multocida* and *Cryptococcus sp.*, with MIC values of 3.125 mg/ml each. Identification of the phytochemical present utilizing LCMS or GCMS can provide a potent broad spectrum antimicrobial agent with minimum side effects.

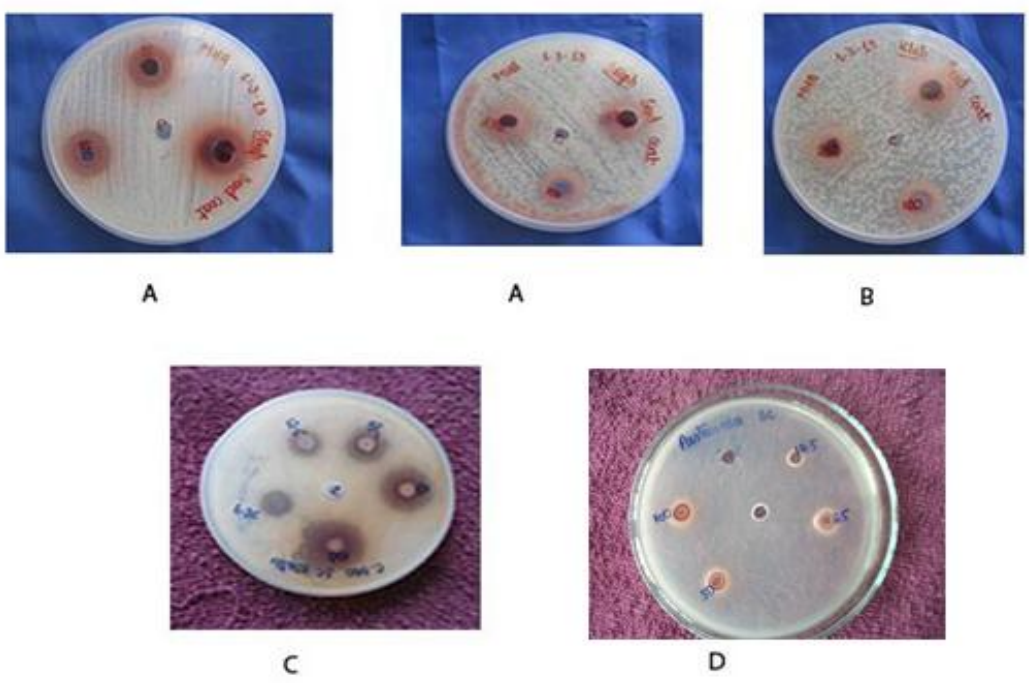

Fig. 1. Antimicrobial Assay of Seed coat extracts showing the inhibition zones against various pathogens
A-S. aureus B- K. pneumoniae, C- C. neofromans, D- P. maltocida

3.3 Acute oral toxicity

No mortality was detected in all groups of animals treated with the extract. Also no untoward clinical signs were noticed in any of the animals treated with the extract during the entire period of observation. Hence it could be interpreted that the extract is safe for use internally as well as for topical application.

IV CONCLUSION

The present study confirms the use of seed coat extract for various human and animal infections suggestive of bacterial and fungal infestation. A well marked clear margin of sensitivity proves the seed coats capability to fight against infections. A detailed study into the phytochemical constituents, their isolation and identification and further testing is required to identify the potent antimicrobial molecules present in the seed coat. This will provide a natural molecule that may have a molecular target different from those that are already used against bacteria and fungi, providing development of a drug without resistance.

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