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Antimicrobial Activity of *Tamarindus Indica* by Disc Diffusion and Phytochemical Analysis Method

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Abstract

The phytochemical analysis and antibacterial activity of aqueous pulp extract of Tamarindus indica were studied. The aqueous pulp extract of this plant was obtained using hot water extraction method. The antibacterial activity of aqueous pulp extract of this plant was carried out against four bacteria; Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Salmonella typhi by disc diffusion method. Phytochemical constituents present in the extract were found to include saponins (2.2%), alkaloids (4.32%) and glycosides (1.591%). Aqueous pulp extract of Tamarindus indica showed antibacterial activity against all the tested bacteria in the order of sensitivity as Staphylococcus aureus, Eseherichia coli, Pseudomonas aeruginosa with the exception salmonella typhi. The antibacterial activity of aqueous pulp extract on Staphylococcus aureus was sensitive at 80, 120, 140,160 and 180 mg mL-1 of extract with 0.2, 0.3, 0.6, 0.8 and 10.0 mm zones of inhibition while Escherichia coli revealed 0.2. 0.2, 0.4 and 0.6 mm zones of inhibition at 120, 140,160 and 180 mg mL-1 of extract, respectively. Pseudomonas aeruginosa was only sensitive at 140, 160 and 180 mg mL-1 of the extract with 0.4, 0.6 and 0.8 mm zones of inhibition.

Key words: Pulp extract, Tamarindus indica, rats, aques, zones of inhibition, antibacterial activity

1. Introduction

Tamarindus indica is a plant that is used in traditional medicine for the treatment of *cold, fever, stomach disorder, diarrhea and jaundice* and as *skin cleanser*. To evaluate the scientific basis for the use of the plant, the antimicrobial activities of extracts of the stem bark and leaves were evaluated against some common gram negative and gram-positive bacteria and fungi.

The study also investigated the chemical constituents of the plant and the effect of temperature and pH on its antimicrobial activity. The antimicrobial activity of the concentrated extracts was evaluated by determination of the diameter of zone of inhibition against both gram negative and gram-positive bacteria and fungi using the paper disc diffusion method.

The activity of the plant extracts was not affected when treated at different temperature ranges (4ºC, 30ºC, 60ºC and 100ºC), but was reduced at alkaline pH. Studies on the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts on the test organisms showed that the lowest MIC and the MBC were demonstrated against ***Salmonella paratyphi, Bacillus subtilis and Salmonella typhi*** and the highest MIC and MBC was exhibited against ***Staphylococcus aureus*.**

Tamarindus indica is a multipurpose tree widely available in the tropics, is of great importance in traditional medicine. The leaves and bark of the plants have been utilized for the treatment of body pain, yellow fever and stomach disorders traditionally. Compounds such as carvacrol, cinnamaldehyde, epicathechin, lupeol, tartaric acid are components of tamarind also reported antibacterial, antifungal, antiviral, antioxidant, carminative, digestive and laxatives activities of tamarind.

The modern day review article is an exquisite attempt to demonstrate the extreme therapeutic potential of tamarind fruit (*Tamarindus indica*), particularly its pulp, seed, and leaf extract, against lifestyle related chronic disorders. The rapid transition in the diet patterns and also the varying lifestyle of the people has made its way forth, a momentous upsurge in a number of chronic as well as degenerative diseases.

An excess of foods having functional and nutraceutical significance has come into view recently. These foods have emerged as effective therapeutical remedies against these disorders owing to their natural phytochemical constituents present in them, in abundance. *Tamarindus indica* serves as a proverbial herbal medicine in each and every part of the world that is known to mankind. Also, the tamarind kernel powder (TKP) is of immense commercial significance in some of the major, leading industries of the World.

2. Materials and Methods:

Plant Material

Plant materials were collected from the local area of Kadayalumoodu, Kanyakumari district, Tamilnadu and were identified and authenticated at the Biological Sciences Department, GD PUBLIC SCHOOL (CBSE), and BODINAYAKANUR.Tamilnadu State, India. Preparation of Extracts this was carried out as earlier described with slight modifications.

Preparation of Extract

The freshly collected stem bark and fresh mature leaves were chopped into pieces and shade dried at room temperature (32-35o C) to constant weight for 5 days. 50g of each of the plant parts were coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender. The powder was transferred into closed containers. Each of the powdered air-dried plant material was extracted with water, acetone and ethanol. 25g of each powdered sample was mixed in a conical flask with 100ml of deionised distilled water or organic solvent, plugged, then shaken at 120 rpm for 30 minutes and kept for 24 h.

Phytochemical Analysis

After 24 h, each of the extracts was filtered rapidly through four layers of gauge and then by a more delicate filtration through Whatman no1 filter paper. The resulting filtrates were then concentrated in a rotary evaporator and subsequently lyophilized to dryness. The yield of powder was 51% from water extracts, 32% from acetone and 17% from ethanol extracts for the stem bark while the respective values of 49%, 32% and 19% (w/w) were obtained for the leaves.

Microbiological Analysis

Test Organisms Bacterial and fungal isolates used for this work. They included *Escherichia coli, Proteus mirabilis, Pseudomonas aerugenosa, Salmonella typhi, Salmonella paratyphi, Shigella flexnerri* for gram negative bacteria and *Staphylococcus aureus, Bacillus subtilis and Streptococcus pyogenes* for gram positive bacteria, all clinical isolates obtained from the Microbiology Laboratory of the Specialist Hospital Government medical college, Kanyakumari, Tamilnadu, India, Fungal isolates used included *Aspergillus flavus, A. fumigatus, A. niger and the yeast Candida albicans* and were laboratory isolates obtained from the Microbiology Laboratory of Government medical college hospital, Kanyakumari, Tamilnadu, India All the bacterial strains were suspended in nutrient broth and incubated at 37o C for 48 h. Nutrient agar (NA) and Potato dextrose agar (PDA) were used for testing the antibacterial and antifungal activity respectively. Phytochemical analysis the freshly prepared extracts were subjected to standard phytochemical analyses to test for the presence of the phytoconstituents tannins, saponins, sesquiterpenes, alkaloids and phlobatamin.

Determination of Antimicrobial Activity

Antimicrobial activity of the aqueous and organic extracts of the plant sample was evaluated by the paper disc diffusion method. For determination of antibacterial activity, bacterial cultures were adjusted to 0.5 McFarland turbidity standard and inoculated onto Nutrient agar (oxoid) plates (diameter: 15cm). For the determination of antimycotic activity, all the fungal isolates and Candida albicans were first adjusted to the concentration of 106 cfu/ml.

Cultures of Candida albicans were suspended in sterile solution of 0.9% normal saline and the spores of the other filamentous fungi were suspended in Tanquay buffer and all the cultures were inoculated onto Sabroud Dextrose Agar plates. Sterile filter paper discs (diameter 6mm for bacteria and 13mm for fungi) impregnated with 100µl of extract dilutions reconstituted in minimum amount of solvent at concentrations of 50 and 100mg/ml were applied over each of the culture plates previously seeded with the 0.5 McFarland and 106 cfu/ml cultures of bacteria and fungi respectively. Bacterial cultures and those of Candida albicans were then incubated at 37o C for 18 h while the other fungal cultures were incubated at room temperature (30 – 32o C) for 48 h.

Paper discs impregnated with 20µl of a solution of 10mg/ml of ciprofloxacin and cotrimoxazole (for bacteria) and nystatin and amphoteracin B (for fungi) as standard antimicrobials were used for comparison. Antimicrobial activity was determined by measurement of zone of inhibition around each paper disc. For each extract three replicate trials were conducted against each organism. Determination of MIC and MBC the minimum inhibitory concentration (MIC) of the extracts was estimated for each of the test organisms in triplicates.

To 0.5ml of varying concentrations of the extracts (20.0, 18.0, 15.0, 10.0, 8.0, 5.0, 1.0 0.5, 0.05 and 0.005mg/ml), 2ml of nutrient broth was added and then a loopful of the test organism previously diluted to 0.5 McFarland turbidity standard for (bacterial isolates) and 106 cfu/ml (for fungal isolates) was introduced to the tubes. The procedure was repeated on the test organisms using the standard antibiotics (ciprofloxacin and cotrimoxazole for bacteria and nystatin and amphoteracin B for fungal isolates).

A tube containing nutrient broth only was seeded with the test organisms as described above to serve as control. Tubes containing bacterial cultures were then incubated at 37o C for 24 h while tubes containing fungal spore cultures were incubated for 48 h at room temperature (30 – 32o C). After incubation the tubes were then examined for microbial growth by observing for turbidity.

To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient agar (for bacteria) and saboraud dextrose agar (for fungi) by streaking. Nutrient agar and saboraud agar only were streaked with the test organisms respectively to serve as control.

Plates inoculated with bacteria were then incubated at 37o C for 24 hours while those inoculated with fungi were incubated at room temperature (30 – 32o C) for 48 h. After incubation the concentration at which no visible growth was seen was noted as the minimum bactericidal concentration. Effect of Temperature and pH on antimicrobial activity of extracts Five milliliters of 100mg/ml of acetone extracts were constituted in test tubes and treated at 4, 30, 60 and 100o C in a water bath for 30minutes and tested for antimicrobial activity.

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To determine the effect of pH, acetone extracts were treated at pH ranges of 2.5 to 10 using 1N HCl and 1N NaOH solutions respectively in series of test tubes for 30minutes. After 30 minutes of treatment, each of the treated extracts were neutralized (pH 7) using 1N HCl and 1N NaOH as the case may be, and then tested for antimicrobial activity.

3. Result

Phytochemical constituents present in the plant extract included tannins, saponins, sesquiterpenes, alkaloids, and phlobatamins. Results of the antimicrobial activity of the plant extracts are shown in Table 1, 2, 3. The result shows

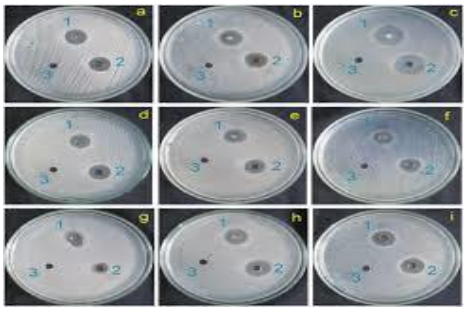

Table1. Results of antimicrobial activities of extracts of Tamarindus indica

Gram-negative aerobes	Imipenem	Meropenem	Ceftazidime	Piperacillin/tazobactam	Gentamicin	Ciprofloxacin
Acinetobacter anitratus	0.25	1.0	8	16	1	8
Citrobacter freundii	1	0.13	32	16	1	0.5
Enterobacter aerogenes	1	0.13	16	32	1	0.25
Enterobacter cloacae	1	0.25	16	32	8	0.25
Escherichia coli	0.13	0.03	1	1	8	0.13
Haemophilus influenzae (BLN)	0.5	0.06	0.06	0.13	8	0.016
H influenzae (BLP)	0.5	0.06	0.06	0.25	8	0.016
Klebsiella pneumoniae	0.25	0.03	0.25	4	4	0.25
Klebsiella species	0.5	0.06	0.25	2	4	0.25
Moraxella catarrhalis	0.06	0.008	0.5	2	2	0.06
Morganella morganii	4	0.25	16	4	4	0.13
Neisseria gonorrhoeae (PS, PR)	0.25	0.03	0.03	1	16	0.008
Neisseria meningitidis	0.03	0.016	0.25	0.25	8	0.008
Proteus mirabilis	2	0.13	0.13	0.5	4	0.13
Proteus vulgaris	4	0.25	0.25	2	4	0.06
Proteus rettgeri	1	0.12	4	4	32	8
Providencia stuartii	2	0.25	4	4	16	8
Pseudomonas aeruginosa	4	2	8	8	16	2
Burkholderia cepacia	8	8	16	128	128	8
Salmonella species	0.12	0.03	0.5	2	0.5	0.06
Serratia marcescens	2	0.25	4	2	16	2
Shigella species	0.25	0.06	0.5	4	1	0.06
Stenotrophomonas maltophilia	128	128	16	256	64	16
Yersinia enterocolitica	0.25	0.03	0.5	2	2	0.13

Adapted from references 1,3-11,14,16-18,31-47. In vitro susceptibility of imipenem and meropenem: susceptible 4 µg/mL or less, intermediate 8 µg/mL, resistant 16 µg/mL or more. BLN Beta-lactamase negative; BLP Beta-lactamase positive; PR Penicillin-resistant; PS Penicillin-susceptible

Table 2(a) Phytochemical Investigation of Tamarindus indica

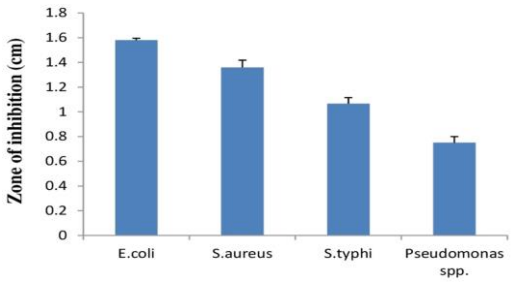

Table3. Results of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ethanolic extracts of Tamarindus indica

International Journal For Academic Research and Development
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2021

Organisms	Inhibition Zones (cm)				
	1 µg/ml	5 µg/ml	10 µg/ml	25 µg/ml	Gentamicin (25 µg/ml)
S. paratyphi A	0.1	0.2	0.6	1.2	2.8
S. typhi	0.1	0.2	0.55	1.2	2.5
E. coli	0	0	0	0	2.5
S. aureus	0	0	0.1	0.5	2.4
MRSA	0	0	0.3	0.5	2.4
V. cholerae	0	0	0	0.3	1.9
S. paratyphi B	0.2	0.3	0.8	1.3	2.8
P. aeruginosa	0	0.3	0.4	1.0	2.6
B. subtilis	0	0.2	0.8	1.1	2.6
P. alkalifaciens	0.3	0.6	0.8	1.3	3.8
P. mirabilis	0.4	0.6	0.9	1.1	3.4
C. fulvum	0.52	0.53	0.55	0.55	1.1
N. crassa	0.96	0.95	0.95	0.92	0.89
A. niger	0.57	0.58	0.60	0.60	0.20

Samples	Phytochemicals						
	Alkaloid	Cardiac glycosides	Flavonoids	Reducing sugar	Saponin	Tannin	Terpenoid
LOW	+	-	+	+	+	-	+
LWW	+	-	+	+	+	-	+
LHW	+	-	+	+	+	-	+
LET	+	-	+	+	+	-	+
POW	+	-	+	+	+	-	+
PWW	+	-	+	+	+	-	+
PHW	+	-	+	+	+	-	+
PET	+	-	+	+	+	-	+
SOW	+	-	+	+	+	-	+
SWW	+	-	+	+	+	-	+
SHW	+	-	+	+	+	-	+
SET	+	-	+	+	+	-	+

LOW = Leaf Ordinary Water; LWW = Leaf Warm Water; LHW = Leaf Hot Water; LET = Leaf Ethanol; POW = Pulp Ordinary Water; PWW = Pulp Warm Water; PHW = Pulp Hot Water; PET = Pulp Ethanol; SOW = Seed Coat Ordinary Water; SWW = Seed Coat Warm Water; SHW = Seed Coat Hot Water; SCET = Seed Coat Ethanol

Both gram positive and gram-negative organisms. The highest activity (diameter of zone of inhibition 27mm) was demonstrated by the acetone extracts of stem bark against Proteus mirabilis while the lowest activity (diameter of zone of inhibition 2mm) was demonstrated by the water extract against Staphylococcus aureus. The leaf extracts generally showed lower activity against the test organisms compared to the stem bark extracts

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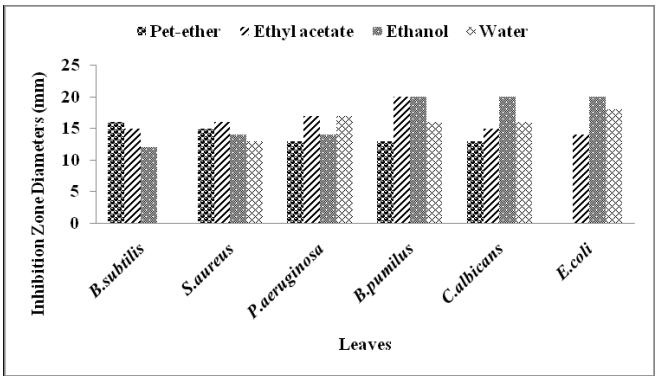

Figure1. Inhibition zone of Diameter in leaves

Result of the effect of temperature and pH on the plant extracts showed that various temperature ranges of 4, 30, 60 and 100o C had no effect on the antimicrobial activity of the extracts (Fig 1), but the activity slightly increased at acidic pH (2 to 6).

While at alkaline pH the activity of the plant extracts reduced. Results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are shown in Table 2. The result showed that *Staphylococcus aureus* had the highest MIC (18 mg/ml) and MBC (17.5 mg/ml), while the lowest MIC of 8 mg/ml was shown by *Salmonella paratyphi* and *Bacillus subtilis* respectively. *Salmonella typhi* had MIC and MBC values of 10 mg/ml for the stem bark

extracts. The MIC and MBC values were generally lower for the leaf extracts against the test organisms compared to those of the stem bark extracts

4. Discussion

The Phytochemical screening of the *Tamarindus indica* leaves and fruits extracts indicated the presence of alkaloid, tannin, saponin, glycoside, flavonoid, anthraquinone, reducing sugar, terpenoid, and phenols. The presence of the above phytochemicals in the plant parts was responsible for its antibacterial activity. Flavonoids have been shown to possess anti-inflammatory, anti-hepatotoxic and antimicrobial activities. Saponins are known to possess antibacterial activities whilst tannins play an important role in wound healing and also possess some antimicrobial activities.

According to this study, Alkaloid is also present in both the extracts. Alkaloid consists of large group of nitrogenous compound which are widely used as anticancer anesthetics and Central Nervous Stimulants. Alkaloids are known to play some metabolic roles and control development in living system. It also interferes with cell division, hence the presence of alkaloids in the *Tamarindus indica* leaves and fruits could account for their use as antimicrobial agents.

In contrast the cold-water extract of the leaves and stem bark, each was active against 16.7%; while the ethanolic extract of each was active against 75% of the test strains. Photochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and herbivores. This may therefore explain the demonstration of antimicrobial activity by the stem bark and leaf extracts of Tamarindus indica. The demonstration of antibacterial activity against both gram positive and gram-negative bacteria may be indicative of the presence of broad-spectrum antibiotic compounds.

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