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Antimicrobial activity of red *Tamarindus indica* L on *Fusarium oxysporum*, pathogen of *Azadirachta indica*

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Abstract
Aim: To determine the phytochemical composition and antimicrobial properties of tamarind extracts on *Fusarium oxysporum* pathogen of *Azadirachta indica* diseased leaves.
Study Design: Completely Randomized Design (CRD).
Methodology: The phytochemical constituents in ordinary, warm and hot water as well as Methanolic extracts of tamarind pulp were screened. The Zone of Inhibition (ZOI) diameter (mm), Minimum Inhibitory Concentration (MIC) against *Fusarium oxysporum* pathogen were determined. Data were analyzed using ANOVA at P = 0.05.
Results: The Methanolic tamarind fruit pulp extract was obtained using the Soxhlet extraction method. The total number of compounds identified in methanol extract tamarind pulp was 10. The major phytoconstituents present in the methanol extract of *Tamarindus indica* L were Tannins, saponins, flavonoids, carbohydrates, reducing sugar, Glycosides, Alkaloides and proteins. Test for Anthraquinones and phytosterols was negative which indicates that they are absent in the methanolic extract of Tamarind pulp. Antimicrobial activity was carried out against the pathogen *Fusarium oxysporum* which Identified from *Azadirachta indica*, infected leaves through morphological evidence. The results represented as the antimicrobial activity of the tamarind extracts against *Fusarium oxysporum* at different concentrations at 25%, 50%, 75%, 100% and positive control referred to as treatments T_1, T_2, T_3, T_4, and T_5, the maximum zone of inhibition was observed at Positive Control i.e., T_5 (15.00 mm) which is more than the extracts which obtained from *Tamarindus indica* and T_4 is 100% concentration of crude is (13.66 mm) followed by (12.00 mm) at 75% of T_3, (8.00 mm) at 50% of T_2, and minimum inhibition was at 25% of T_1 (5.6 mm). Due to the presence of phytochemicals this zone of inhibition may be resulted. Minimum zone of 5.00 mm was observed in *Fusarium oxysporum at* 25% dose and a maximum of 15.00 mm zone at positive control T_5 dose.

Keywords: *Tamarindus indica*, antimicrobial activity, zone of inhibition, pulp extract, minimum inhibitory concentration, phytochemical analysis

Introduction
Tamarind, commonly called Imli in Hindi, is known as Chincha or Amlika in Ayurveda [1]. It is botanically identified as a *Tamarindus indica* L.
Tamarindus indica L. belongs to a Dicotyledonous family of large flowering plants; Fabaceae subfamily Caesalpiniaceae; which is composed of over 700 genera and spans over 19,000 species known to man (Maiti *et al*., 2004; Lewis *et al*., 2005) [2].
Its ethno-medicinal uses include being utilized as a laxative, expectorant, blood tonic, for treating bile disorders, as an antiscorbutic, as a component of cardiac and blood sugar medication. According to folklore, the fruit pulp is used to remedy swellings, sore throat, rheumatism, alcoholic intoxication and sunstroke (El-Siddig *et al*., 2006; Siddig *et al*., 2006; Teklehaimanot, 2008) [3].
T. indica is rich in nutrients and plays an important role in human nutrition, mainly in the developing countries [4]. It contains a high level of protein with many essential amino acids, which help to build strong and efficient muscles. It is also high in carbohydrates, which provides energy, and is rich in minerals, such as potassium, phosphorus, calcium, and magnesium. *T. indica* can also provide smaller amounts of iron and vitamin A [5]. *Tamarindus indica* has numerous applications in traditional medicine, all parts of the plant have therapeutic uses [6]. The plant parts have been extensively studied in terms of pharmacological activity of its major compounds and results indicate potent antibacterial, antifungal, hypoglycaemic, cholesterolemic (Khanzada *et al*., 2008) [7].

Materials and Methods

Collection of samples

The Telangana Forest Academy in Dulapally, Hyderabad, and the Forest College and Research Institute in Mulugu, red tamarind fruits that were collected in September 2021. Seeds were taken out, and pulp was manually scraped. Prior to analysis, non-plant debris, dirt, and infected pulp were removed.

Extraction of *Tamarind* Pulp

Methanol was used to thoroughly extract 50 g of tamarind pulp using a Soxhlet equipment at a set temperature. The extracts were lyophilized (4KBTXL-75; Vir Tis Benchtop K, New York, USA) to remove any remaining water molecules, and the lyophilized powders were stored at 20 °C until further use for the evaluation of antimicrobial activity. The extracts were filtered and concentrated to dryness under reduced pressure using a rotary vacuum evaporator (RE300; Yamato, Japan).

Qualitative Phytochemical Analysis

Using a traditional method, a qualitative analysis of the methanolic extract of *Tamarindus indica* L pulp was carried out to determine the presence and absence of several phytochemicals. Utilizing the techniques described by Trease and Evans (1989) [9], Harbone (1998), Sofowora (1993) [11]; and Sahira and Cathrine (2015) [12], phytochemicals such as alkaloids, saponins, tannins, flavonoids, carbohydrates, and sterols were examined.

Collection of diseased samples

Infected neem leaves were Collected and conserved for further research at the Mulugu Forest College and Research Institute in Telangana.

Isolation

The diseased leaves were Surface sterilized at a 0.2 percent solution of mercuric chloride for one minute. The bits were aseptically transferred to sterilised petri plates containing Potato dextrose agar medium after being thoroughly rinsed in sterile distilled water three times to remove any signs of mercuric chloride. The Petri plates were incubated for seven days at room temperature (271 °C). Pure colonies were separated and Transfered to PDA slants for future use after 7 days. The isolated fungus were identified.

Antimicrobial studies

Agar well diffusion method was used to evaluate the amount of antimicrobial activity (Kudi *et al*. 1999) [13]. The fungus was cultured on potato dextrose agar. The culture of the appropriate fungi. 6 mm wells were made in the cork using a flamed cork borer, and 0.1 ml of the extract was aseptically poured to each well using a sterile syringe. After 72 hours, the diameter of the zone was measured to determine the inhibition zone. For comparison of antifungal activity, fluconazole (300 g/well) was utilised as the benchmark (Gobdi *et al*. 1992) [15]. There were four replications of the experiment.

Results & Discussion

The major phytoconstituents present in methanol extract of *Tamarindus indica* L were Tannins, saponins, flavonoids, carbohydrates, reducing sugar, glycosides, alkaloides and proteins. Test for Anthraquinones and phytosterols was negative which indicates that they are absent in the methanolic extract of tamarind pulp (Table-1).

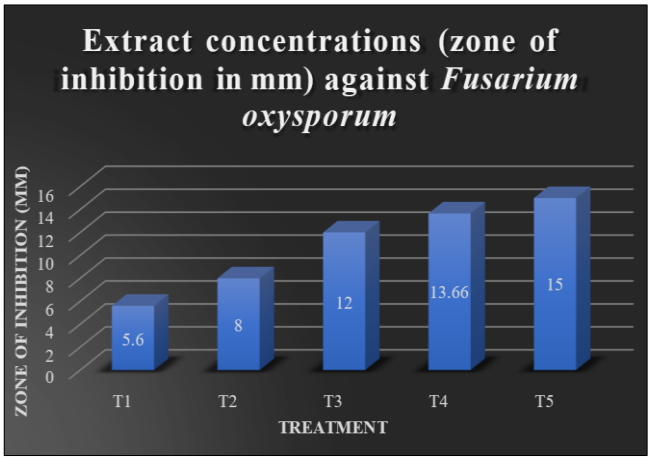
Table 1: Phytochemicals of tamarind. (+ = Present; = Absent)

Sr. No.	Tests	In methanol extract of fruit pulp
01	Tannins	+
02	Saponins	+
03	Flavonoids	+
04	Carbohydrates	+
05	Reducing Sugar	+
06	Anthraquinones	-
07	Glycosides	+
08	Alkaloids	+
09	Phytosterols	-
10	Proteins	+

Table 2: Tamarindus *indica* Pulp Extract concentrations (zone of inhibition in mm) against *Fusarium oxysporum* Pathogen identified from *Azadirachta indica* diseased leaves.

Fungal Pathogen	Concentration (Extracts) and Inhibition zones)				Positive control (Flucanozole)
	25%	50%	75%	100%	
Fusarium oxysporum	5.6 mm	8.00 mm	12.00 mm	13.66 mm	15.000 mm

S.E(m) ± 0.211 and C.D. @ 5% - 0.673



Zone of inhibition in (mm) of *Fusarium oxysporum*

Tamarind pulp extract Application was carried out against the Neem fungus *Fusarium oxysporum*. The results represent the antimicrobial activity of the tamarind extract against *Fusarium oxysporum* at different concentrations at 25%, 50%, 75%, 100% and positive control referred as treatments T_1, T_2, T_3, T_4, and T_5. The maximum zone of inhibition was observed at positive control fluconazole (15.000 mm) followed by at 100% (13.66 mm), at 75% (12.000 mm), at 50% (8.000 mm) at 25% (5.6 mm) zone of inhibition. Lowest inhibition was at 25% i.e., is treatment-1 and highest at positive control i.e., Treatment-5.

Conclusion

Tamarind extract inhibited *Fusarium oxysporum as* it is evident from the good inhibition zone suggesting its antimicrobial activity. The highest inhibition was created

through positive control. The phytochemicals identified from red tamarind may be responsible for inhibiting *Fusarium oxysporum in vitro.*

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