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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(11): 970-972

www.thepharmajournal.com
Received: 20-07-2022
Accepted: 19-10-2022

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Antifungal activity of red *Tamarindus indica* L on *Rhizoctonia solani*, pathogen of *Tectona grandis*

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Abstract
The Research work was conducted to investigate the phytochemical analysis and antimicrobial activity of methanolic extract of Red *Tamarindus indica* L fruit pulp on *Rhizoctonia solani* pathogen of *Tectona grandis.* The Methanolic tamarind fruit pulp extract was obtained using Soxhlet extraction method. The total number of compounds identified in methanol extract tamarind pulp was 10. The major phytoconstituents present in methanol extract of red *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 *Rizoctonia solani* which Identified from *Tectona grandis* infected leaves through morphological evidence. The results represented as the antimicrobial activity of the red tamarind extract against *Rizoctonia solani* 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 100% i.e., T4 (14.333mm) which is more than the positive control T_5 (fluconazole which is 13mm) fallowed by (11.333mm) at 75 % of T_3, (7.667mm) at 50 % of T_2, and minimum inhibition was at 25 % of T_1 (5.000mm). Due to presence of phytochemicals this zone of inhibition may be resulted. A Minimum zone of 5.00mm was observed in *Rhizoctonia solani* at 25 % dose and maximum of 14.333mm zone at 100 % dose.

Keywords: *Tamarindus indica*, antimicrobial activity, Zone of inhibition, Pulp extract, Minimum inhibitory concentration, Phytochemical Analysis

Introduction
Tamarindus indica L. (Red Tamarind) is a dicotyledonous plant in the Leguminosae family and the Caesalpiniaceae subfamily (Khanzada *et al*., 2008) [9]. Red *Tamarindus indica* L. is a versatile plant that is also known as IMLI/Indian date. The fruit's pulp has long been used as a spice in Asian cuisine, particularly in southern India. Various parts of the tree are used in the pharmaceutical and textile industries, as well as for fodder, lumber, and fuel. (Jaspher *et al*. 2017) [6]. It is a tropical tree primarily used for its fruits, which are eaten fresh or processed, used as a seasoning or spice, and as medicine beverages. The fruit of *Tamarindus indica* is high in minerals such as potassium, phosphorus, calcium, and magnesium. It has the highest levels of protein and carbohydrate of any fruit (BAIF, 2002) [1], but it has lower levels of ironand vitamin A. (Khanzada *et al*., 2008) [9]. It also has edible leaves that are high in fat, fiber, vitamins, proteins, flavonoids, and essential oils, as well as fat, fiber, vitamins, proteins, flavonoids, and essential oils (Razali *et al*., 2012) [14].

The red pigment (anthocyanin) from half-ripen red variety tamarind has a natural and appealing red colourant, that can be used as a source of natural red food colourant in the future (Kaur *et al* 2006) [8]. The red tamarind is a rare mutant that contains rosy red pigments that give it its red colour, and at the unripe stage the fruits contain anthocyanins (Lewis *et al*. 1964) [13].

It is a rich source of most essential amino acids and phytochemicals, has been reported to have anti-disease properties (Kuru 2014) [11]. This has been used as a medicinal plant for centuries; the fruit, which has been described as curative in numerous pharmacopoeias, is the most valuable part of the plant. The presence of polyhydroxylated chemicals, many of which are flavonoid in nature, has been linked to leaf protection (Joyeux *et al*., 1995) [7].

Tamarind leaves are utilized as antibacterial agents because they have a high concentration of polyphenols (Gomathi *et al*., 2011) [3]. The phytochemicals and the antimicrobial activities of tamarind are analyzed and reported in this paper.

Materials and Methods

Collection of samples

Ripen red tamarind fruits were collected from two locations at Telangana Forest academy, Dulapally, Hyderabad and Forest College and research institute Mulugu September 2021. During Seeds were removed and pulp was hand scrapped. Non-plant material, dirt and infected pulp was removed prior to analysis.

Extraction of Tamarind Pulp

50 g tamarind pulp was exhaustively extracted with methanol, using Soxhlet apparatus at a controlled temperature. The extracts were filtered and concentrated to dryness under reduced pressure using rotary vacuum evaporator (RE300; Yamato, Japan), lyophilized (4KBTXL-75; Virs Tis Benchtop K, New York, USA) to remove traces of water molecules and the lyophilized powders were stored at 20°C until further use directly for the assessment of antimicrobial activity.

Qualitative phytochemical analysis

Qualitative analysis for the presence and absence of different phytochemicals was done using conventional method in methanolic extract of *Tamarindus indica* L pulp. Phytochemicals such as alkaloids, saponins, tannins, flavonoids, carbohydrates, and sterols were analyzed using the methods given by (Trease and Evans 1989); Harbone, 1998); Sofowora, 1993) and Sahira and Cathrine, 2015) [17, 5, 16 15].

Collection of diseased sample

Infected leaves of Teak were collected in paper bags and these were dried and preserved for further studies from Forest College and Research institute, Mulugu in Telangana.

Isolation

The infected leaves were cut into small bits and surface sterilized with 0.2 percent Mercuric chloride solution for 1 minute. The bits were washed thoroughly in sterile distilled water 3 times to remove traces of Mercuric chloride and then aseptically transferred to sterilized petri plates containing Potato dextrose agar medium (Potato-200g, dextrose-20g, agar 15g). The Petri plates were incubated at room temperature (27 ± 1 ℃) for 7 days. After 7 days, pure colonies were isolated and transferred to PDA slants for further use. The fungi thus isolated were identified.

Antimicrobial studies

Antimicrobial activity was estimated by Agar well diffusion method (Kudi *et al.* 1999) [10]. Potato dextrose agar was used to culture the fungus. The plates were inoculated with 24 h culture of respective fungi. With the help of a flamed corn borer 6 mm wells were cut and to each of the well 0.1 ml of the extract were aseptically added with the help of sterile syringe. Inhibition zone was recorded by measuring the diameter of the zone after 72 h. Flucanozole (300 g/well) was used as standard for comparison of antifungal activity (Gobdi *et al.* 1992) [2]. The experiment was performed in four replications.

Results & Discussion

The major phytoconstituents present in methanol extract of *Tamarindus indica* Linn 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 *Rhizoctonia solani* from *Tectona grandis* leaves

Fungal Pathogen	Concentration (Extracts) and Inhibition zones)				Positive control (Fluconazole)
	25 %	50 %	75 %	100 %	
Rizoctonia solani	5.000mm	7.667mm	11.333mm	14.333mm	13.000mm

S.E (m) ± 0.258 and C.D.@5% - 0.824

Application was carried out against the Teak fungus *Rizoctonia solani*. The results represent the antifungal activity of the tamarind extract. The maximum zone of inhibition was observed at 100% (14.333mm) which is more than the positive control (fluconazole) and minimum inhibition was at 25 % (5.000 mm). The results were similar to the (Gupta and Bansal, 2003) [4] said the methanolic extract has shown highest inhibition against the *Rhizoctonia solani*.

Conclusion

Phytochemical analysis of methanolic extract of tamarind pulp represents the presence of tannins, saponins, flavonoids, carbohydrates, reducing sugar, glycosides, alkaloides and proteins. Tamarind extract inhibited *Rizoctonia solani* as it is evident from the *good* inhibition zone suggesting its antimicrobial activity. (Kuru 2014) [12] Reported anti-disease properties of Tamarind. Gomathi *et al* (2011) [3] attributed the antimicrobial activity to high concentration of polyphenols. The phytochemicals identified from red tamarind may be responsible for inhibiting *Rizoctonia solani in vitro.*

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