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Antifungal activity and phytochemical analysis of the alcoholic and aqueous extract of the Aerial part of the plant Tamarindus indica L.

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Abstract

Tamarindus indica is fruit tree which belong to the family leguminosae (Fabeaceae) a medicinal plant commonly known as imli. Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases. Tamarind has been used for centuries as a medicinal plant. . Due to their antimicrobial, an antifungal and antiseptic effect, have an extensive ethnobotanical use in many areas. Aerial plant parts - stem, bark and fruit of Tamarindus indica were tested for their antifungal property in vitro using well diffusion method against five fungal strains Candida albicans, Candida glabrata, Candida krusei, Candida sp.and Aspergillus niger. Air dried powder of stem and bark sample ethnolic, methanolic and aqueous crude extracts, was tested at 50ug, 100ug and 200ug concentrations for antifungal activity. The phytochemical analysis revealed the presence of many active constituents.

Keywords: *Tamarindus indica*, Antimicrobial activity, Phytochemical constituent, Inhibition zone, Well diffusion method.

Introduction

Tamarindus indica L. (Tamarind), dicotyledonous tree, belongs to family leguminosae and subfamily caesalpiniaceae[1]. For centuries *T. indica* has been reported to be used as a medicinal plant for the treatment of diseases such as cold, fever, stomach disorder, diarrhea, jaundice and skin cleanser[2]. This plant has numerous bioactive molecules, hence has great therapeutic value including anti diabetic, antimicrobial and antivenomeic property[3]. Antimicrobial property of various areal parts, leaf, stem bark and fruit of *Tamarindus* have been reported[4-6]. Due to their antimicrobial, antifungal and antiseptic effect, have an extensive ethnobotanical use in many areas[7]. Studies of many medicinal plant indicated they contain substances like peptide, unsaturated long fatty acids, aldehydes, alkaloids, essential oils, phenols and water or ethanol soluble compounds[8]. However, studies on anti fungal property are very limited[6,9]. Present study reports the phytochemical analysis and antifungal property of stem, bark, and fruit, aqueous as well as alcoholic extracts of *Tamarindus*.

Material and methods

In the present study, phytochemical analysis and anti fungal property of alcoholic and aqueous extracts of aerial parts of *Tamarindus*-stem, bark and fruit were carried out. The Plant material was collected from Gwalior region M.P in 2012-2013 and 2013-2014 different seasons in bulk.

Extraction procedure: The anti fungal property of stem, bark and fruit of *Tamarindus indica* was analyzed using three solvents extracts i.e. Ethanol (70%), methanol (70%) and Aqueous. All the solvent extracts were prepared using soxhlet apparatus. About 5grams of finely grounded powder of the samples as soxilated in 200ml of solvent and the concentrated extract collected was vacuum dried.

0.025g of each sample was dissolved completely in 5ml DMSO and used immediately for anti fungal studies. The extracts were used at 50µg, 100µg and 200µg concentrations for the present work.

Clinically isolated strain *Candida sp.* and the type cultures - *Candida albicans* (MTCC 3017), *Candida glabrata* (MTCC3019), *Candida krusei* (MTCC9215), and *Aspergillus niger* (MTCC 478) were used for the work. All the fungal cultures were maintained on Nutrient Agar medium.

Phytochemical analysis: All the extracts were screened for the presence of phytochemicals - Alkaloids, Saponins, Tannins, Flavonoids, Carbohydrates, and Sterols using the methods given by researchers[9,10].

Evaluation of antimicrobial activity of the plant extracts: *In vitro* sensitivity test was used to study antifungal activity of *Tamarind* extracts. This was determined by Agar Well Diffusion method[11]. All fungal strains mycelium was inoculated in Nutrient broth with 5% glucose and incubated for 5 hours. Turbidity of the cultures was adjusted to that of 0.5 McFarland standards[12].

Six wells of 5.0 mm size were created at equal distance in the solidified Muller Hinton agar (MHA) medium. Then fungal

cultures were spread plated by Kirbybaur method on a sterile MHA plate (Hi Media) so as to achieve a confluent growth. With the help of micropipette 50µg, 100µg and 200µg of plant extract was poured into the wells. The plates were allowed to stand for 1h or more for diffusion to take place and then incubated at 37^0C for 24 h. The zone of inhibition (Inhibition zone, IZ) was recorded to the nearest size in mm[13]. The level of sensitivity was classified as + (5.0mm – 7.0mm); ++ (7.1 – 9.00mm); +++ (9.1mm – 11.00mm); ++++ (11.1mm and above). Each experiment was carried out in three replicates and each experiment was repeated twice. The data was pooled, mean zone of inhibition and standard error was calculated. The antimicrobial property of plant extracts was analyzed and compared with the standard antifungal drugs (Fluconazole, Itrokenazole, Metrocanazole, Ketocanazole and streptomycin).

Results and discussion

Phytochemical analysis of Tamarindus extracts: Experimental results on qualitative analysis of various phytochemical constituents in *Tamarindus indica* stem, bark and fruit- ethanolic, methanolic and aqueous extracts are given in the Table-1 shows that Flavonoids, Tannins, Saponins and Carbohydrates are present in all the nine extracts. Phytosterols were present only in ethanol and water extracts of Fruit. While glycosides were absent in methanol and aqueous extracts of stem. Alkoloids were absent in aqueous extracts of stem and bark. Proteins gave positive test for ethanol extract of stem and all the three extracts of fruit. Reducing sugars were present only in six out of nine extracts of *Tamarindus. Fruit e*thanol and aqueous extracts and stem water extract gave positive test for Anthroquinones (Table-1).

In vitro sensitivity test for standard drugs: The *in vitro* sensitivity of all the five fungal cultures to five standard anti fungal drugs, including streptomycine, were analyzed and the level of sensitivity and zone of inhibition diameter are given in the Table-2.

Fungal cultures *C. albicans* and *C. glabrata* showed high to very high sensitivity against streptomycin and Fluconazole. *C. albocans recorded* growth inhibition up to 10.2 mm and 18.2 mm and *C. glabrata* recorded growth inhibition up to 12.4mm and 11.0mm against Strepotomycin and Fluconazole respectively. *C. albicans* and *C. glabrata* were also inhibited by drug Metroconazole and they demonstrated 6.4 mm and 10.4 mm of inhibition zone respectively against this drug. For the rest of the drugs these fungal cultures recorded resistance. *C. krusei* was sensitive to only itraconazole and showed 5.8 (+) mm zone of inhibition. Similarly, *Candida sp.* was resistant for all the drugs except for ketocanazole. It recorded 6.8±0.84mm zone of inhibition. While *C. glabrata* has demonstrated resistance against ketacozole and Itacozole but was sensitive to Streptomycin (12.4±1.14mm), Fluconazole (11.0±1.58mm), metroconazole (10.4±1.82mm) (Table-2). The fungal culture *A. niger* demonstrated high sensitivity to Fluconazole (16.2 mm IZ) and minimum sensitivity to Metroconazole (5.4 mm IZ). But *A. niger* was resistant to rest of the drugs tested (Table-2).

Table-1: Phytochemical qualitative profile of *Tamarindus indica* stem and bark ethanolic , methanolic and aqueous extracts.

Phytochemical Name	Stem			Bark			Fruit		
	EE	ME	AE	EE	ME	AE	EE	ME	AE
Alkaloids	+	+	-	+	+	-	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+
Phytosterols	-	-	-	-	-	-	+	-	+
Glycosides	+	-	-	+	+	+	+	+	+
Protein	+	-	-	-	-	-	+	+	+
Reducing sugars	+	+	+	+	-	-	+	+	+
Anthroquinones	-	-	+	-	-	-	+	-	+

+ present; - absent (EE: Ethanolic Extract; ME: Methanolic extract; AE: Aqueous extract).

Table-2: Differential sensitivity of five fungal cultures against standard antifungal.

Antifungal drug (100 µg)	Diameter of inhibition zone (in mm)									
	C. albicans		*C. glabrata*		*C. krusei*		*Candida sp.*		*A. niger*	
Streptomycin	10.2±8.5	+++	12.4±1.14	++++	-	-	15.2±0.84	++++	-	-
Fluconazole	18.2±1.3	++++	11.0±1.58	+++	14±7.91	++++	11.6±0.55	++++	16.2±0.84	++++
Itraconazole	-	-	-	-	5.8±5.31	+	-	-	-	-
Ketoconazole	-	-	-	-	-	-	6.8± 0.84	+	-	-
Metroconazole	6.4±1.14	+	10.4±1.82	+++	-	-	-	-	5.4±0.55	+

Sensitivity levels- : + = 5.0 to 7.0 mm; ++ = 7.1 to 9.0; +++ = 9.1 to 11.0, ++++ = 11.1 above.

Analysis of In vitro Anti fugnal property of Tamarindus extracts: Data on inhibition zone size and the level of sensitivity against ethanol, methanol and aqueous extracts of *T. indica.* Stem, bark and fruit, against fungal strains *C. albicans, Candida glabrata*, *Candida krusei*, *Candida sp.* and *A. niger* is given in the Table-3.

The result presented in the Table-3 clearly shows that *Tamarindus indica* plant aqueous extracts of stem, bark and fruit have completely failed to inhibit the growth of the all tested cultures. However, the ethanolic and methanolic extracts have limited antifungal property against *C. albicans, C. glabrata*, *C. krusei*, *Candida sp.* and *A. niger.* Further, alcoholic extracts did not record anti mycotic property at 50µg concentration against these five cultures (Table-3).

C. albicans has shown growth inhibition to bark methanolic extract and fruit ethanol as well as methanol extracts. At 200µg concentration, fruit ethanolic extract of *Tamarindus* recorded 8.0mm of inhibition zone. At this concentration both bark and fruit extracts inhibited *C. albicans* growth up to 6.0 mm and 5.6mm respectively (Table-3).

Interestingly, the *C. glabrata* culture showed no sensitivity to any of the *Tamarindus* extracts we have tested, indicating its resistance to stem, bark and fruit extracts (Table-3).

C. krusei culture showed sensitivity to methanolic and ethanolic extracts of bark and ethanolic extract of stem. Bark extract demonstrated low to very high antifungal property. At 100µg concentration, ethanolic and methanolic extracts recorded inhibition of *C. krusei* growth up to 6.5mm and 7.5mm in diameter (Table-3). The maximum zone of inhibition was recorded with bark methanolic extract (12.0±1.14 mm) followed by ethanolic extracts of bark (11.5±0.71mm) and stem (11.0±0.0mm) at 200 µg concentration (Table-3).

Candida sp. Isolated from clinical sample recorded sensitivity to ethanol (stem and bark) and methanol (bark) extracts of *Tamarindus*. Its growth was inhibited up to 7.5mm (100µg) and 14.0mm (200µg) with ethanol stem extract. The bark ethanolic extract exhibited low (+) ability to inhibit *Candida sp* growth *in vitro*. It has resulted only 6.5mm zone of inhibition at both 100 and 200µg concentrations (Table-3). However, the methanol extract of bark demonstrated high (+++) to very high (++++) growth inhibition efficiency. They recorded 9.5±0.71 mm and 11.5±0.71 mm inhibition zone *in vitro* cultures (Table-3).

The fungal culture *A. niger* recorded sensitivity to *Tamarindus* plant stem ethanolic (100, 200µg), bark methanolic (200µg) and also to fruit, both ethanolic and methanolic (200µg) extracts. The maximum zone of inhibition was recorded with stem ethanolic extract (9.2±0.45 mm) followed by bark methanolic extract (8.4±0.55mm). The fruit ethanolic and methanolic extracts demonstrated *A. niger* growth inhibition up to 7.2 and 7.0mm diameter respectively (Table-3).

Discussion: Medicinal plants have enormous ability to synthesize wide variety of secondary metabolites with antimicrobial potential[14-17]. Qualitative and quantitative analysis of various phytochemicals presence in different extracts of *Tamarindus* aerial parts have been worked out[2,18-21].

Revealed the presence of Alkaloids, glycosides, flavonoids, reducing sugars, saponins and tannins in leaf and fruit ethanolic, methanolic and water aqueous extracts of *Tamirindus*[22]. These findings are in accordance with our results. Uchechukwu *et al* have reported presence of carbohydrates, reducing sugars, tannins and saponins leaf, bark and fruit ethanolic and water extracts of *Tamarindus* and also reported absence of Alkaloids in bark extracts[18]. Similar results were also reported by Gupta *et al* [23]. Our present results for flavonoids and alkaloids are in accordance to earlier studies. However, unlike in earlier reports, reducing sugars were not observed in the bark methanolic and aqueous extracts and also alkaloids in bark aqueous extract. Earlier report of Uchechukwu *et al* have shown the absence of anthroquinones in water extracts of all extracts tested, as similar to our results[18]. We further observed that these compounds were absent in methanolic extracts of stem, bark and fruit parts and also in ethanolic extract of bark (Table-1).

Table-3: Antifungal activity of stem, bark and fruit of *T. indica* plant against *C. albicans, C. glabrata, C. krusei, Candida sp.* and *A. niger.*

Strains	Solvent	Stock (0.025mg/1ml)	Diameter of inhibition zone (in mm)					
			Stem		bark		fruit	
C. albicans	Ethanol	Amount	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		20µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		40µl	0.0±0.0	-	0.0±0.0	-	8.0±0.71	++
	Methanol	10µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		20µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		40µl	0.0±0.0	-	6.0±1.22	+	5.6±0.89	+
	Aqueous	10µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		20µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		40µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
C.glabrata	Ethanol	10µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		20µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		40µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
	Methanol	10µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		20µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		40µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
	Aqueous	10µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		20µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		40µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
C.krusei	Ethanol	10µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		20µl	6.5±0.71	+	6.5±0.71	+	0.0±0.0	-
		40µl	11±0.0	+++	11.5±0.71	++++	0.0±0.0	-
	Methanol	10µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		20µl	0.0±0.0	-	7.5±0.71	++	0.0±0.0	-
		40µl	0.0±0.0	-	12±1.14	++++	0.0±0.0	-
	Aqueous	10µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		20µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		40µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
Candida sp.	Ethanol	10µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		20µl	7.5±0.71	++	6.5±0.71	+	0.0±0.0	-
		40µl	14±1.41	++++	6.5±9.19	+	0.0±0.0	-
	Methanol	10µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		20µl	0.0±0.0	-	9.5±0.71	+++	0.0±0.0	-
		40µl	0.0±0.0	-	11.5±0.71	++++	0.0±0.0	-
	Aqueous	10µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		20µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		40µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
A. niger	Ethanol	10µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		20µl	5.0±0.0	+	0.0±0.0	-	0.0±0.0	-
		40µl	9.2±0.45	+++	0.0±0.0	-	7.2±0.84	++
	Methanol	10µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		20µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		40µl	0.0±0.0	-	8.4±0.55	++	7.0±0.71	+
	Aqueous	10µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		20µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
		40µl	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-

Sensitivity levels : +=5.0 to 7.0 mm; ++ = 7.1 to 9.0; +++ =9.1 to 11.0, ++++=11.1 above.

According to Srinivasan *et al*, demonstration of antimicrobial compound may be indicative of the presence of broad spectrum antibiotics for pathogens that are so prevalent in recent times. Limited literature is available on antifungal property studies in *Tamarindus*[24]. Marjorie, observed that extract of stem bark was more effective than other parts of this plant like leaf extract to *A. niger*[25]. According to Doughari, stem bark of *T. indica* (water, acetone and ethanolic) extracts did not show any antimycotic activity against *A. niger* and *C. albicans*[2]. Leaf water and fluid extracts are also reported to have no influence on *C.albicans* growth[26]. Aqueous extract had antifungal activity with highest IZ was found to be in fungi- *C. albicans* and *A. niger* studied by Aram *et al*, which contradicts the earlier reports of Doughari and Nehad *et al* [2,19,27]. According to Adeola *et al*, *A. niger* and *C. albicans* were resistant to all the extract except methanol extract of the pulp showed activity on *A. niger* at higher concentration[28]. According to Dipali *et al*, Tamarind pulp extract showed very lower zone of growth inhibition against *A.niger*[29]. Similarly Gupta *et al*, noted that out of the ten fungi tested only *Aspergillus sp.* and *Penicillium sp* found to be partially sensitive to aqueous-ethanol (50%) extract of *Tamarindus* fruit[23]. Our resent study agrees with earlier reports statement of no antifungal activity in aqueous extract of *Tamarindus* leaf. Further we also observed that all aqueous extracts failed to inhibit not only *C. albicans* and *A. niger* but also they were ineffective against *C. krusei, candida sp* and also *C. glabrata.* Our studies also agree with Adeola *et al*, and in addition we also noticed that both methanol and ethanol extracts of leaf, stem, bark and fruit at 100 and 200µg concentrations exhibited antifungal property against *C. albicans, C.krusei, candida sp* and *A.niger* (Table-3)[28].

Conclusion

In conclusion*, Tamarindus indica* ethanol and methanol (70%) extracts of leaf, stem, bark and fruit, with different phytochemical constituents has demonstrated its broad spectrum antifungal property, which is almost at par with the standard antifungal drugs (100µg). The results have shown the possibility of this plant being used in drug development for human beings for the treatment of various daily normal causes. However, the phytochemicals detected need further qualitative and quantitative analysis and individually for the antimicrobial action.

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