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Studies on the antibacterial activity and phytochemical screening of *Tylophora indica* linn on opportunistic bacterial pathogens coinfected with HIV

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ABSTRACT

Tylophora indica is an extensively used Indian traditional medicine to cure various human ailments, and acts as a folk remedy for the treatment of bronchial asthma, inflammation, bronchitis, allergies, rheumatism and dermatitis. It also seems to be a good remedy in traditional medicine as anti psoriasis, seborrheic dermatidis, anaphylactic, leucopenia. The roots are suggested to be a good natural preservative of food. Antibacterial activity of ethyl acetate and methanol extracts of plant was investigated by well-diffusion method against bacterial pathogens associated with HIV. The plant extracts showed better inhibitory activity against the tested organisms. Methanolic leaf extract of *Tylophora indica* showed highest inhibitory activity when compared with all treatments. Phytochemical screening of the methanolic leaf extract of the plant revealed the presence of tannins, saponins, flavonoids, carbohydrates and alkaloids. Thin Layer Chromatography(TLC) value of the leaf extract showed the highest Retention Factor(RF) value. This study creates social awareness among the HIV patients who were infected by various opportunistic bacterial pathogens.

Keywords: *Tylophora indica*, Well-diffusion method, HIV, Antibacterial activity, Thin Layer Chromatography(TLC), Retention Factor(RF) and Social awareness.

INTRODUCTION

Plants are known to contain innumerable biologically active compounds [1] possess antibacterial [2] properties. Nature has a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. It is reported that over 50% off all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the Pharmaceutical industry. People living with AIDS are prone to developing other illnesses and infections because of their suppressed immune systems called Opportunistic Infections or OIs [3]. Many bacterial pathogens, including *Mycobacterium, Staphylococcus, Streptococcus, Shigella, Camphylobacter, Listeria and Leginoella, Haemophilus, Pseudomonas, Rhodococcus and Salmonella* are most common in persons infected with HIV. Many infectious pathogens associated with HIV are resistant to synthetic drugs, hence an altenative therapy is very much needed.

Plant Description:

Tylophora indica is a climbing perennial plant that grows in India, commonly called as antmool in Ayurveda. The leaves of *Tylophora* have traditionally used as a folk medicine, it has been used for respiratory problems such as asthma, allergies, bronchitis and common cold. It is also believed by some have laxative and other purgative properties. The leaves and roots of *Tylophora indica* have emetic, cathartic, laxative, expectorant, diaphoretic and purgative properties and other reported activities include immunomodulator activities [4] and antiamoebic activity [5]. In this study, *T.indica* used as an alternative remedy for the treatment of bacterial pathogens coinfected with HIV. It has also been used for the treatment of allergies, cold and dysentery, hay fever and arthritis. It has reputation as an alternative and as a blood purifier, often used in rheumatism and syphilitic rheumatism. Root or leaf powder is used in diarrhea, dysentery and intermittent fever [6]. The roots are suggested to be a good natural preservative of food. The plant has been reported to contain 0.2-0.46% alkaloids viz., Tylophorine, tylophorinine[7], tylophorinidine, septicine, sotylocrebrine[8] tylphoricine,sterols, flavonoids [9] was resins and tannins. It also seems to be a good remedy in traditional medicine as anti psoriasis, seborrheic , anaphylactic, leucopenia and as an inhibitor of the Schultz-Dale reaction [10]. The objective of the present study is to create social awareness among the HIV patients for the use of available medicinal plant *T.indica* against the bacterial pathogens associated with the deadly HIV.

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MATERIALS AND METHODS

Plant collection and identification

Fresh leaves, stems and root materials of *T.indica* L were collected from the Herbal Garden of PRIST University, Thanjavur, Tamil Nadu, India. The identity of the plant confirmed with the help of a Botanist. The plant materials were cleaned, shade dried and powdered. Fresh plant materials were washed with tap water, air dried, homogenized to a fine powder and stored in air-tight bottles.

Plant extraction and Phytochemical Screening:

The crude plant extracts were obtained by using Soxhlet apparatus. 2 types of solvents has been used namely ethylacetate and methanol. For the collection of crude extract classical method was performed by using rotatory shaker. Qualitative Chemical tests were carried out on the methanolic extract of the plant using standard procedures to identify the phytoconstituents as described by Trease and Evans(1978) [11] and Sofowara (1993) [12]. Phytochemical screening and Antimicrobial activity were done in Armats Biotech Private Ltd., Chennai.

Bacterial strains

Bacterial strains used in this study were isolated from clinical cases of suspected symptomatic HIV patients namely *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus* and *Salmonella typhi.* All the strains were confirmed by cultural and biochemical characteristics [13].

Antibacterial activity

The antibacterial assay of ethyl acetate and methanolic extracts was performed by agar well diffusion method[14]. 3 types of concentrations were used namely 10mg/10ml, 10mg/1ml and 50mg/1ml of plant extract dissolved in Dimethylsulfoxide. In 10mg/10ml , 3 types of concentrations used such as [for 10-100mg] 10mg/10µl, 50mg/50 µl, 100mg/100 µl. In 10mg/1ml 3 types of concentrations used were [for 100mg/1000mg], 250mg/25 µl, 500mg/50 µl and 1000mg/100 µl. In 50mg/1ml, three types of concentrations used namely [for 1000-2000mg], 1250mg/25 µl, 1500mg/30 µl, 2000mg/40 µl. For each concentration, controls were included that comprised pure solvents instead of the extracts. The plates were then incubated at 37^0 for 24 hours. The diameter of inhibition zones was measured.

Table 1: Isolated Bacteria from HIV positive patients

Bacteria	Percentage (%)	CD_4 Count/µL
Klebsiella pneumoniae	22	300-380
Escherichia coli	16	350-470
Staphylococcus aureus	12	480-530
Pseudomonas aeruginosa	10	500-670
Salmonella typhi	4	300-380
Total	64	

Table 2:Morphology, Staining and Biochemical charcteristics of isolated bacteria

Name of the Organism	Gram staining	Acidfast staining	Morphology	Motility	Biochemical tests Indole	MR	VP	Citrate	Urease	Nitrate reduction test	H_2S Production Test	Carbohydrate fermentation test
K. pneumoniae	_	_	Bacilli	+	_	_	+	+	+	+	_	+(A,G)
E.coli	_	_										
	Bacilli	+	+	+	_	_	_	+	_	+(A,G)		
S. aureus	+	_	Cocci	_	_	+	_	_	+	+	_	+(A)
P. aeruginosa	_	_	Bacilli	+	_	_	_	+	_	+	+	_
S. typhi	_	_	Bacilli	+	_	+	_	+	_	+	_	+(A,G)

K. pneumoniae =Klebsiella pneumoniae; E.coli = Escherichia coli* ; *S.aureus = Staphylococcus aureus; P.aeruginosa=Pseudomonas aeruginosa; S.typhi=Salmonella typhi

Table 3: Antibacterial activity of *Tylophora indica* L Ethyl acetate Root extract

Organisms	Zone of inhibition(mm) Concentration(mg/ml)								
	10mg/10ml(10-100mg)			10mg/10ml(100-1000mg)			10mg/10ml(1000-2000mg)		
	10mg	50mg	100mg	250mg	500mg	1000mg	1250mg	1500mg	2000mg
	10µl	50 µl	100 µl	25 µl	50 µl	100 µl	25 µl	30 µl	40 µl
K. pneumoniae	R	17	18	14	15	16	13	14	16
E.coli	R	13	14	17	12	14	12	13	14
S.aureus	R	11	12	11	12	14	11	12	13
P.aeruginosa	R	13	14	11	12	13	12	13	14
S.typhi	R	13	15	11	12	13	11	12	13

R-Resistant.

Table: 4. **Antibacterial activity of *Tylophora indica* L Ethyl acetate Leaf extract**

Organisms	Zone of inhibition (mm) Concentration(mg/ml)								
	10mg/10ml(10-100mg)			10mg/1ml(100-1000mg)			50mg/1ml (1000-2000mg)		
	10mg	50mg	100mg	250mg	500mg	1000mg	1250mg	1500mg	2000mg
	10µl	50 µl	100 µl	25 µl	50 µl	100 µl	25 µl	30 µl	40 µl
K.pneumoniae	R	15	16	14	16	17	16	18	19
E. coli	R	16	17	11	12	13	14	16	17
S.aureus	13	14	15	13	16	19	15	16	18
P.aeruginosa	14	16	18	14	17	18	14	15	17
S.typhi	13	15	16	12	14	15	13	15	16

R-Resistant.

Thin layer chromatography(TLC)

TLC is used to separate the compound present in the crude plant extract. Here the solvent used were 5% and 10% methanol in chloroform. 1mg/ml concentration of the crude stem extract was spotted on the TLC plates and dried. It was then run with both ultraviolet and iodine chamber. The Retention Factor(RF) value was calculated by using the following formula

RF= Distance travelled by solute/Distance traveled by solvent. The compounds from the spots were scrabbed and used for further screening.

Bioautography:

Bioautography is a rapid aid in the bioassay guided isolation and fractionation of antibacterial compounds and fractions. In this approach, the activity of plant extract against bacteria is determined on chromatograms in accordance with the bioautography procedure [15].

RESULTS AND DISCUSSION:

Isolated bacterial pathogens and their identification:

Table 1 showed the isolated bacteria with CD4 counts. Five major bacterial pathogens were known to be isolated from the samples. *Klebsiella pneumoniae*(22%) showed highest incidence followed by, *Escherihcia coli*(16%), *Staphylococcus aureus*(12%) *Pseudomonas aeruginosa*(10%) and *Salmonella typhi*(4%) known to be found among the HIV patients. Table 2 showed the morphology, staining and biochemical characteristics of the isolated bacterial pathogens. Infectious diseases are the leading cause of death across the world. As a global concern the antibiotic resistance by pathogens has emerged. Many of the antibiotics have been out of use as multidrug resistant pathogens have emerged [16].

Antibacterial activity of *Tylophora indica* L:

Table 3 represented the Results for ethyl acetate root extract of *T.indica*. For 10-100mg, highest zone of inhibition 18mm was noted in the concentration of 100µl for *K.pneumoniae*. 10 µl of 10-100mg showed resistant to all bacteria. For 100-1000mg, highest zone about 17mm was observed in 10µl concentration for *E.coli* followed by 16mm for 100µl concentration for the *K.pneumoniae*. For 1000-2000mg, maximum zone about 16mm was noted in 40µl concentration *S.aureus.* Other concentrations showed moderate inhibition to all isolated organisms. Natural products, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Table 4 represented Results for methanolic leaf extract of *T.indica*. For 10-100mg, highest zone of inhibition 18mm was noted in the concentration of 100µl for *P.aeruginosa*, followed by 17mm in 100µl concentration for *E.coli*., 10 µl concentration showed resistant to *K.pneumoniae* and *E.coli*. For 100-1000mg, highest zone about 19mm was observed in 100µl concentration for *S.aureus* followed by 18mm for 100µl concentration *P.aeruginosa.* For 1000-2000mg, maximum zone 19mm was noted in 40µl concentration for *K.pneumoniae* followed by 18mm in the same concentration for S.aureus and 30µl concentration for *K.pneumoniae*. Other concentrations showed moderate inhibition to all isolated organisms. Table 5 represented Results for methanolic root extract of *T.indica*. For 10-100mg, highest zone of inhibition 16mm was noted in the concentration of 100µl for S.*typhi* followed by 14mm in 100µl concentration for *P.aeruginosa* and *K.pneumoniae.* 10µl concentration showed resistant to *E.coli*, *S.aureus* and *P.aeruginosa*. For 100-1000mg, highest zone about 15mm was observed in 100µl concentration for *S.typhi.* all con-

Table: 5. Antibacterial activity of *Tylophora indica* L Methanolic Root extract

Organisms	Zone of inhibition (mm) Concentration(mg/ml)								
	10mg/10ml(10-100mg)			10mg/1ml(100-1000mg)			50mg/1ml(1000-2000mg)		
	10mg 10µl	50mg 50 µl	100mg 100 µl	250mg 25 µl	500mg 50 µl	1000mg 100 µl	1250mg 25 µl	1500mg 30 µl	2000mg 40 µl
K. pneumoniae	12	13	14	11	12	13	12	13	14
E. coli	R	11	12	12	13	14	12	13	14
S.aureus	R	11	12	12	13	14	11	12	13
P.aeruginosa	R	13	14	11	12	13	11	13	14
S. typhi	11	13	16	13	14	15	13	14	15

R-Resistant.

Table: 6. Antibacterial activity of *Tylophora indica* L Methanolic Leaf extract

Organisms	Zone of inhibition(mm) Concentration(mg/ml)								
	10mg/10ml(10-100mg)			10mg/1ml(100-1000mg)			50mg/1ml(1000-2000mg)		
	10mg 10µl	50mg 50 µl	100mg 100 µl	250mg 25 µl	500mg 50 µl	1000mg 100 µl	1250mg 25 µl	1500mg 30 µl	2000mg 40 µl
P.aeruginosa	12	13	17	12	24	28	13	14	15
K.pneumoniae	11	13	15	22	24	25	13	14	16
E.coli	R	12	13	13	18	20	13	13	15
S. aureus	12	13	14	R	16	18	13	14	15
S. typhi	11	12	15	11	13	14	12	14	15

R-Resistant.

Table 7:Phytochemical Screening of the Stem Methanol extract of *Tylophora indica* L

S.No.	Phytochemical analysed	Result	Observation
1.	Saponins	+	Appearance of foam
2.	Tannins	+	blue black color
3.	Test for Phenolic compounds		
	i) Ferric chloride test	+	No color change.
	ii) Gelatin test	-	No precipitate.
	iii) Lead acetate test	-	No precipitate.
4.	Carbohydrate tests Molish's test	-	Violet ring.
5.	Anthraquinones	-	No color change.
6.	Alkaloids	+	White creamy precipitate.
7.	Protein and aminoacids		
	i) Millon's test	-	No precipitate.
	ii) Biuret test	-	No color change.
8.	Flavonoids Alkaline reagent test	+	Appearance of yellow color
9.	Glycosides Borntragers test	-	No color change.
10.	Fixed oils and fats Saponification	+	No color change.

+ —— Positive *- —— Negative*

centrations of 100-1000mg showed resistance to *S.typhi.* For 1000-2000mg, maximum zone 15mm was noted in 40µl concentration for *S.typhi.* Other concentrations showed moderate inhibition to all isolated organisms. Table 6 represented Results for methanolic leaf extract of *T.indica*. For 10-100mg, highest zone of inhibition 17mm was noted in the concentration of 100µl for *P.aerginosa*, followed by 15mm in 100µl concentration for *K.pneumoniae* and *S.typhi.* For 100-1000mg, highest zone about 28mm was observed in 100µl concentration for *P.aeruginosa* followed by 25mm in 100µl concentration for the same spp. For 1000-2000mg, maximum zone 16mm was noted in 40µl concentration for *K.pneumoniae*. Other concentrations showed moderate inhibition to all isolated organisms. Methanolic leaf extract of *Solanum trilobatum* showed highest inhibition rate when compared with all treatments. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and reemerging infectious diseases. Therefore, much attention has been given to traditional medicine in order to look for new leads to develop better drugs to treat resistant bacteria [17].

TLC:

RF value of the highest antimicrobial activity showed stem methanol extract of *Solanum trilobatum* L was calculated by the TLC method as 0.9.

Phytochemical screening of *Tylophora indica* L

Table 7 showed the phytochemical screening results for *T.indica*. The plant showed positive results for Saponins, Tannins, Alkaloids, Flavanoids, Phenolic compounds, fixed oils and fats.

Administered orally, the antibacterial compounds of *T.indica* may be able to control wide range of microorganisms. The growth of many fungi, yeasts, bacteria, and viruses were inhibited by the phytochemical constituents isolated from *T.indica* [15]. They have been reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects [16].

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