Srivani Ramesh. *et al*. / *International Journal of Phytopharmacology.* 3(2), 2012, 139-146.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

SCREENING OF ANTIBACTERIAL PROPERTIES OF INDIAN MEDICINAL PLANTS AGAINST MULTI DRUG RESISTANT DIABETIC FOOT ULCER ISOLATES

Arumugam Suresh[a], Gopal Muthu[a], Gopalakrishnan Suresh[b], Ravikanth Premnath[c], Prakasam Gopinath[a], Anand Moses[d], Srivani Ramesh*[a]

[a]Department of Microbiology, Dr. ALM PG IBMS, University of Madras, Taramani, Chennai, India.
[b]Department of Biotechnology, Guindy Campus, University of Madras, Chennai, India.
[c]Department of Biotechnology, Anna University, Trichy, India.
[d]Department of Diabetology, KMCR&H, Chennai, India.

ABSTRACT

Fifteen percent of all diabetic patients develop a foot ulcer at some point in their lives, which is highly susceptible to infection. Diabetic foot infection is polymicrobial in nature; most of the bacteria have multidrug resistance. Thus, there is a need for new treatment strategies and recently much attention have been paid to extracts and biologically active compounds isolated from plant species. Crude extract from five different medicinal plants (*Ficus racemosa, Gymnema slyvestre, Cinnamomum zeylancium, Terminalia chebula* and *Momordica charantia)* were studied for their antibacterial activity against the multidrug resistant isolates of diabetic foot ulcer along with controls by agar dilution method. Partial purification was carried out for effective antimicrobial active plant. Among the plant extracts, highest antibacterial activity were expressed by *T. chebula*, followed by *F. racemosa, G. slyvestre, C. zeylancium* and *M. charantia* and the minimum inhibitory concentration of *T. chebula* against the DFU isolates were between as 0.5-4mg/ml. Partial purification of *T. chebula* was carried out by chromatographic techniques. Gallic acid was found to be the principle compound for antibacterial activity determined by HPLC. Development of newer formulation from *T. chebula* could be considered as an alternative therapeutic strategy for the management of diabetic foot infections.

Keywords:- *Terminalia chebula*, Gallic acid, Medicinal plant, Antibacterial activity, Plant extract, Diabetic foot ulcer.

INTRODUCTION

Diabetes and foot problems are almost synchronous. The trio of problem leading on to the diabetic foot is neuropathy, vascular changes and infections, which constitute the diabetic foot syndrome. Infection complicates the pathological picture of diabetic foot and plays a main role in the development of moist gangrene. The infection leads to the early development of complication even after a trivial trauma, the disease progress and becomes refractory to antibacterial therapy (Viswanathan *et al*., 2002).

DFU is polymicrobial in nature. Many studies have reported on the bacteriology of DFU over 25 years, but with varied results from different places. Earlier studies (Lipsky *et al*., 1990; Dang *et al*., 2003; Lipsky *et al*., 2004) have found *Staphylococcus aureus* as the main causative pathogen but recent investigation reported a predominance of gram negative aerobes (Gadepalli *et al*., 2007; Shakil and Khan, 2010).

The development of multi drug resistance is mainly owing to the indiscriminate use of antibiotics for the treatment of infectious diseases (Davis, 1994; Service,

Corresponding Author

Dr. Srivani Ramesh
E.Mail id: dr.srmicro@gmail.com

1995). In addition to this problem, antibiotics are also associated with adverse effects on the host including hypersensitivity, and immune-suppression (Ahmad *et al*., 1998). This necessitates the evaluation of newer therapeutic strategies for treatment of infections. Therefore, we evaluated five Indian medicinal plants for their antibacterial activity.

Several studies were conducted on antimicrobial activity of plant extracts worldwide. (De Boer *et al*., 2005; Nair *et al*., 2005). Much attention has been paid to extracts and biologically active compounds isolated from plant species used in Siddha, Ayurvedic medicines against most of the infections and other conditions (Essawi *et al*., 2000).

In India, thousands of species of plants were known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. Herbal medicine is one of the main therapeutics followed in the in developing countries, because of feasibility, acceptability with minimal side effects and traditional value. Notably, the usages of plant extracts for the treatment of microbial infections were recently implemented in developed countries too (Parekh *et al*., 2005). With this background, the present study was planned to isolate and characterize microbial pathogens from diabetic foot infection and to study their current trends of antibiotic resistance and five different medicinal plants were evaluated for their antibacterial properties against isolates from DFU.

MATERIALS AND METHODS

Plant material

The plants were collected from Indian Medicinal Practitioner Co-Operative Pharmacy and Stories Ltd., (IMCOPS) Thiruvanmiyur, Chennai. The Taxonomic identities of these plants were confirmed with Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai. The Ethno botanical information of the screened plants was given in Table-1.

Table 1. Ethno botanical information of screened plants

Plant name	Family	Common Name	Parts used
Ficus racemosa	Moraceae	Indian fig tree	Bark
Cinnamomum zeylancium	Lauraceae	Cinnamom	Bark
Terminalia chebula	Combretaceae	Kadukai	Fruits
Momordica charantia	Cucurbitaceae	Bitter melon	Fruits
Gymnema sylvestre	Asclepiadaceae	Sirukunjan	Whole Plants

Preparation of Extract

The plant materials washed under running tap water, shade dried and then homogenized to fine powder and stored in airtight bottles. Fifty grams of dried powder mixed with 100ml of Methanol, Isopropanol, Chloroform, Diethyl Ether and Hexane (Qualigens, India.) kept on rotary shaker at 190-220 rpm for 24hours. After overnight incubation, the supernatant was filter through Whatman No.1 filter paper and the filtrate has dried to evaporate the organic solvent at room temperature. The sediment extract was weighed and dissolved in 10% Dimethyl Sulfoxide (DMSO). It was stored at 4°C in airtight bottles for further studies (Agaoglu *et al.*, 2007)

Twenty-five grams of dried powder was mixed 200 ml of double distilled water containing 0.5N NaOH and kept at 80ºC for 3 hours. Whatman No.1 filter paper was used for filtration and filtrate is dried, weighed and dissolved with 10 % DMSO. It was stored at 4°C in airtight bottles for further studies (Wafaa *et al*., 2007).

Evaluation of extracts for antibacterial activities

Agar well dilution method

The antibacterial activities of the extracts were determined using the well diffusion method. The bacteria used in the study were multi-drug resistant DFU isolates. The isolates consisted five strains of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*, with appropriate Control strain (*Staphylococcus aureus* ATCC 5923, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603 and *Enterococcus fecalis* ATCC 25922).

The inoculum size of the test strain standardized according to the Clinical and Laboratory Standards Institute (CLSI, 2005) guidelines. Susceptibility tests were performed by a modified agar-well diffusion method. One ml volume of the standard suspension of test bacterial strain was spread evenly on Mueller Hinton Agar plate using a sterile cotton swab and the plates were allowed to dry at room temperature. Subsequently 6-mm diameter wells were bored in the agar and a 20µl volume of each extract was transferred into wells. After holding the plates at room temperature for 2 h to allow diffusion of the extract into the agar, the plates were incubated at 37°C for 24hours. Inhibition Zone Diameter was measured to the nearest millimeter (mm). Vancomycin (1µg/ml) was used as experimental positive control and 10% DMSO, 0.5N NaOH were used as negative control (Chattopadhyay *et al*., 2007).

Microbroth dilution method

Minimal inhibitory concentration (MIC) determination was done by micro broth dilution method. In brief, 0.1g/ml concentration of plant extracts was prepared. 136µL of Muller Hinton Broth (MHB) with

64µl of respective plant extract suspension were added in the first and 100µl of Muller Hinton Broth in the rest of the well was dispensed in the microtitre plate. In addition, 100µl of mixture first well serially diluted up to the last well except control to get a concentration of 64-0.25mg/ml. Organism and broth controls were maintained, the test organisms were adjusted to 0.5 McFarland standards and 10µl was inoculated into all the wells and incubated at 37˚C for 24hours. MIC/MBC was determined based on the growth observed in the lowest dilution of the well (Kannan *et al*., 2009).

Identification of antibacterial compound

Thin layer chromatography (TLC)

The concentrated extracts *T. chebula* were subjected to TLC analysis to observe the fraction present in the extract according to the procedure of Irouegbu and Nkere, 2005.

Column chromatography of selected plant extracts

The column chromatographies of the selected extracts were performed using the procedure described by Irouegbu and Nkere, 2005. The column separation was carried out with a glass column of internal diameter 40mm and length of 1.0m. A 250g of Silica Gel with a particle size 0.200mm was wet packed using the Methanol/Chloroform (2:3) solvent system determined from Thin Layer Chromatography. After settling, the methanolic extracts of selected plants were gradually poured on the column. The extract was allowed to drain completely into the gel before the eluent Methanol/Chloroform (2:3) was continuously used to elute the stationary phase. The eluent was collected as different fractions in 2-minute intervals. Each fraction was further assessed for antimicrobial activity using agar well diffusion method.

Identification of Antimicrobial compound by HPLC

The equipment consisted of a Hewlett-Packard HP 1100 Series HPLC equipped with auto sampler; Injection volume is 10µl, and a Varian model DAD detector. Chromatogram was carried out on a MetaChem PolarisTM Amide C18 column, 5µm, 4.6 x 250 mm. Mobile Phase is 0.1% phosphoric acid:acetonitrile (85:15) gradient at a flow rate of 1ml/min. Calibration curves for the gallic acid was obtained from standard solutions containing concentration was 7.5mg/100ml Calibrations were determined at 280 nm, and analyses were conducted at 35°C **(**Yang *et al*., 1993**).**

RESULT

Agar well diffusion method

The extracts of the medicinal plants tested were found to have effective antibacterial activity against a group of microorganisms that were isolated from diabetic foot infection. The aqueous extract (28mm) of *T. chebula* shows the highest activity against control and DFU isolates of *S. aureus* followed by methanol and Isopropanol extract (25 and 25mm respectively), less activity was observed for other extracts of *T. chebula.*

For *P. aeruginosa* the highest activity was observed in methanol (27mm) and isopropanol (27mm) extract of *T. chebula* followed by hexane extract (26mm) and other extract does not show any significant activity. Aqueous (32mm) extract followed by methanol (26mm) and isopropanol (25mm) showed the highest activity for *E. faecalis* ATCC 25922 and DFU isolates. Methanol and isopropanol showed the highest activity for *K. pneumoniae* ATCC 700603 and DFU isolates, less activity observed for other extracts. This result was shown in Table-2.

Ficus racemosa extracts showed antibacterial activity against standard strains and clinical isolates of DFU but it showed lesser activity when compared to activity of *T. chebula*. The zone of inhibition of various extracts of *Ficus racemosa* for Diabetic foot ulcer isolates is as follows: methanol (21 mm) and aqueous (19mm) for *P. aeruginosa*; methanol (21mm) for *S. aureus*; methanol (20mm), aqueous (20mm) and isopropanol (19mm) for *Enterococcus faecalis*; isopropanol (21mm), methanol (20mm) and aqueous (20mm) for *Klebsiella pneumoniae.* Less activity was observed for other extracts of the plants *M. charantia*, *Gymnema sylvestre* and *Cinnamomum zeylancium* for the Diabetic foot ulcer isolates except for the Control strains (Table-2).

Micro broth dilution method

Results of MIC and Minimum Bactericidal Concentration (MBC) were given in the Table-3. The MIC values of methanol and isopropanol extracts of *T. chebula* were found to be 2mg/ml, against both *standard strain and S. aureus. P. aeruginosa* ATCC 27853 (0.5mg/ml)*; P. aeruginosa* (1mg/ml), *K. pneumoniae* ATCC 70060 (2mg/ml); *K. pneumoniae* (4mg/ml). The MIC value of Hexane extract of *T. chebula* was observed to be 2mg/ml against both *P. aeruginosa* ATCC 27853 and DFU isolates; 2mg/ml against *E. faecalis* ATCC 25922 and 4mg/ml against *E. faecalis* DFU isolates. The MIC value of aqueous extract of *T. chebula* was observed to be 2mg/mL for *E. faecalis* ATCC 25922 and 4mg/ml for *E. faecalis* DFU isolates.

The MBC value of Methanol, Hexane, Aqueous and Isopropanol extracts of *T. chebula* were same compared with MIC dilution (Table-3).

Identification of antibacterial compound

Thin layer chromatography

TLC performed to determine the best solvent for the column chromatography. The methanolic extracts of *T. chebula* was separated by TLC on Silica Gel G using

different solvent systems; Methanol alone, Methanol/Chloroform (2:3), Methanol/Acetic Acid (1:1), n-Hexane/Methanol/Acetone (1:1:1) and Methanol/Acetone (5:1). After each separation, the TLC plates exposed to iodine vapor in an iodine chamber to visualize the spots. Methanol/Chloroform (2:3) solvent system, which yield 4 bands to elute compound from TLC.

Column chromatography of selected plant extracts

The eluents collected as different fractions in 30 minutes intervals. Eight fractions further assessed for antimicrobial activity using agar diffusion method. The concentration of each fraction adjusted to 10 mg/ml before assaying antimicrobial activity against a set of microbial strains. The antimicrobial activity observed between fractions 2-5 against standard strains shown in Table-4.

Identification of Antimicrobial compound by HPLC

In column chromatography, the antimicrobial activity observed only in the fraction F4 among F1-F8 fractions. Fractions F2-F5 pooled and subjected to HPLC, it exhibited peak on the chromatogram. It was compared with standard Gallic acid by retention time with it was identified as a gallic acid. It was shown in Fig. 1. Total amount of Gallic acid present in the Methanol extract of *T. chebula* is 2.07%

The eluted gallic acid tested against control strain by MIC showed in Table-5, the micro-broth dilution method was done. Dilution range of 1 to >512 µg/ml for gallic acid separated from column was used. According to micro-broth dilution method, 64 µg/ml for *S. aureus* ATCC 25923 and *E. faecalis* ATCC 25922, 256 µg/ml for *P. aeruginosa* ATCC 27853 and 128 µg/ml for *K. pneumoniae* ATCC 700603.

Table 3. MIC and MBC of *T. chebula* extracts on DFU isolates

Bacterial isolates	***Terminalia chebula* MIC and MBC in mg/ ml**							
	Methanol		**Isopropanol**		**Aqueous**		**Hexane**	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus ATCC 25923	2	2	2	2	NA	NA	NA	NA
P. aeruginosa ATCC 27853	0.5	0.5	1	1	NA	NA	2	4
K. pneumoniaee ATCC 700603	2	4	4	4	NA	NA	NA	NA
E. faecalis ATCC 25922	NA	NA	NA	NA	2	2	2	2
Five Multidrug resistant Diabetic Foot Ulcer Isolates								
S. aureus	2	2	2	4	NA	NA	NA	NA
P. aeruginosa	1	1	1	1	NA	NA	2	4
K. pneunmoniae	4	4	4	4	NA	NA	NA	NA
E. faecalis	NA	NA	NA	NA	4	4	4	4

** NA- *Not applicable*

Table 4. Screening all the chromatographic fractions for anti-microbial activity (methanol (2): chloroform (3), fraction time 10min.)

Bacterial isolates	*T. chebula* Zone of inhibition (mm)								
	F1	F2	F3	F4	F4	F5	F6	F7	F8
S. aureus ATCC 25923	-	-	10	18	7	-	-	--	--
P. aeruginosa ATCC 27853	-	-	8	19	8	-	-	-	-
K. pneumoniaee ATCC 700603	-	8	10	19	10	6	-	-	-
E. faecalis ATCC 25922	-	-	12	22	10	6	-	-	-
"F"-fraction									

Table 5. MIC and MBC of *T. chebula* extracted compound gallic acid against control strain

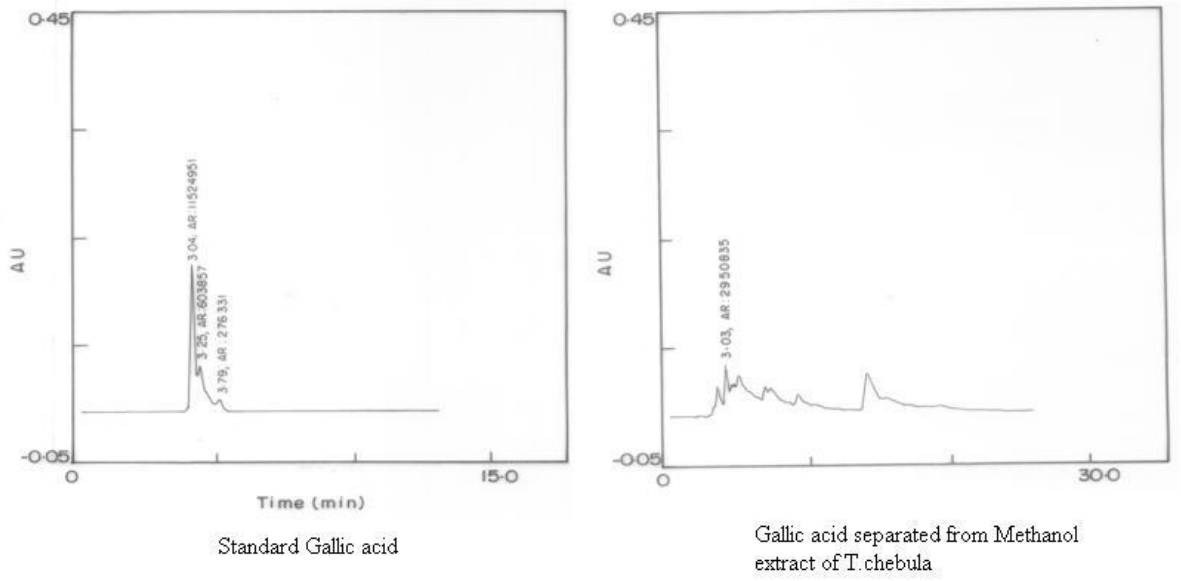
Bacterial isolates	Gallic acid(µg/ml)	
	MIC	MBC
S. aureus ATCC 25923	64	64
P. aeruginosa ATCC 27853	256	256
K. pneumoniaee ATCC 700603	128	128
E. faecalis ATCC 25922	64	64

Table 2. Antibacterial screening of Medicinal plants

Multidrug resistant bacterial pathogens	Zone of Inhibition (mm)																											
	Terminalia chebula						*Momordica charantia*						*Cinnamomum zeylancium*						*Ficus racemosa*						*Gymnema slyvestre*					
	M	I	D	C	H	A	M	I	D	C	H	A	M	I	D	C	H	A	M	I	D	C	H	A	M	I	D	C	H	A
***P. aeruginosa* ATCC 27853**	28	28	17	0	27	23	14	18	14	12	8	10	10	7	15	10	0	0	21	17	13	9	10	19	16	13	11	12	0	10
P. aeruginosa 1	27	26	15	0	26	22	0	9	0	0	0	0	10	7	15	10	0	0	18	20	15	10	10	17	0	0	0	0	0	0
P. aeruginosa 2	27	26	16	0	26	22	8	7	8	0	8	6	10	6	14	9	0	0	11	12	8	8	9	10	0	0	0	0	0	0
P. aeruginosa 3	26	27	15	0	26	21	7	6	8	3	8	6	10	6	14	8	0	0	18	20	14	8	10	17	0	0	0	0	0	0
P. aeruginosa 4	27	27	15	0	26	22	7	6	0	0	8	6	10	6	14	9	0	0	18	20	14	9	10	17	0	0	0	0	0	0
P. aeruginosa 5	26	27	15	0	26	22	3	0	8	0	0	0	10	6	14	8	0	0	18	20	14	8	10	17	0	0	0	0	0	0
***S. aureus* ATCC 25923**	25	25	15	20	20	28	25	25	18	0	0	20	10	0	0	0	0	11	21	17	13	17	10	16	10	10	10	10	0	0
S. aureus 1	25	25	15	18	0	28	0	0	0	0	0	0	10	0	0	0	0	0	16	18	17	16	10	0	0	0	0	0	0	0
S. aureus 2	24	25	14	18	0	28	0	0	0	0	0	0	10	0	0	0	0	0	16	18	16	14	10	0	0	0	0	0	0	0
S. aureus 3	25	24	15	20	0	27	0	0	0	0	0	0	10	0	0	0	0	0	10	10	15	15	10	0	0	0	0	0	0	0
S. aureus 4	25	25	15	20	0	28	0	0	0	0	0	0	10	0	0	0	0	0	16	18	10	15	10	0	0	0	0	0	0	0
S. aureus 5	25	25	15	20	0	28	0	0	0	0	0	0	10	0	0	0	0	0	16	18	20	16	10	0	0	0	0	0	0	0
***E. faecalis* ATCC 25922**	28	20	17	0	22	34	11	12	0	14	0	11	12	0	0	0	0	14	20	19	10	0	0	20	12	12	14	0	0	12
E. faecalis 1	26	20	15	0	20	32	11	12	0	14	0	11	11	0	0	0	0	12	20	19	10	0	0	20	0	0	10	0	0	0
E. faecalis 2	25	20	14	0	19	30	11	12	0	14	0	11	11	0	0	0	0	12	20	19	10	0	0	20	0	0	10	0	0	0
E. faecalis 3	26	20	15	0	18	31	11	12	0	14	0	11	11	0	0	0	0	12	20	19	10	0	0	20	0	0	10	0	0	0
E. faecalis 4	25	20	15	0	19	30	11	12	0	14	0	11	11	0	0	0	0	12	20	19	10	0	0	20	0	0	10	0	0	0
E. faecalis 5	26	20	14	0	20	31	11	12	0	14	0	11	11	0	0	0	0	12	20	19	10	0	0	20	0	0	10	0	0	0
***K. pneumoniae* ATCC 700603**	27	25	17	15	25	16	0	0	10	0	0	0	0	0	10	0	0	12	20	21	10	10	10	20	11	10	0	10	0	10
***K. pneumoniae* 1**	26	24	16	12	25	16	0	0	0	0	0	0	0	0	0	0	0	0	18	18	10	10	10	0	0	0	0	0	0	0
***K. pneumoniae* 2**	20	23	16	13	22	12	0	0	0	0	0	0	0	0	0	0	0	0	18	18	10	10	10	0	0	0	0	0	0	0
***K. pneumoniae* 3**	27	25	17	15	20	13	0	0	0	0	0	0	0	0	0	0	0	0	15	16	8	7	0	0	0	0	0	0	0	0
***K. pneumoniae* 4**	22	16	15	14	15	18	0	0	0	0	0	0	0	0	0	0	0	0	14	13	15	10	10	0	0	0	0	0	0	0
***K. pneumoniae* 5**	22	21	13	12	0	20	0	0	0	0	0	0	0	0	0	0	0	0	18	18	10	10	10	0	0	0	0	0	0	0

M-Methanol, I-Isopropanol, C-Chloroform, D-Diethyl Ether and H-Hexane, shaded no.-showed highest activity of medicinal plant against bacterial pathogen

Figure 1. Identification of gallic acid with standard gallic acid by HPLC



Standard Gallic acid

Gallic acid separated from Methanol extract of T.chebula

DISCUSSION

The crude extract from 5 different medicinal plants *Ficus racemosa, Gymnema sylvestre, Cinnamomum zeylancium, Terminalia chebula* and *Momordica charantia* were studied for their antibacterial activity. The extracts (Methanol, Chloroform, Isopropanol, Aqueous and hexane) of the medicinal plants were made by standard procedure, tested against multi drug resistant microorganisms that were isolated from DFU. Not all the solvents exhibited activity against the bacterial isolates only the following extract showed. Aqueous (32mm) extract of *T. chebula* followed by methanol (26mm) and isopropanol (25mm) showed the highest activity for *E. faecalis* ATCC 25922 and DFU isolates of *E. faecalis*. The highest activities were observed in methanol (27mm) and isopropanol (27mm) extracts against *P. aeruginosa*. Methanol and isopropanol extracts showed 25 and 25mm respectively against *S. aureus*.

This was similar to an earlier study by Kannan *et al*., 2009 in which alcoholic extract of *T. chebula* exhibited greater activity than the aqueous and hexane extracts against gram positive and gram-negative bacteria. Two possibilities that may account for the higher antibacterial activity, the nature of biological active components (Alkaloids, Flavonoids, Essential oil, Tarpenoids, Tannins, etc.) and the stronger extraction capacity of ethanol that may have yielded a greater number of active constituents responsible for antibacterial activity.

F. racemosa extracts showed antibacterial activity against standard strains and clinical isolates of DFU but it showed lesser activity when compared to *T. chebula*. The zone of inhibition of various extracts of *F. racemosa* for Diabetic foot ulcer isolates is as follows: methanol and aqueous showed 21 mm, 19mm respectively for *P. aeruginosa*; methanol (21mm) for *S. aureus*; methanol (20mm), aqueous (20mm) and isopropanol (19mm) for *E. faecalis*; isopropanol (21mm), methanol (20mm) and aqueous (20mm) for *K. pneumonia.*

F. microcarpa and *F. cordata,* having antibacterial activity, also they characterized the active phenolic compound. Mousa *et al*., 1994 reported that antibacterial activity of *F. racemosa*. Less activity was observed for other extracts of the plants *M. charantia, G. sylvestre* and *C. zeylancium* for the diabetic foot ulcer isolates compared with control strains.

MIC was done to *T. chebula* extracts, we have found the MIC value of 2mg/ml for methanol extracts of *T. chebula* against standard stains, and DFU isolates of *S. aureus* and *K. pneumoniae* ATCC 70060. *P. aeruginosa* ATCC 27853 showed 0.5mg/ml, *P. aeruginosa* were 1mg/ml, and 4mg/ml for *K. pneumoniae*. The MIC value of hexane extract of *T. chebula* was observed to be 2mg/ml against both *P. aeruginosa* ATCC 27853 and DFU isolates of *P. aeruginosa*; 2mg/ml against *E. faecalis* ATCC 25922 and 4mg/ml against DFU isolates *of E. faecalis*. The MIC value of aqueous extract of *T. chebula* was observed to be 2mg/ml for *E. faecalis* ATCC 25922 and 4mg/ml for *E. faecalis*.

The MIC value of our methanol, hexane, aqueous and isopropanol extracts of *T. chebula* were compared with Acharyya *et al*., 2009, they have also shown 0.5mg-2.0mg/ml MIC for bacterial pathogen, similar to that of our study. This antibacterial activity study also revealed the stronger extraction capacity of solvents like ethanol, methanol. These compounds were

shown to contain phenolics and flavonoids, which possesses antimicrobial activity (Kannan *et al*., 2009). Grosvenor *et al*., 1995 has shown that the basis of the differences in susceptibility might be due to the differences in the cell wall composition of gram positive and negative bacteria that the drug-resistant strains of bacteria sensitive to the tested plant extracts. And has clearly indicated that antibiotic resistance does not interfere with the antimicrobial action of plant extracts and these extracts might have different modes of action on test organisms.

Five plants in this study were also screened previously against other test strains by Ahmad *et al*., 1998; the present study also showed similar results with varying degrees of potency. Nimri *et al*., 1999 has showed that the difference in potency may be due to the stage of collection of the plant sample, different sensitivity of the test strains and method of extraction.

The presence of various phyto-constituents was detected by phytochemical investigation. The tests performed on methanolic extracts of *T. chebula* for the results of phytochemical analysis showed the presence of constituents such as Alkaloids, carbohydrates, tannins, and phenolic compounds, flavonoids, saponins, proteins and amino acids, and steroids. Tannic acid represents the major constituent of the fruit of *T. chebula* (Naik *et al*., 2004). Some studies reported that tannic acid is bacteriostatic or bactericidal to some Gram positive and negative pathogens. Gallic acid is one of the chemical that had antimicrobial properties (Panizzi *et al*., 2002) and in some as gallate esters. The antibacterial activity of methanolic extract of *T. chebula* and the results of TLC are agreement with the results of Sato *et al*., 1997, Ahmad *et al*., 1998, and Bonjar, 2004.

To locate the major active constituents responsible for antibacterial activity against bacterial pathogens, TLC-bioautography was performed from *T. chebula* plant extracts. Methanolic extracts of *T. chebula* were fractionated for the active compounds. Fractions were pooled and analyzed by HPLC and found to be gallic acid and are confirmed with standard gallic acid. HPLC fingerprint analysis of medicinal plant is an efficient measurement for identifying and assessing the stability of the crude extracts. The HPLC analysis of the *T. chebula* extracts showed prominent peaks with retention time of 3.03 minutes. Peak has been identified to be gallic acid using standard solutions under similar condition. The retention time for gallic acid in standard solution was also found to be 3.03 min. HPLC analysis of *T. chebula* extract was also performed by Naik *et al*., 2003; they recorded retention times of 3.76 min, and identified them as gallic acid. Saleem *et al*., 2002, also have documented the medicinal property of *T. chebula* Retz.

The beneficial effects of *T. chebula* extracts on the wound healing process were shown by a study conducted by Suguna *et al*., 2002. They have topically administrated the alcoholic extract of leaves of *T. chebula* on dermal wounds of rats. The treated wounds showed faster healing due to improved rates of contraction and a decreased period of epithelisation. The total protein DNA and collagen content were shown to be increased in granulation tissue. They showed antibacterial activity of *T. chebula* extracts against *S. aureus* and *Klebsiella spp.*

CONCLUSION

A new drug formulation program me should be undertaken to develop modern drugs with the compounds isolated from *T. chebula.* Extensive investigation needed to study their mechanism of action, pharmaco therapeutics, toxicity with proper standardization and clinical trials. As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, development of modern drugs from *T. chebula* could be considered for the management of Diabetic foot infections.

ACKNOWLEDGMENTS

The authors acknowledge the support of University Grand Commission, for the grant of Major Project in 2008-11.

REFERENCES

Acharyya S, Patra A, Bag PK. Evaluation of the antimicrobial activity of some medicinal plants against enteric bacteria with particular reference to multi-drug resistant *Vibrio cholerae*. *Tropical Journal of Pharmaceutical Research*, 8, 2009, 231-237.

Agaoglu S, Dostbil N, Alemdar S. Antimicrobial activity of some spices used in the meat industry. *Bulletin of the Veterinary Institute in Pulawy*, 51, 2007, 53-57.

Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. *Journal of Ethnopharmacology*, 62, 1998, 183-193

Chattopadhyay RR, Bhattacharyya SK, Medda C, Chanda S,Datta S, Pal NK. Antibacterial activity of black myrobalan (Fruit of *Terminalia chebula* Retz.) against uropathogen Escherichia coli. *Pharmacognosy Magazine*, 11, 2007, 212-215.

Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 15th informational supplement. CLSI document M100-S15. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 25, 2005, 1.

Dang CV, Kim JW, Gao P, Yustein J. The interplay between MYC and HIF in cancer. *Nature Reviewes Cancer,* 8, 2008, 51–56.

Davis J. Inactivation of the antibiotics and the dissemination of resistance genes. *Science*, 264, 1994, 375-382.

De Boer HJ, Kool A, Broberg A. Antifungal and antibacterial activity of some herbal remedies from Tanzania. *Journal of Ethnopharmacology*, 96, 2005, 461-469.

Essawi T, Srour M. Screening of some Palestinian medicinal plants for antibacterial activity. *Journal of Ethnopharmacology*, 70, 2000, 343-349.

Gadepalli R, Dhawan B, Sreenivas V, Kapil A, Ammini AC, Chaudhry R. A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. *Diabetes Care,* 29, 2006, 1727–1732.

Grosvenor PW, Gothard PK, William MC, Supriono A, Gray DO. Medicinal plants from Riau province, Sumatra, Indonesia. *Journal of Ethnopharmacology*, 45, 1995, 75-95.

Iroegbu, CU, Nkere CK. Evaluation of the antibacterial properties of *Picralima nitida* stembark extracts. *International Journal of Molecular Medicine and Advance Science*, 1(2), 2005, 182-189.

Kannan P, Ramadevi SR, Hopper W. Antibacterial activity of *Terminalia chebula* fruit extract. *African Journal of Microbiology Research*, 3(4), 2009, 180-184.

Lipsky BA, Berendt AR, Deery HG, Embil JM, Joseph WS, Karchmer AW, Lefrock JL, Lew DP, Mader JT, Norden C, Tan JS. Diagnosis and Treatment of Diabetic foot Infections. *Journal of Guidelines for Diabetic foot Infections*, 39(1), 2004, 885 - 2342.

Lipsky BA, Pecorano RE, Larson SA. Outpatient management of uncomplicated lower extremity infections in diabetic patients. *Archives of Internal Medicine*, 150, 1990, 790-797.

Mousa O, Vuorela P, Kiviranta I, AbdelWahab S. Hiltunen R, Vuorela H, *Journal of Ethnopharmacology*, 41(1-2), 1994, 71-76

Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohoni DP, Biyanic MK, Mohan H. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. *Phytochemistry,* 63, 2003, 97-104.

Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected Indian medicinal flora. *Turkish Journal of Biology*, 29, 2005, 41-47.

Nimri LF, Meqdam MM, Alkofahi A. Antibacterial activity of Jordanian medicinal plants. *Pharmaceutical Biology,* 37(3), 1999, 196-201.

Panizzi L, Caponi C, Catalano S, Cioni PL, Morelli, I. In vitro antimicrobial activity of extracts and isolated constituents of *Rubus ulmifolius*. *Journal of Ethnopharmacology*, 79(2), 2002, 165–168.

Parekh J, Jadeja D, Chanda S. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turk J Biol,* 29, 2005, 203-210.

Saleem A, Sinkkonen J, Ka¨hko¨nen, M, Klika KD, Pihlaja K. The structural and conformational analysis of chebulinic acid and 2, 4-chebuloyl-b-D-glucopyranose isolated from the fruit of *Terminalia chebula* Retz.: Antioxidant activities of *T. chebula* compounds. *European Journal of Organic Chemistry,* 2002.

Service RF, Antibiotics that resist resistance. *Science*, 270, 1995, 724-727.

Shakil S, Khan A. Infected foot ulcers in male and female diabetic patients: a clinico-bioinformative study. *Annals of Clinical Microbiology and Antimicrobials*, 9, 2010, 2.

Suguna L, Surjeet S, Sivakumar P, Padmavathi S, Gowri C. *Phytotherepy Research*, 16, 2002, 227.

Viswanathan V, Jasmine JJ, Snehalatha C, Ramachandran A. Prevalence of pathogens in diabetic foot infection in South Indian type 2 diabetic patients. *Journal of the Association of Physicians of India,* 50, 2002, 1013–6.

Wafaa AH, Hassan A, Nefisa MA. Biological and anti-microbial activities of aqueous extracts from neem tree. *Journal of applied science research* 3(10), 2007, 1050-1055.

Yang CS, Wang ZY. Tea and Cancer. *Journal of the National Cancer Institute*, 85, 1993, 1038-1049.