In Vitro Study of Antimicrobial Activity of Different Plant Parts of *Terminalia Chebula* Against Pathogenic Bacteria

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ABSTRACT

T. chebula is one of the most versatile plants having a wide spectrum of pharmacological and medicinal activities. Although the plant possess a number of pharmacological activities due to the presence of bioactive compounds, very little work has been done on the potential medicinal applications of this plant against the diseases particularly on multidrug resistant bacterial pathogens. The activity of different solvent extracts (Di-Chloromethane , Metahnol, Benzene, Petroleum Ether) of leaves, bark, seed and fruit pulp of T. chebula against Escherichia coli and Staphylococcus aureus was analysed. Pathogenic microbes confirmed that the plant contains bioactive compounds that exhibit measurable antimicrobial activity against these bacteria.Among the solvents used,methanol and benzene extracts of leaves, seed and fruit showed the highest activity in comparison to bark. All plant parts exhibited antimicrobial activity in different solvents against the bacterial strain S. aureus and E.coli.

Key words: *Antimicrobial, Antimicrobial activity, T.chebula, Zone of inhibition.*

1. INTRODUCTION

Medicinal plants have long been exploited as evident by literature on antimicrobial, anti-inflammatory, anti cancerous and anti helmintic properties and many more to be discovered in upcoming times (**Samy *et al.,* 2000**). Secondary metabolites extracted from different plant species have tremendous opportunities in antimicrobial prospects against a broad spectrum of bacterial and fungal strains. These plant based therapeutics are attracting the scientific community over past 20 years for being natural with no adverse side effects and in turn being more cost effective. Moreover they are biodegradable and pose minimum environmental hazards (**Evans *et al.,* 1986**).The ancient knowledge and promising potential of medicinal plants should be taken in account for identification of bioactive compounds for the development of formulations which in turn will help the society and mankind. *Terminalia chebula* Retz.commonly known as harad belongs to family *Combretaceae* found in the forests of Northern India ,Uttar Pradesh, Bengal and very common in existence in Southern part of India. The plant is a medium to large sized tree distributed throughout tropical and subtropical Asia including China. Tribal people in Tamilnadu, Karnatka routinely used harad to cure several ailments like fever, cough diarrhoea, gastroenteritis, skin disease, urinary tract infection (**Dash, 1991**).The antimicrobial activity of this plant has been reported against several bacterial strains (**Malckzadeh et al, 2001; Bag et al., 2009**) using fruit pulp .The plant part (fruit pulp) have been tested against *H.pylori, X.campestris* pv.citri and *S.typhi* The plant fruit pulp also reported to be effective

against a number of dermatophytes and yeasts (**Dutta *et al.*, 1998**). The fruit of the plant possess complex antimicrobial compounds to cure disease like digestive and cardiovascular ailments along with pathogenic bacteria. Our present research compared the potential of different plant parts which include leaves, bark and seed along with fruit pulp to evaluate antimicrobial activity against pathogenic bacteria. So in future the other parts of the plants can be used for selective infection and can further be exploited for active principle detection responsible for antimicrobial property.

2. MATERIAL AND METHOD

2.1 Plant and culture collection

The bark, leaves, seeds and fruit pulp of ***Terminalia chebula*** used in the present study for antimicrobial activity were procured from Botany Department of Kurukshetra University, Kurukshetra, Haryana, India. The human pathogenic microorganisms were procured from Microbial Type Culture Collection (MTCC): Institute of Microbial Technology (IMTECH), Chandigarh; which included Gram-negative bacteria: *Escherichia coli* (MTCC 5704) *and* Gram-positive bacteria: *S. aureus* (MTCC 3160).

2.2 Preparation of extracts of leaves, bark, seed and fruit pulp of *Terminalia chebula*

2.2.1 Preparation of *Terminalia chebula* bark extract

The bark of *Terminalia chebula* were oven dried at 50-65ºC for 4-5 hour and grounded into fine powder. The 20 gm of this powder was soaked in 100 ml of Petroleum ether, methanol, Benzene and Di-chloromethane; and incubated for 72 hr. at room temperature. The extract was filtered with Whatman filter paper. The extra solvent from the filtrate was evaporated by using water bath at 45-50°C. The residual powder after solvent extraction was dissolved in DMSO and stored at 4° C.

2.2.2 Preparation of *Terminalia chebula* leaves extract

The leaves of *Terminalia chebula* were thoroughly washed with water then allowed for oven drying at 50-65ºC for 20-40 min due to its fragile structure and grounded into fine powder. The 20gm of this powder was soaked in 100 ml of Petroleum ether, methanol, Benzene and Di-chloromethane; and incubated for 72 hr. at room temperature. The extracts were filtered with Whatman filter paper. The extra solvent from the filtrate were evaporated by using water bath at 45-50°C. The residual powder after solvent extraction was dissolved in DMSO and stored at 4° C

2.2.3 Preparation of *Terminalia chebula* fruit pulp extract

The fruit of *Terminalia chebula* were thoroughly washed with water then allowed for oven drying at 50-60-ºC for 3-4 hours and grounded into fine powder. The 20 gm of this powder was soaked in 100 ml of Petroleum ether, methanol, Benzene and Di-chloromethane; and incubated for 72 hr. at room temperature. The extracts were filtered with Whatman filter paper. The extra solvent from the filtrate were evaporated by using water bath at 45-50°C. The residual powder after solvent extraction was dissolved in DMSO and stored at 4° C.

2.2.4 Preparation of *Terminalia chebula* seed extract

The seed of *Terminalia chebula* were thoroughly washed with water then allowed for oven drying at 50-60-ºC for 4-5 hours and grounded into fine powder. The 20gm of this powder was soaked in 100 ml of Petroleum ether, methanol, Benzene and Di-chloromethane; and incubated for 72 hr. at room temperature. The extracts were filtered

with Whatman filter paper. The extra solvent from the filtrate were evaporated by using water bath at 45-50°C. The residual powder after solvent extraction was dissolved in DMSO and stored at 4° C.

2.3 Antimicrobial activity of plant extracts by Agar Well Diffusion Assay (Pereze et al., 1990)

The antimicrobial activities of plant extracts were evaluated by agar well diffusion assay (Pereze et al., 1990). The microbial inoculums were inoculated aseptically spread uniformly on surface of pre solidified Mueller Hinton Agar (MHA) plates with the help of sterile glass spreader or sterile cotton swabs. A well of about 6.0 mm diameter was aseptically punctured using a sterile cork borer. The cut agar was carefully removed by the use of sterile forceps. DMSO was used as a negative control. The petriplates were kept in laminar for 30 minutes for pre-diffusion to occur then petriplates were incubated overnight at 37 ° C for 24 hours. The antimicrobial spectrum of extract was determined in terms of zone sizes (inhibition zone diameters) around each well. Zones were measured by high media zone scale.

3. RESULTS AND DISCUSSION

Different solvent extracts prepared using different plant parts were found to possess significant antimicrobial activity against Gram Positive and Gram Negative bacteria compared to standard.

The extracts of different plant parts have been assessed for antimicrobial activity against *E. coli* and *S. aureus*. The antimicrobial activity of different solvent extracts of leaves, bark seed and fruit pulp at three different concentrations (5mg/ml, 2.5mg/ml and 1.25mg/ml) is shown in Table 1 & 2.The agar disc diffusion method was used to evaluate the antimicrobial activity by measuring the inhibition zones against the test microorganism.

Table 1. Inhibition zone diameters (in mm) of different plant extract of *T.chebula* in various solvents and in three dilutions against *E.coli.*

Solvent	Leaves			Bark			Fruit Pulp			Seed		
	D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3
Methanol (A)	20	18	16	23	15	13	17	15	13	18	15	13
Di-Chloromethane (B)	14	15	13	15	13	-	25	20	16	14	13	-
Petroleum Ether (D)	-	-	-	-	14	-	18	15	13	16	-	21
Benzene (E)	14	13	12	-	-	-	**38**	20	16	19	16	12

Table 2. Inhibition zone diameters (in mm) of different plant extract of *T.chebula* in various solvents and in different dilutions against *S.aureus.*

Solvent	Leaves			Bark			Fruit Pulp			Seed		
	D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3
Methanol (A)	25	22	20	23	16	15	23	18	17	18	15	13
Di-Chloromethane (B)	19	18	15	17	14	13	22	17	15	16	12	-
Petroleum Ether (D)	18	13	12	-	-	-	24	18	14	14	-	-
Benzene (E)	19	16	16	14	12	-	**34**	29	20	16	13	-

Methanolic extracts of leaf exhibited the highest antibacterial efficacy against tested pathogen at 5mg/ml concentration resulting in a inhibition zone of 20mm which decrease with decreasing concentration(2.5,1.25 mg/ml) to 16mm taken for MIC assay,while the di- cholroethane and benzene extracts of leaf resulted in comparable zone of inhibition as shown in Fig 1 and 2. As compared the petroleum ether extract did not show any activity against the pathogen studied. **Ghosh et al. (2011)** reported similar findings in five different medicinal plants viz;*T. bellerica*,*T. chebula*, *E. officinalis*, *Punica granatum* and *Lawsonia inermis*.The methanolic leaf extracts of *Terminalia chebula* exhibited higher antimicrobial potential amongst all plant parts used followed by fruit extracts showing effective antimicrobial activity. Similarly methanolic extract of bark resulted in a zone of 23mm and efficacy goes on decreasing with decrease in concentration.

Fig 1. Antimicrobial activity of solvent Di-Chloromethane of *T.chebula* leaves against *E.coli*

Fig 2. Antimicrobial activity of solvent benzene of *T.chebula* leaves against *S.aureus*

Fig 3. Antimicrobial activity of solvent Dichloroethane of *T.chebula* leaves against *E.coli*

Dichloroethane extracts exhibited antimicrobial activity at 5 and 2.5mg/ml ,while petroleum ether and benzene extract of bark did not show any activity against *E. coli* as shown in Fig. 3. Stem bark (2.5 mg/ml) methanolic extract of *Ficus carica* was tested against *B. subtilis*, *S. aureus*, *B. megaterium*, *P. aeruginosa* and *E. coli*. using agar well diffusion assay.The methanolic extract of bark tissue were found to be most effective against all the six micro organisms tested **(Al Yousuf *et al.*, 2012).** Similar findings were reported using leaf in *Convolvulus arvensis* where

the crude and solvent soluble extracts of leaves and stem from Peshawer region showed highest potential as compared to plant collected from other regions against *B. subtilis*, *P.aeruginosa* and *E.coli*. (**Raza *et al.*, 2010**).

The fruit pulp exhibited prominent antibacterial activity in benzene extract with 38mm zone of inhibition at 5mg/ml concentration followed by 25mm in dichloromethane 18mm in petroleum ether which is comparable to 17mm in methanolic extracts as shown in Fig 4, 5 and 6. Similar results were reported in *C.auriculata* **(Samy and Ignachimuthu, 2000)** exhibiting significant antimicrobial activity against *E. coli* and *S.aureus* at a concentration of 6mg/ml which is a higher concentration than present investigation.. The result reported were in constrast to the earlier reults where methanolic extracts have been found to me more effective as compared to other solvents (**Ghosh *et al.*, 2011**). Fruit pulp extract showed a minimum inhibition zone of 13mm in methanolic extracts when taken as MIC assay. **Chauhan *et al.* (2011)** used petroleum ether, chloroform,ethyl acetate and methanol as organic solvents to prepare plant extracts.in *Cassia fistula* against *S. aureus*, *Streptococcus epidermidis*, *E. coli* and *K. pneumonia*.The methanolic extracts from fruit exhibited fair antimicrobial activity against all pathogens tested.

Fig 4. Antimicrobial activity of solvent Di-Chloromethane of *T.chebula* fruit pulp against *E.coli*

Fig 5. Antimicrobial activity of solvent Benzene of *T.chebula* fruit pulp against *E.coli*

Fig 6. Antimicrobial activity of solvent petroleum ether of *T.chebula* fruit pulp against *S.aureus*

The methanol, di-chloromethane and benzene extracts prepared using the leaves, bark, seed and fruit gave better result as compared to petroleum ether. Methanolic extract of seeds resulted in the maximum inhibition zones of 18mm, 15mm and 13mm while the benzene extracts resulted in 19mm, 16mm and 12mm zone of inhibition, at 5, 2.5 and 1.25 mg/ml concentration respectively which clearly indicate that the seeds have tremendous potential to be used against fruit pulp. **Muthukumar *et al.* (2014)** evaluated antimicrobial analysis against two gram positive and two gram negative pathogenic bacterial strains using leaf extracts of *Wedellia caledulacea* Less (*Asteraceae*). They reported the potential of hot and cold aqueous extracts along with ethanolic extracts using disc diffusion assay. As in India most of the decoctions are prepared in water so the finding needs to explore the viability of organic solvents in preparation of extracts but the ethanolic extracts of plant were observed to be more effective than the hot and cold aqueous extracts which depicts the importance of organic solvents to be employed for extract prepations in

antimicrobial analysis. Nair and his co associates in 2005 reported the similar findings in preparing extracts using different solvent extracts.

In comparison to *E.coli* the methanolic extract of leaves against *S. aureus* resulted in zones of 25mm, 22mm and 20mm at three different concentrations as shown in Table-2 which indicates the potential efficacy of leaf tissue in comparison to fruit pulp. The benzene and dichloromethane extracts exhibited zones of 19 at 5mg/ml followed by 16mm,18mm at 2.5mg/ml which in turn followed by 16mm and 15mm at a concentration of 1.25mg/ml.

The methanolic extract of bark was highly efficient against *S.aureus* (23mm) at a concentration of 5mg/ml. The benzene and dichloromethane extract did exhibited zone of inhibition against tested pathogen but using petroleum ether as solvent negligible results observed.

The methanolic extract of fruit pulp showed less efficacy as compared to benzene extracts which resulted prominent antibacterial activity confirmed from a zone of 34mm at 5mg/ml concentration which is comparable to *E.coli*. which is followed by a comparable zone of inhibition using other three solvent systems.

The seeds of plant also showed mild activity against *S.aureus* resulting in zones of 18mm, 15mm and 13mm at three different concentration which is followed by a comparable zone of inhibition of 16mm and 13mm using benzene and dichloroethane as solvent extracts. In comparison the petroleum ether extract exhibited antibacterial activity only at 5mg/ml concentration.

Similar reports have been obtained where the plant fruits have been tested for different ailments. **Kannan *et al.* (2009)** reported the ethanolic extracts of *Terminalia chebula* using fruit extracts to be effective against *S. typhii, S.epidermidis, S.aureus, B.subtilis and P.aeruginosa*. The result indicated the potential of using dry fruit extracts even at 1mg/ml to possess broad spectrum activity .Similarly **Aneja *et al.* (2009)** reported that *Terminala chebula* fruit extracts are effective against five dental caries which in turn were associated with *Streptococcus* spp. Mainly *Streptococcus mutans and Lactobacillus spp*. The bioactive substances from these plants can be employed in the formulation of antimicrobial agents for the treatment of various bacterial infections. The results of present investigation indicate that antibacterial activity varies with plant part and solvent extract concentration. The purification of these phyto constituents and determination of their respective antimicrobial potencies should be the prospect route for examination.

4. CONCLUSION

The present investigation clearly demonstrates the need of the hour to focus on whole plant parts to be investigated for antibacterial analysis. As fruits have been vital source to be consumed the other plant parts like leaves, bark seed, fruit pulp should be taken into consideration for analysis. The study ascertains the material available is in bulk instead of waiting for fruiting season and fruit harvest for development of new drugs. Further the different components of plants could be isolated using HPTLC or preparative HPLC for further analysis and animal model should be investigated for exploration of pharmaceutical field.

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