Extraction, gas chromatography–mass spectrometry analysis and screening of fruits of *Terminalia chebula* Retz. for its antimicrobial potential

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ABSTRACT

Background: *Terminalia chebula* is called the "king of medicines" in Tibet and is always listed first in the Ayurvedic meteria medica because of its extraordinary powers of healing. **Objective:** Identification, isolation and screening of pyrogallol which are responsible for antimicrobial property of fruits of *Terminalia chebula*. **Materials and Methods:** Ethyl acetate fraction of fruits of *Terminalia chebula* was subjected to Gas chromatography–mass spectrometry (GC-MS) for the components present in the extract. **Results:** Sixty four constituents were identified out of which kaempferol-3-O-rutinoside flavonoid and Vitamin E has been detected for the first time in fruits of this plant. Pyrogallol (46.26%) which was the major component of the extract in GC-MS analysis was isolated and screened for antimicrobial activity against selected test pathogens by Disc Diffusion Assay. Crude ethyl acetate fraction of the fruits was showing the same activity potential as was observed for pure pyrogallol which was the major component as per GC-MS analysis. The most sensitive species among the bacteria was *Enterobacter aerogenes* with highest inhibition zone (IZ = 31 mm; AI = 1.409 ± 0.046) even at minimum inhibitory concentration (0.039 mg/ml). **Conclusion:** Hence activity shown by crude ethyl acetate fraction might be due to pyrogallol present in the extract. On the basis of results it can be advocate that achieved crude ethyl acetate fraction can be explored for preparing antimicrobial drugs in future for the infectious caused by the pathogens tested in the study.

Key words: Disc diffusion assay, *Enterobacter aerogenes*, GC-MS, kaempferol-3-O-rutinoside, pyrogallol, *Terminalia chebula*

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INTRODUCTION

Terminalia chebula Retz is a medicinal plant belonging to family Combretaceae. It is commonly called as black myrobalan. The fruits of *T. chebula* are commonly used in treatment of various ailments such as allergy, vomiting, urinary tract infections, cardiac diseases, digestive problems, bleeding, cancer, skin disorders and diabetes mellitus.[1] It also possesses antioxidant activity and free radical scavenging property. Antimicrobial activitiy of *T. chebula* have also been reported in many research publications.[2,3] *Pyrogallol* (benzene-1,2,3-triol) is a polyphenol is known to display fungicidal/fungistatic properties.[4] Moreover, its derivatives are biologically active components of plants and plant products.[5, 6,] Current study involves the GC-MS analysis of the ethyl acetate extracts of fruits of *T. chebula*, selection of most active compound identification *and* screening for antimicrobial activity against selected pathogens. The aim was to determine whether the activity of the plant species is due to individual compound or group of compounds, in addition the aim was to isolate to the most appropriate economical method of extracting the active fraction from fruits of *T. chebula* which is widely used commercially for herbal medicine.

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MATERIALS AND METHODS

Plant material

Fruits of T. chebula were collected in the month of October from the University of Agriculture Sciences Gandhi Krishi Vignyan Kendra, Bangalore and the specimen of the plant

was identified at the Department of Botany, University of Rajasthan. The sample specimen with No. RUBL20868 was submitted in the 'Herbarium' of Botany Department, University of Rajasthan.

Extraction procedure

Fruits were separately shade dried and finely powered using a mixer. Twenty grams of finely powdered sample was soxhlet extracted with ethyl acetate on a water bath for 24 h and filtered. Obtained extract was dried in vacuum and stored at 4°C. The chemical composition of the ethyl acetate fractions were got analyzed by GC/MS.

Gas chromatography-mass spectrometry analysis (GC-MS)

GC-MS technique was used in this study to identify the phytocomponents present in the extracts. This was carried out at Jawaharlal Nehru University, New Delhi, India. The GC-MS used was a Schimadzu QP2010PLUS system. All the conditions used in GC-MS method were recorded in Table 1. Identification of the peaks was based on computer matching of the mass spectra with the National Institute of Standards and Technology (NlST 08 and NIST 08s) library and by direct comparison with published data.[7]

Table 1: GC-MS method

Column Oven Temp.:	100.0°C	
Injection Temp.:	270.00°C	
Injection Mode:	Split	
Flow Control Mode:	Linear Velocity	
Pressure:	169.6 kPa	
Total Flow:	16.3 mL/min	
Column Flow:	1.21 mL/min	
Linear Velocity:	28.9 cm/sec	
Purge Flow:	3.0 mL/min	
Split Ratio:	10.0	
High Pressure Injection:	OFF	
Carrier Gas Saver:	OFF	
Splitter Hold:	OFF	
Oven Temp. Program		
Rate	Temperature(°C)	Hold Time(min)
–	100.0	2.00
5.00	250.0 1	0.00
15.00	300.0	25.00
[GC Program]		
IonSourceTemp:	250.00°C	
Interface Temp.:	280.00°C	
Solvent Cut Time:	7.00 min	
Detector Gain Mode:	Relative	
Detector Gain:	0.00 kV	
Threshold:	1000	
[MS Table]		
Start Time:	7.00 min	
End Time:	70.32 min	
ACQ Mode:	Scan	
Event Time:	0.50 sec	
Scan Speed:	1250	
Start m/z:	40.00	
End m/z:	600.00	
Sample Inlet Unit:	GC	

Identification of pyrogallol (phenol) by chromatography

Dried ethyl acetate extract of fruits dissolved in ethyl acetate, was chromatographed two-dimensionally on silica gel coated (0.2-0.3 mm) plates. These plates were developed in an organic solvent mixture of acetic acid- chloroform (1:9) *and* ethyl acetate-benzene (9:11) *and* separately on cellulose MN300 in benzene-methanol-acetic acid (45:8:4) *and* 6% aqueous acetic acid.[8] One spot (Rf 0.8) in one direction *and* (Rf 0.15) second direction was observed which indicate the presence of pyrogallol in the ethyl acetate extracts of fruit. Preparative TLC of the extract was carried out on silica gel coated and activated (0.4-0.5-mm thick) glass plates in the selected solvents. Spot was marked in each plate and was collected and eluted with ethyl acetate. Elutes were pooled, dried in vacuum and rechromatographed to test the purity of the isolated compound.

The isolated compound was crystallized, weighed and subjected to melting point and infra-red spectral studies on Perkins Elmer model 555 spectrophotometer in KBr pellets. Pyrogallol (formula $C_6H_6O_3$; m.w. 126; m.p. 131° - 135°) was identified in the fruit of plant.

ANTIMICROBIAL ASSAY

Selected test microorganisms

Pathogenic microorganisms selected for study include seven bacteria, viz., Escherichia coli (MTCC no. 46), *Pseudomonas aeruginosa* (MTCC 1934), *Proteus mirabilis* (MTCC 3310), *Raoultella planticola* (MTCC 2271), *Enterobacter aerogens* (MTCC 2822), *Bacillus subtilis* (MTCC 121), *Staphylococcus aureus* (MTCC 3160) and three fungal strains, viz., *Candida. albicans* (MTCC 183), *Aspergillus flavus* (MTCC 277) and *Aspergillus niger* (MTCC 282). Selected microorganisms were procured from IMTECH, Chandigarh, India. Bacterial strains were grown and maintained on "Muller- Hinton Agar Medium" (Beef extract 2.0 g; Peptone 17.5 g; Starch 1.5 g; Agar 17.0 g; in 1000 ml of distilled water; Final pH 7.4 ± 0.2) at 37 ± 2° while fungal strains were grown on "Sabouraud Dextrose Agar Medium" (Peptone 10 g; Dextrose 20 g; Agar 20 g in 1000 ml of distilled water; pH adjusted to 6.8-7.0 at 27 ± 2°C).

Screening

Disc diffusion assay (DDA) was performed for antimicrobial screening.[9] MH agar (for bacteria) and SD agar (for fungi) base plates were seeded with the standard inoculum size of bacteria, yeast and fungi (1×10^8 CFU/ml for bacteria, 1×10^7 CFU/ml for yeast and 1×10^6 CFU/ml for dermatophytic fungi). Sterile filter paper discs (6 mm in diameter) were impregnated with 100 µl of each of the extract (10 mg/ml concentration) to give a final concentration of 1 mg/disc, left to dry in vaccuo to remove residual solvent, which might interfere with

the determination. Extract discs were then placed on the seeded agar plates. Each extract was tested in triplicate along with standard drugs streptomycin (1 mg/disc) for bacteria, itraconazol (1 mg/ml) for *A. niger* and *A. flavus* and *Clotrimazole* (1 mg/ml) for C. *albicans*, respectively. The plates were kept at 4°C for 1 h for diffusion of extract, thereafter were incubated at 37 ± 2°C for 24 h; 27 ± 2°C for 48 h and 27 ± 2°C for 5-7 days for bacteria, yeast and fungus, respectively. Zone of inhibition (IZ) or depressed growth of microorganisms was measured and the 'Activity Index' (AI) for each extract was calculated.

Minimum inhibitory concentration and minimum bactericidal/fungicidal concentration

Minimum inhibitory concentration (MIC) was determined for plant extract showing antimicrobial activity against test pathogens in disc diffusion assay. Broth microdilution method was followed for determination of MIC values.[10] Plant extracts were resuspended in acetone (which has no activity against test microorganisms) to make 10 mg/ml final concentration and then was added to broth media of 96-wells of microtiter plates using two-fold serial dilution. Thereafter, 100-µl inoculum of standard size was added to each well. Bacterial and fungal suspensions were used as negative control, while broth containing standard drug was used as positive control. The microtiter plates were incubated at 37 ± 2°C for 24 h for bacteria, 27 ± 2°C for 48 h for yeast and 27 ± 2°C for 5-7 days for fungi. Each extract was assayed in duplicate and each time two sets of microtiter plates were prepared, one was kept for incubation while another set was kept at 4°C for comparing the turbidity in the wells of microtiter plate. The MIC values were taken as the lowest concentration of the extracts in the well of the microtiter plate that showed no turbidity after incubation. The turbidity of the wells in the microtiter plate was interpreted as visible growth of microorganisms. The minimum bacterial/fungicidal concentration (MBC/MFC) was determined by subculturing 50 µl from each well showing no apparent growth. Least concentration of extract showing no visible growth on subculturing was taken as MBC/MFC.

RESULTS

Phytochemical analysis

The results of GC-MS analysis of the ethyl acetate fraction of fruits of *T. chebula* identified the various compounds through the NIST08L [database are listed in Table 2]. The active principle, area of the peak concentration (%), retention time

Table 2: Components identified in ethyl acetate fraction of fruits of *T. chebula* by GC-MS analysis

Peak	Retention time (s)	Area %	Chemical Formula	Mol. Weight (g/mol)	Name of compound
1.	11.680	0.43	$C_{11}H_{22}O$	170	2-Undecanone
2.	13.770	0.67	$C_{12}H_{24}$	168	Cyclododecane
3.	14.929	46.21	$C_6H_6O_3$	126	Pyrogallol
4.	16.871	2.73	$C_{14}H_{22}O$	206	Phenol
5.	18.465	3.68	$C_{18}H_{36}$	252	9-Octadecene
6.	18.609	0.61	$C_{16}H_{34}$	226	Hexadecane
7.	19.995	0.43	$C_{16}H_{32}$	224	Cyclohexane
8.	20.372	1.93	$C_{15}H_{30}O$	226	8-Pentadecanone
9.	22.351	0.24	$C_{50}H_{102}$	702	Triacontane
10.	22.845	5.13	$C_{20}H_{40}$	280	9-Eicosene
11.	22.956	0.35	$C_{14}H_{30}$	198	Tetradecane
12.	23.798	0.23	$C_{18}H_{36}O$	268	Oxirane
13.	24.272	0.29	$C_{16}H_{34}O_2$	258	1,16-Hexadecanediol
14.	24.398	0.41	$C_{13}H_{26}$	182	Heptylcyclohexane
15.	24.598	3.03	$C_{19}H_{38}O$	282	10-Nonadecanone
16.	24.687	0.53	$C_{20}H_{28}O_4$	332	Phthalic acid
17.	25.582	2.02	$C_{17}H_{34}O_2$	270	Hexadecanoic acid
18.	25.840	0.25	$C_{43}H_{88}$	604	Tritetracontane
19.	26.839	6.02	$C_{20}H_{40}$	280	9-Eicosene
20.	28.440	1.30	$C_{17}H_{34}O$	254	9-Heptadecanone
21.	28.819	0.96	$C_{19}H_{34}O_2$	294	9,12-Octadecadienoic acid
22.	28.909	2.47	$C_{19}H_{36}O_2$	296	9-Octadecenoic acid
23.	29.341	1.48	$C_{19}H_{38}O_2$	298	Octadecanoic acid
24.	29.575	0.27	$C_{44}H_{90}$	618	Tetratetracontane
25.	29.998	0.87	$C_{20}H_{36}O_2$	308	Linoleic acid ethyl ester
26.	30.079	0.56	$C_{20}H_{38}O_2$	310	9-Octadecenoic acid ethyl ester

Table 2: Contd...

Peak	Retention time (s)	Area %	Chemical Formula	Mol. Weight (g/mol)	Name of compound
27.	30.145	0.52	$C_{20}H_{34}O_2$	306	9,12,15-Octadecatrienoic acid
28.	30.472	2.98	$C_{23}H_{46}$	322	1-Tricosene
29.	31.952	0.13	$C_{20}H_{38}$	278	1,19-Eicosadiene
30	32.359	0.43	$C_{22}H_{37}F_7O_2$	466	Heptafluorobutyric acid
31.	32.819	0.22	$C_{21}H_{42}O_2$	326	Eicosanoic acid
32.	33.042	0.24	$C_{12}H_{26}O$	186	1-Octanol
33.	33.457	0.23	$C_{16}H_{34}O$	242	1-Decanol
34.	33.977	2.34	$C_{28}H_{56}$	292	Cyclooctacosane
35.	35.947	0.18	C_9H_{16}	124	1H-Indene
36.	36.238	0.60	$C_{29}H_{53}F_5O_2$	528	Hexacosyl pentafluoropropionate
37.	36.849	0.17	$C_{41}H_{77}F_5O_2$	696	Octatriacontyl pentafluoropropionate
38.	37.258	0.17	$C_{34}H_{70}$	478	Tetratriacontane
39.	37.671	0.58	$C_{24}H_{38}O_4$	390	1,2-Benzenedicarboxylic acid
40	38.566	0.73	$C_{23}H_{46}$	322	9-Tricosene
41.	40.027	0.49	$C_{20}H_{26}N_2O_2$	326	Ibogamin-9(17H)-o
42.	41.815	0.47	$C_{38}H_{69}F_7O_2$	690	Tetratriacontyl heptafluorobutyrate
43.	42.717	0.15	$C_{36}H_{65}F_7O_2$	662	Dotriacontyl heptafluorobutyrate
44.	42.873	0.08	$C_{25}H_{50}O_2$	382	Tetracosanoic acid
45.	43.146	0.09	$C_{35}H_{72}$	492	Pentatriacontane
46.	44.171	0.66	$C_{22}H_{41}F_3O_2$	394	Eicosyl trifluoroacetate
47.	44.831	0.15	$C_{30}H_{50}$	410	Squalene
48.	45.767	0.87	$C_{28}H_{49}F_7O_2$	550	Tetracosyl heptafluorobutyrate
49.	46.201	0.14	$C_{38}H_{69}F_7O_2$	690	Tetratriacontyl heptafluorobutyrate
50.	47.172	0.34	$C_{22}H_{37}F_7O_2$	466	Heptafluorobutyric acid
51.	48.708	0.77	$C_{21}H_{44}O_3S$	376	Sulfurous acid
52.	49.347	0.22	$C_{29}H_{58}O_2$	438	Octacosanoic acid
53.	49.744	0.27	$C_{29}H_{50}O_2$	430	Vitamin E
54.	50.309	0.14	$C_{28}H_{49}F_7O_2$	550	Tetracosyl heptafluorobutyrate
55.	51.060	0.11	$C_{27}H_{54}O_2$	410	Hexacosanoic acid
56.	52.109	0.46	$C_{41}H_{77}F_5O_2$	696	Octatriacontyl pentafluoropropionate
57.	53.000	1.00	$C_{31}H_{62}O_2$	466	Triacontanoic acid
58.	54.225	0.04	$C_{26}H_{47}F_5O_2$	486	Tricosyl pentafluoropropionate
59.	54.714	0.06	$C_{20}H_{40}O_2$	312	Acetic acid
60.	55.242	0.04	$C_{28}H_{56}O_2$	424	Heptacosanoic acid
61.	56.573	0.30	$C_{37}H_{69}F_5O_2$	640	Tetratriacontyl pentafluoropropionate
62.	57.871	0.05	C25 H50 O2	382	Tetracosanoate
63.	59.546	0.19	C27H30O15	594	*Kaempferol-3-O*-rutinoside
64.	60.576	0.30	C10H18O4	202	Ethanedioic acid

(RT), molecular weight *and* molecular formula are presented in the table. Figure 1 represents the gas chromatograms of the extract which shows 64 distinct peaks identified in GC-MS. The major components in the ethyl acetate fraction as identified by GC-MS was pyrogallol (1,2,3-benzenetriol). Mass spectrum of pyrogallol is shown in Figure 2. The GC-MS spectrum gives the structure of the compound, molecular formula ($C_6H_6O_3$), molecular weight 126.0 [Figure 2].

A new flavonoid Kaempferol-3-O-rutinoside was identified for the first time in the fruits. Other compounds identified in the extracts are Phenol (2.73%), 9-Octadecene (3.68%), 9-Eicosene (5.13%), Hexadecanoic acid (2.02%), 9,12-Octadecadienoic acid (0.96%), 9-Octadecenoic acid (2.47%), Eicosanoic acid (0.22%), 1,2-Benzenedicarboxylic acid (0.58%), Tetracosanoic acid (0.08%), Vitamin E (0.27%), Ethanedioic acid (0.30%). Figure 3 shows the chemical structure of pyrogallol, kaempferol-3-O-rutinoside and vitamin E.[11] observed gallic acid, chebulic acid, 1,6-di-O-galloyl-D-glucose, punicalagin, 3,4,6-tri-O-galloyl-D-glucose, casuarinin, chebulanin, corilagin, neochebulinic acid, terchebulin, ellagic acid, chebulagic acid, chebulinic acid, and 1,2,3,4,6-penta-O-galloyl-D-glucose) in the fruit of *T. chebula* Retz. by RP-HPLC method. Tannins contain phenolic carboxylic acid like gallic acid, ellagic acid, chebulic acid and gallotannins such as 1,6 di-Ogalloyl- β-D-glucose,

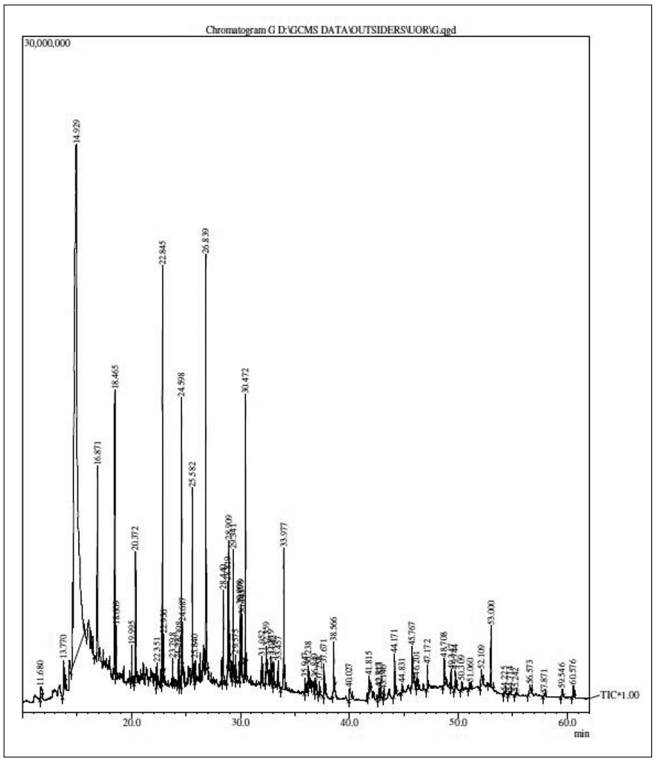

Figure 1: Chromatogram of ethyl acetate fraction of fruits of *T. chebula* by GC-MS analysis

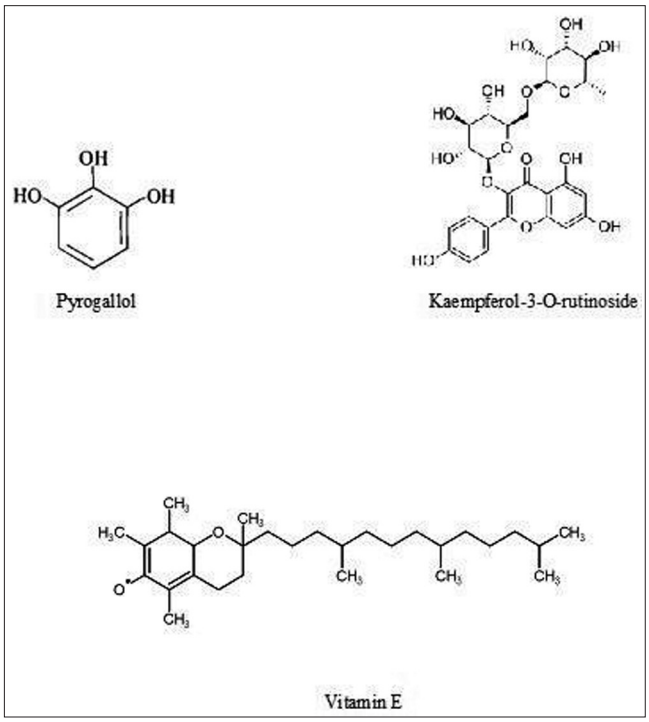

Figure 3: Chemical structure of molecules in ethyl acetate fraction of fruits of *T. chebula*

3,4,6 tri-*O*-galloyl-β-D-glucose, 2,3,4,6 tetra-*O*-galloyl-β-D-glucose, 1,2,3,4,6 penta-*O*galloyl- β-D-glucose. Ellagitannin such as punacalagin, casurarinin, corilagin and terchebulin

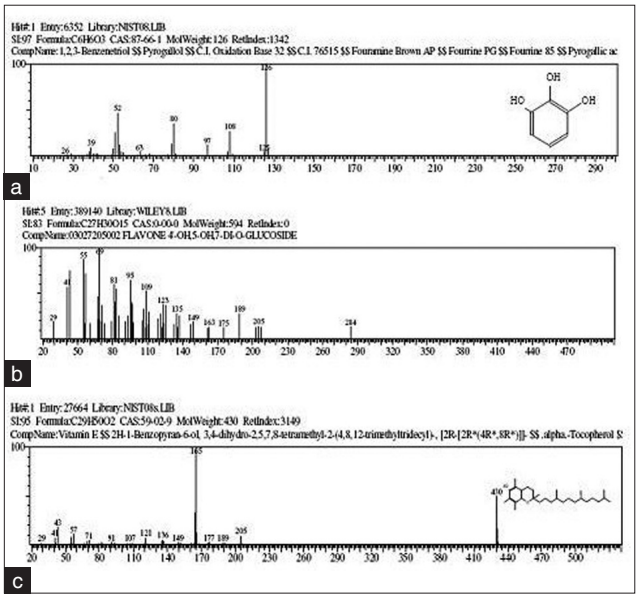

Figure 2: Mass spectrums (a) The GC-MS spectrum gives the structure of the pyrogallol, molecular formula ($C_6H_6O_3$), molecular weight 126.0 (b) Mass spectrum of Kaempferol-3-O-rutinoside (c) The GC-MS spectrum gives the structure of the vitamin E, molecular formula ($C_{29}H_{50}O_2$), molecular weight 430

and others such as chebulanin, neochebulinic acid, chebulagic acid and chebulinic acid reported in literature.[12]

Antimicrobial screening

Present investigation clearly indicates the presence of highest percentage of pyrogallol in ethyl acetate fraction of fruits of *T. chebula*. Pyrogallol has been reported to have various biological activity like allelochemic, antibacterial, abortifacient, anticlastogen, antidermatitic, antilupus, antimutagenic, antioxidant, antipsoriac, antiseptic, CNSActive, candidicide, cardiovascular, ecbolic, fungicide, insulin-sparing, irritant, nephrotoxic, nigrifacient, pesticide, prostaglandin-synthesis-inhibitor from Dr. Duke's phytochemical and ethnobotanical database.[13] Pyrogallol present in the ethyl acetate fraction was identified and eluted by PTLC screened for antimicrobial activity along with ethyl acetate fraction. Antimicrobial activity (assessed in terms of inhibition zone and activity index) of the plant extracts, tested against selected microorganisms was recorded in Table 3. Results reveal that the inhibition zone produced by pyrogallol against selected pathogens was similar to the ethyl acetate fraction of the fruits. In both cases the highest activity was showed against *E. aerogens* (Pyrogallol: IZ = 31 mm, AI 0. 1.409 ± 0.046; Ethyl acetate fraction: IZ = 29 mm, AI = 1.313 ± 0.026). Against *P. mirabilis* both pyrogallol *and* ethyl acetate fraction showed same activity. (IZ = 19 mm, AI = 0.760 ± 0.061). Both the test extracts are not active against *P. aeruginosa* and *A. niger*. MIC *and* MBC/MFC values were evaluated for pyrogallol *and* ethyl acetate fraction (shown activity in "Disc Diffusion Assay") and recorded in Table 4.

Table 3: Antimicrobial activity of crude ethyl acetate extract and pure pyrogallol of *T. chebula* by Disc Diffusion Assay

Test Microorganisms	Ethyl acetate extract		Pryogallol	
	IZ	AI	IZ	AI
E. coli	16	0.15 ± 0.022	15.50	0.596 ± 0.173
P. aeruginosa	–	–	–	–
P. mirabilis	19	0.760 ± 0.061	19	0.760 ± 0.061
R. planticola	19.83	0.661 ± 0.015	19	0.633 ± 0.033
E. aerogens	29	1.313 ± 0.026	31	1.409 ± 0.046
B. subtilis	14	0.777 ± 0.032	13.33	0.740 ± 0.120
S. aureus	13.83	0.658 ± 0.029	14.25	0.678 ± 0.036
A. niger	–	–	–	–
A. flavus	26	1.733 ± 0.039	24.75	1.650 ± 0.050
C. albicans	26	1.875 ± 0.072	25	1.786 ± 0.072

IZ = Inhibition zone in mm (mean value; include 6-mm diameter of disc), AI = Activity Index (IZ developed by extract/ IZ developed by standard), ± = SEM, (-) = No activity Extracts assayed in triplicate, IZ of standard drug Streptomycin against *E. coli* (26 mm), *P. aeruginosa* (20 mm), *P. mirabilis* (25 mm), *R. planticola*(30 mm), *E. aerogens* (22 mm), *B. subtilis* (18 mm) *S. aureus* (21 mm), IZ of standard drug itraconazol against *A. niger* (10 mm) *and A. flavus* (15 mm). IZ of standard drug Clotrimazole against *C. albicans* (14 mm)

Discussions and conclusions

In the present investigation MIC 0.039 mg/ml was recorded against *P. mirabilis, E. aerogens, A. flavus* by ethyl acetate fraction *and* against *E. coli, R. planticola*, *E. aerogens, A. flavus, C. albicans* by pyrogallol. Pyrogallol (1,2,3-Trihydroxybenzene) is allochemical which contains 3 hydroxyl groups belong to phenolic compounds of plants. The phenolic hydroxyl group has a wide range of cellular activities that have not been clearly investigated. At present there is intense interest in polyphenols which are present in the diet as part of fruits, tea, coffee and wine[14] since they have been shown to protect cells from oxidative stress.[15] In addition, these compounds show a wide spectrum of action involving antitumor, antivirial, antibacterial, cardioprotective, prooxidant and antimutagenic activity.[16,17] The presence of the hydroxyl group and a system of delocalized electron play an important role in the antimicrobial activity.[18] A characteristic feature of a phenolic hydroxyl group is its significantly greater acidity than that of an aliphatic hydroxyl groups.[19] Hydroxyl group and a system of delocalized electrons might be responsible for strong antimicrobial activity. It was described earlier that the hydroxyl group (bound to a benzene ring) is important for the activities of some antimicrobial compounds and that these activities are enhanced by the presence of alpha-beta double bonds.[20] Pyrogallol has been reported to be an effective antimicrobial agent and its toxicity is attributed to the three hydroxyl groups present in its structure.[21,22]

In the conclusion, it is showed that the antimicrobial activity of ethyl acetate fraction was due to pyrogallol which is present in higher quantity in fruits. Results of the present study reveal that all compound tested, inhibited the growth of selected bacteria and fungi, indicating broad spectrum bioactive nature of selected plant. In the present scenario when existing antibiotics are gradually becoming ineffective against pathogenic microorganisms, such studies should highly be encouraged, so that new and alternative sources for future antibiotics may be explored well in advance.

Table 4: MIC and MBC/MFC values of crude ethyl acetate extract and pure pyrogallol of *T. chebula* by Disc Diffusion Assay

Test Microorganisms	Ethyl acetate extract		Pryogallol	
	MIC	MBC/MFC	MIC	MBC/MFC
E. coli	0.078	0.156	0.039	0.078
P. aeruginosa	–	–	–	–
P. mirabilis	0.039	0.078	0.078	0.156
R. planticola	0.078	0.156	0.039	0.156
E. aerogens	0.039	0.039	0.039	0.039
B. subtilis	0.078	0.156	0.156	0.312
S. aureus	0.156	0.312	0.078	0.078
A. niger	–	–	–	–
A. flavus	0.039	0.039	0.039	0.078
C. albicans	0.078	0.312	0.039	0.039

MIC = Minimum Inhibitory Concentration (mg/ml), MBC/MFC = Minimum Bactericidal/Fungicidal Concentration (mg/ml)

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