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

Geeta Singh, Padma Kumar

*Department of Botany, Laboratory of Plant Tissue Culture and Secondary Metabolites, University of Rajasthan, Rajasthan, India*

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**ABSTRACT**

**Background:** *Terminalia chebula* is called the “king of medicines” in Tibet and is always listed first in the Ayurvedic materia 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 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*

**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 activity 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 the most appropriate economical method of extracting the active fraction from fruits of *T. chebula* which is widely used commercially for herbal medicine.

**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 based on computer matching of the mass spectra with the National Institute of Standards and Technology (NIST 08 and NIST 08s) library and by direct comparison with published data.[7]

**Table 1: GC-MS method**

| Parameter                          | Specification               |
|------------------------------------|-----------------------------|
| 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 : 1.00                |
| 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. 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 C₆H₄O₃; 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; pH adjusted to 6.8-7.0 at 27 °C) 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 × 10⁶ CFU/ml for bacteria, 1 × 10⁷ CFU/ml for yeast and 1 × 10⁶ CFU/ml for dermatophytic fungi). Sterile filter paper discs (6 mm in diameter) were impregnated with 100 μl each of the extract (10 mg/ml concentration) to give a final concentration of 1 mg/disc, left to dry in vacuum 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 bacterical/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

| Peak | Retention time (s) | Area % | Chemical Formula | Mol. Weight (g/mol) | Name of compound |
|------|-------------------|--------|------------------|---------------------|------------------|
| 1    | 11.680            | 0.43   | C₁₁H₂₂O          | 170                 | 2-Undecanone     |
| 2    | 13.770            | 0.67   | C₁₂H₂₄            | 168                 | Cyclododecane    |
| 3    | 14.929            | 46.21  | C₁₈H₃₄O₂         | 126                 | Pyrogallol       |
| 4    | 16.871            | 2.73   | C₁₄H₂₆O          | 206                 | Phenol           |
| 5    | 18.465            | 3.68   | C₁₈H₃₆            | 252                 | 9-Octadecene     |
| 6    | 18.609            | 0.61   | C₁₆H₃₄            | 226                 | Hexadecane       |
| 7    | 19.995            | 0.43   | C₁₈H₃₂            | 224                 | Cyclohexane      |
| 8    | 20.372            | 1.93   | C₂₀H₃₈O          | 226                 | 8-Pentadecanone  |
| 9    | 22.351            | 0.24   | C₂₀H₄₀            | 702                 | Triacetone       |
| 10   | 22.845            | 5.13   | C₂₅H₄₀            | 280                 | 9-Eicosene       |
| 11   | 22.956            | 0.35   | C₁₄H₂₀            | 198                 | Tetradecane      |
| 12   | 23.798            | 0.23   | C₁₆H₃₈O          | 268                 | Oxirane          |
| 13   | 24.272            | 0.29   | C₁₈H₃₆O₂         | 258                 | 1,16-Hexadecanediol |
| 14   | 24.398            | 0.41   | C₁₈H₃₆O₂         | 182                 | Heptylcyclohexane |
| 15   | 24.598            | 3.03   | C₂₀H₄₀O          | 282                 | 10-Nonadecanone  |
| 16   | 24.687            | 0.53   | C₂₀H₄₀O          | 332                 | Phthalic acid    |
| 17   | 25.582            | 2.02   | C₂₀H₄₀O₂         | 270                 | Hexadecanoic acid |
| 18   | 25.840            | 0.25   | C₂₂H₄₄O          | 604                 | Tritetracontane  |
| 19   | 26.839            | 6.02   | C₂₂H₄₀            | 280                 | 9-Eicosene       |
| 20   | 28.440            | 1.30   | C₂₀H₃₈O          | 254                 | 9-Heptadecanone  |
| 21   | 28.819            | 0.96   | C₁₈H₃₄O₂         | 294                 | 9,12-Octadecadienoic acid |
| 22   | 28.909            | 2.47   | C₁₈H₃₄O₂         | 296                 | 9-Octadecanoic acid |
| 23   | 29.341            | 1.48   | C₁₈H₃₄O₂         | 298                 | Octadecanoic acid |
| 24   | 29.575            | 0.27   | C₂₀H₄₀            | 618                 | Tetracontane     |
| 25   | 29.928            | 0.87   | C₂₀H₃₈O₂         | 308                 | Linoleic acid ethyl ester |
| 26   | 30.079            | 0.56   | C₂₀H₃₈O₂         | 310                 | 9-Octadecanoic 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   | C₁₈H₃₆O₂         | 306                | 9,12,15-Octadecatrienoic acid |
| 28.  | 30.472            | 2.98   | C₁₈H₃₈           | 322                | 1-Tricosene       |
| 29.  | 31.952            | 0.13   | C₂₀H₄₀           | 278                | 1,19-Eicosadiene  |
| 30.  | 32.359            | 0.43   | C₂₁H₄₄F₂O₂       | 466                | Heptfluorobutyric acid |
| 31.  | 32.819            | 0.22   | C₂₁H₄₆O₂         | 326                | Eicosanoic acid   |
| 32.  | 33.042            | 0.24   | C₁₉H₃₉O          | 186                | 1-Octanol         |
| 33.  | 33.457            | 0.23   | C₁₉H₄₀O          | 242                | 1-Decanol         |
| 34.  | 33.977            | 2.34   | C₂₁H₅₈           | 292                | Cyclooctacosane   |
| 35.  | 35.947            | 0.18   | C₂₁H₄₃           | 124                | 1H-Indene         |
| 36.  | 36.238            | 0.60   | C₂₀H₃₆F₂O₂       | 528                | Hexacosyl pentafluoropropionate |
| 37.  | 36.849            | 0.17   | C₁₉H₄₇F₂O₂       | 696                | Octatriacontyl pentafluoropropionate |
| 38.  | 37.258            | 0.17   | C₁₉H₄₆           | 478                | Tetratriacontane  |
| 39.  | 37.671            | 0.58   | C₁₉H₄₆O₄         | 390                | 1,2-Benzenediacarbonylic acid |
| 40.  | 38.566            | 0.73   | C₁₉H₄₈           | 322                | 9-Tricosene       |
| 41.  | 40.027            | 0.49   | C₁₉H₄₉N₂O₂       | 326                | Ibogamin-9(17H)-o |
| 42.  | 41.815            | 0.47   | C₁₉H₄₉F₂O₂       | 690                | Tetracontyol heptfluorobutyrate |
| 43.  | 42.717            | 0.15   | C₁₉H₄₉F₂O₂       | 662                | Dotriacontyl heptfluorobutyrate |
| 44.  | 42.873            | 0.08   | C₁₉H₄₉O₂         | 382                | Tetracosanoic acid |
| 45.  | 43.146            | 0.09   | C₁₉H₅₂           | 492                | Pentatriacontane  |
| 46.  | 44.171            | 0.66   | C₁₉H₄₇F₂O₂       | 394                | Eicosyl trifluoracetate |
| 47.  | 44.831            | 0.15   | C₁₉H₅₀           | 410                | Squalene          |
| 48.  | 45.767            | 0.87   | C₁₉H₄₈F₂O₂       | 550                | Tetracosyl heptfluorobutyrate |
| 49.  | 46.201            | 0.14   | C₁₉H₄₉F₂O₂       | 690                | Tetracontyol heptfluorobutyrate |
| 50.  | 47.172            | 0.34   | C₁₉H₄₉F₂O₂       | 466                | Heptfluorobutyric acid |
| 51.  | 48.708            | 0.77   | C₁₉H₄₉O₅S        | 376                | Sulforus acid     |
| 52.  | 49.347            | 0.22   | C₁₉H₄₉O₂         | 438                | Octacosanoic acid |
| 53.  | 49.744            | 0.27   | C₁₉H₄₉O₂         | 430                | Vitamin E         |
| 54.  | 50.309            | 0.14   | C₁₉H₄₉F₂O₂       | 550                | Tetracosyl heptfluorobutyrate |
| 55.  | 51.060            | 0.11   | C₁₉H₄₉O₂         | 410                | Hexacosanoic acid |
| 56.  | 52.109            | 0.46   | C₁₉H₄₇F₂O₂       | 696                | Octatriacontyl pentafluoropropionate |
| 57.  | 53.000            | 1.00   | C₁₉H₄₉O₂         | 466                | Triacontyol acid  |
| 58.  | 54.225            | 0.04   | C₁₉H₄₉F₂O₂       | 486                | Tricosyl pentafluoropropionate |
| 59.  | 54.714            | 0.06   | C₁₉H₄₉O₂         | 312                | Acetic acid       |
| 60.  | 55.242            | 0.04   | C₁₉H₄₉O₂         | 424                | Heptacosanoic acid |
| 61.  | 56.573            | 0.30   | C₁₉H₄₉F₂O₂       | 640                | Tetratriacontyl pentafluoropropionate |
| 62.  | 57.871            | 0.05   | C₂₅H₅₀O₂         | 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 (C₆H₈O₃), 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, terebeulin, 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-O-galloyl-β-D-glucose,
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 punicalagin, casurarinin, corilagin and terchebulin 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, antielastogen, antidermatitic, antilupus, antimutagenic, antioxidant, antipsoriac, antiseptic, CNSActive, candicidice, cardiovascular, ecobic, fungicide, insulin-sparing, irritant, nephroxic, 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 0.1409 ± 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*           | 26 | 1.733 ± 0.039 | 24.75 | 1.650 ± 0.050 |
| *A. flavus*          | 26 | 1.875 ± 0.072 | 25   | 1.786 ± 0.072 |
| *C. albicans*        | –  | –  | –  | –  |

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

Extraction assayed in triplicate, IZ of standard drug Streptomycin against *E. coli* (14 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* (24 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 since they have been shown to protect cells from oxidative stress. In addition, these compounds show a wide spectrum of action involving antitumor, antiviral, antibacterial, cardioprotective, prooxidant and antimutagenic activity. The presence of the hydroxyl group and a system of delocalized electron play an important role in the antimicrobial activity. A characteristic feature of a phenolic hydroxyl group is its significantly greater acidity than that of an aliphatic hydroxyl groups. 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. Pyrogallol has been reported to be an effective antimicrobial agent and its toxicity is attributed to the three hydroxyl groups present in its structure.

In 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.

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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*      | 0.039 | 0.078 | 0.078 | 0.156 |
| *P. mirabilis*       | 0.078 | 0.156 | 0.039 | 0.039 |
| *R. planticola*      | 0.078 | 0.156 | 0.039 | 0.039 |
| *E. aerogens*        | 0.078 | 0.156 | 0.039 | 0.039 |
| *B. subtilis*        | 0.078 | 0.156 | 0.039 | 0.039 |
| *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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