Research Article

Phytochemical investigation, antioxidant and antibacterial activities, and GC-MS analysis of ethanol extract of *Terminalia chebula* Retz. dried seeds

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Abstract

Objective: *Terminalia chebula* Retz. belongs to the family Combretaceae and it is an important potent medicinal plant used in four categories of medicines such as Ayurveda, Unani, Siddha and Homeopathy. The aim of the present study was to evaluate the antioxidant, antibacterial and anti-diabetic activities of ethanolic dried seed extract of *Terminalia chebula*. **Materials and Methods:** In-vitro antioxidant activities such as Free radical scavenging activity by DPPH[•] method, hydroxyl (OH[•]) radical scavenging, phosphomolybdenum and ferric reducing power activities respectively were performed for the selected source. Phytochemical screening was evaluated based on standardized methods. Also, the antibacterial studies were carried out by Agar well diffusion method in order to study the inhibition effect of ethanolic seed extract against the test bacterial pathogens. Detection of various phyto-constituents were been identified by Gas Chromatography-Mass Spectrometry. **Results:** The IC₅₀ of DPPH[•] radical scavenging activity was 15.56 µg/mL concentration respectively. Also, the RC₅₀ of phosphomolybdenum reduction and ferric reducing power activities were 29.59 and 20.69 µg/mL concentration respectively. Total phenolic and flavonoid content were determined, in which phenols were found to be significantly higher as 48.52±0.36 GAE/mg. The antibacterial activity was effective for ethanolic seed extract by agar well diffusion method, in which the maximum inhibition was against *P. vulgaris* and *M. luteus* (20 mm). The active compounds were identified by GC-MS analysis which revealed the presence of Dodecahydropyrido (1,2-b) isoquinolin-6-one, Corynan-17-ol, 18, 19-didehydro-10-methoxy-acetate (ester), Oleic acid. **Conclusion:** The results prove that *Terminalia chebula* seeds as a potent antioxidant and antibacterial agent. Hence the seeds of *Terminalia chebula* can be formulated as drug and also shall be tested for treating several free radicals related diseases as well as microbial infections in future.

Keywords: *Terminalia chebula*, Dot-plot, DPPH[•] radical, Hydroxyl radical (OH), RC₅₀, alpha amylase

Introduction

Medicinal plants have been selected and are used traditionally as potential and effective drug candidates since they possess definitive drug-like mechanism when compared to chemically-synthesized derivatives. Phyto-medicine refers to making use of plants for effective medicinal purpose for treating, curing of diseases and improve human health system. The

pharmacological property or the efficacy of medicinal plants is due to the presence of secondary metabolites such as Tannins, Alkaloids, Phenolic compounds, Steroids, etc. Each secondary metabolite has its own pharmacologically active and valuable biological properties (Saraswathi et al., 2017; Arvind Kumar Shakya, 2016; Kumar et al., 2015; Maurya et al., 2008; Chopra and Doiphode, 2002).

Antioxidants are compounds that are capable to inhibit or slow down the oxidation, which occurs due to environmental stress or Reactive Oxygen Species. Antioxidants are the first line of defense mechanism in neutralizing the free radicals (Pisoschi and Negulescu, 2011; Litescu et al., 2011). Fruits, vegetables, grains, nuts,

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Figure 1. Dried Seeds of *Terminalia chebula*

pulses when consumed have a great effect in reducing many chronic diseases. The phytochemicals, derived from medicinal plants have significant role as antioxidant-rich molecules (Pellegrini et al., 2006; Hu, 2003; McCullough et al., 2003). Excess production of free radicals starts to form chain reactions, circulates in the human system thereby oxidize the low density lipoproteins (LDL) and slowly moving to lethal condition. The oxidation process gets initiated continuously due to specific substances, until a scavenging or neutralizing mechanism occurs (Campanella et al., 2006; Alam et al., 2013; Ranjeet Sawant et al., 2013).

Terminalia chebula belonging to Combretaceae family is a tree native to North east India & Indio-Burma region (Figure 1). It is a medium-sized deciduous tree with a height of up to 30 m. The leaves are elliptic rhombus, with an acute tip, cordate at the base, glabrous above a yellowish pubescence below. The flowers are monoecious, mono-tonous white to yellow, with a strong odor. The fruit are glabrous, ellipsoids ovoid drupes, yellow to orange brown in colour. *Terminalia chebula*, though contains several phytoconstituents like tannins, flavonoids, sterols, amino acids, fructose, resin, fixed oils etc., however, it is fairly rich in different tannins (approximately 32% tannin content). The chief components of tannin are chebulic acid, chebulinic acid, chebulagic acid, gallic acid, corilagin and ellagic acid (Chattopadhyay and Bhattacharyya, 2007; Kumar, 2006).

Materials and methods

Collection of seed material and preparation of extract

The dried seeds of *Terminalia chebula* were collected from Indian herbal market and were carefully washed with tap water followed by rinsing in distilled water and air-dried at room temperature for few days in order to remove unwanted materials present in the surface of seeds. Then they were ground into semi-

powder by maceration, finally stored in cool and dry place in a clean air-tight container. Extraction of seed material with Ethanol was performed by direct method and condensation in a rotary evaporator was carried out at 40°C-45°C resulting in smooth, semi-viscous brown extract (Harborne, 1998).

Screening of radical scavenging activity by rapid dot-plot DPPH staining method

Drops of DPPH (0.4 mm) solution in methanol were loaded onto a 5 cm × 5 cm TLC plate (silica gel 60 F254; Merck) in each column and allowed to dry for 2 minutes (Soler-Rivas et al., 2000). The first row of TLC plate was considered as control, containing only DPPH. Ethanol extract of dried seeds of *Terminalia chebula* of various concentrations was carefully loaded onto the DPPH spot in second row. The third row of TLC plate was considered as standard reference, where ascorbic acid was carefully loaded onto the DPPH spot. Stained silica gel layer revealing purple background with yellow or white spots indicates radical scavenging capability of the plant extract, whereas no colour change indicates that the plant extract couldn't exhibit radical scavenging activity.

Qualitative phytochemical analysis of *Terminalia chebula*

Screening of phytochemicals for ethanol extract of dried seeds of *Terminalia chebula* was carried out comparatively using standardized methods (Harborne, 1998).

Quantitative estimation of total phenols and flavonoids

Determination of total Phenols

Folin-Ciocalteu reagent method was used to determine the total phenolic compounds with slight modifications (Spanos and Wroslad, 1990). One hundred µL of ethanol extract of dried seeds of *Terminalia chebula* (1 mg/mL) was mixed with 900 µL of distilled water and 1 mL of Folin-Ciocalteu reagent (1:10 diluted with distilled water). After 5 mins, 1 mL of sodium carbonate (20% w/v) solution was added. The mixture was then allowed to stand for 30 mins incubation in dark at room temperature. The absorbance was measured by UV-vis spectrophotometer at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (GAE/mg of extract), which is a common reference compound.

Determination of total flavonoids

The total flavonoid content of ethanol extract of dried seeds of *Terminalia chebula* was determined using aluminium chloride reagent method with slight modifications (Liu et al., 2007). Five hundred µL of extract (1 mg/mL) was mixed

with 0.5 mL of methanol and 0.5 mL of (5% w/v) sodium nitrite solution. Then, 0.5 mL (10% w/v) aluminium chloride solution was added followed by 1 mL of 1M sodium hydroxide. The mixture was incubated for 30 minutes at room temperature and the absorbance was measured by UV-vis spectrophotometer at 510 nm. The result was expressed as (QE/mg of extract) quercetin equivalent.

In vitro Antioxidant activities of ethanol extract of dried seeds of *Terminalia chebula*

(a) Free radical Scavenging activity

The antioxidant activity was determined by DPPH scavenging activity (Khalaf et al., 2008) in which various concentrations of ethanol extract (5-30 µg/mL) was been pipetted out in clean test tubes. Freshly prepared DPPH (1, 1-Diphenyl-2-picryl hydrazyl) solution (1 mL) was added to each tube and the samples were incubated in dark at 37°C for 20 mins and the absorbance was measured by UV-vis spectrophotometer at 517 nm. The data were expressed as the percent decrease in the absorbance compared to the control. Ascorbic acid was used as standard. The percentage inhibition of radical scavenging activity was calculated as,

$$\% \text{ of Radical Scavenging Activity} = [(\text{Control OD} - \text{Sample OD}) / \text{Control OD}] * 100$$

(b) Phosphomolybdenum reduction activity

Total antioxidant capacity can be calculated in which various concentrations of ethanol extract (10-60 µg/mL) from the prepared sample (1 mg/mL) was been pipetted out and 1 mL of the reagent solution was added, followed by incubation in boiling water bath at 95°C for 90 mins. After cooling the sample to room temperature, and the absorbance was measured by UV-vis spectrophotometer at 695 nm (Prieto et al., 1999). A typical blank solution contained 1 mL of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions. Ascorbic acid was used as standard. The percentage of reduction was calculated as,

$$\% \text{ of Phosphomolybdenum reduction} = [(\text{Sample OD} - \text{Control OD}) / \text{Sample OD}] * 100$$

(c) Ferric (Fe³⁺) reducing power activity

The ethanol extract was taken in various concentrations (20-120 µg/mL) and was mixed with 1 mL of phosphate buffer (0.2M, pH-6.6) and 1 mL of potassium ferricyanide (1% w/v), and incubated at 50°C in water bath for 30 mins. Then, 0.5 mL of trichloroacetic acid (10% w/v) was added to the mixture followed by the addition of 0.1 mL of freshly prepared ferric chloride (0.01% w/v) and then centrifuged at 3000 rpm for 10 mins, in requirement if precipitate was observed and the absorbance was measured by UV-vis spectrophotometer at 700 nm (Oyaizu, 1986). Ascorbic acid was used as standard. The percentage of

reduction was calculated as,

$$\% \text{ of Fe}^{3+} \text{ reduction} = [(\text{Sample OD} - \text{Control OD}) / \text{Sample OD}] * 100$$

(d) Hydroxyl radical (OH) scavenging activity

The scavenging activity of ethanol extract of *Terminalia chebula* on hydroxyl radical was measured according to the method (Klein et al., 1981). Various concentrations of ethanol extract (50-300 µg/mL) were added with 1 mL of iron-EDTA solution (0.13% Ferrous Ammonium Sulfate and 0.26% EDTA w/v), 0.5 mL of EDTA solution (0.018% w/v), and 1 mL of dimethyl sulphoxide (DMSO) (0.85% v/v in 0.1M phosphate buffer, pH-7.4). The reaction was initiated by adding 0.5 mL of ascorbic acid (0.22% w/v) and incubated at 80°C-90°C for 15 min in water bath. After incubation the reaction was terminated by the addition of 1 mL of ice-cold TCA (17.5% w/v). 3 mL of freshly prepared Nash reagent was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the colour formed was measured by UV-vis spectrophotometer at 412 nm. Ascorbic acid was used as standard. The percentage of hydroxyl radical scavenging activity was calculated as,

$$\% \text{ of (OH}^\bullet \text{) Scavenging Activity} = [(\text{Control OD} - \text{Sample OD}) / \text{Control OD}] * 100$$

In vitro Anti-diabetic activity of ethanol extract of dried seeds of *Terminalia chebula*

Alpha amylase inhibition method

The anti-diabetic activity was determined by alpha amylase inhibition method in which various concentrations of ethanol extract (50-300 µg/mL) was been pipetted out in clean test tubes (Hansawasdi et al., 2000). 10 µL of freshly prepared amylase enzyme solution (1% w/v) was added to each tube and the samples were incubated in room temperature at 37°C for 10 mins. After incubation period, 0.5 mL of starch (1% w/v) was added and was incubated in room temperature at 37°C for 1 hour. To terminate the enzymatic reaction, 0.1 mL of freshly prepared 1N hydrochloric acid, (v/v) were added to the mixture, followed by the addition of 0.1 mL of freshly prepared iodine solution (1% w/v). The data were expressed as the percent increase in the absorbance was measured by UV-vis spectrophotometer at 565 nm. Acarbose was used as standard. The percentage inhibition of alpha amylase was calculated as,

$$\% \text{ of alpha amylase inhibition} = [(\text{Sample OD} - \text{Control OD}) / \text{Sample OD}] * 100$$

Thin layer chromatography analysis

Thin layer chromatography (TLC) analysis was carried out for

ethanol extract of dried seeds of *Terminalia chebula* on silica gel aluminium sheet (Merck Silica gel 60 F254). The ethanol extract was spotted at 0.5 mm above from the bottom of the TLC plate. The spotted TLC plate was placed in a 100 mL beaker containing solvent mixture (Stahl, 2005). The chromatogram was developed and the spots were visualized under UV light at 254 nm as well as in iodine vapour. The ratio in which distinct coloured bands appeared was optimized and R_f values were calculated.

Screening of crude extract for antibacterial activity

Agar Well diffusion assay

Nutrient agar was prepared and poured in the sterile petri plates and allowed to solidify. 24 hours grown bacterial pathogens *Escherichia coli* (MTCC 443), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Shigella flexneri* (MTCC 1457), *Proteus vulgaris* (MTCC 426), *Klebsiella pneumoniae* (MTCC 109) and *Micrococcus luteus* (MTCC 1538) were swabbed on nutrient agar plates (Eloff, 1998). Then, the stock crude of ethanol extract of dried seeds of *Terminalia chebula* was prepared and varying concentration (250µg, 500µg, 750µg and 1000µg/mL) of ethanol extract was loaded in the wells made using sterile cork borer. Tetracycline (30µg/mL) was used as standard. The plates were then incubated at 37°C for 24 hours. After incubation the inhibition diameter was measured using zone scale.

Identification of bioactive compounds by Gas Chromatography-Mass Spectrometry analysis

The presence of active compounds was screened by thin layer chromatography and the active compounds were identified by gas chromatography and mass spectrometry (GC-MS) method, (TSQ QUANTUM XLS). The name of the instrument is Gas Chromatography-Mass Spectrometry and the instrument made is of Thermo scientific. The software required for analytical studies

is XCALIBUR (ver-2.2) (Saraswathi et al., 2019). The column size is of TG-5MS (30mX0.25mmX0.25µm). The injector temperature and interface temperature (°C) was at 280°C.

Statistical analysis

All the experiments were conducted in triplicates and data given in tables were average of three replicates. All data were reported as the mean ± standard deviation of three replicates.

Results and discussion

Screening of radical scavenging activity by rapid dot-plot DPPH staining method

The results of dot-plot assay showed active spots in which various concentrations of ethanol extract of dried seeds of *Terminalia chebula* were placed in respective rows. The zone exhibiting purple colour indicates that there is no antioxidant (free radical scavenging) activity and the zone exhibiting yellow colour indicates antioxidant activity. From the results obtained (Figure 2), it is evident that ethanol extract of *Terminalia chebula* has effective antioxidant activity (scavenging the free radicals), also compared with standard ascorbic acid.

Qualitative Phytochemical analysis

Results of Phytochemical screening for the ethanol extract (Table 1) revealed the presence of alkaloids, phenols, tannins, diterpenes, flavonoids, terpenoids, etc. Alkaloids play significant role in plant protection and survival, since they have ability to ensure survival against several microbes. Phenolic compounds present in medicinal plants have definitive therapeutic applications such as increased bile secretion, reduction in blood cholesterol level, lipid

Table 1. Qualitative analysis of ethanol extract of dried seeds of *Terminalia chebula*

Phytochemicals	Tests	Results
Alkaloids	Mayer's test	+
	Hager's test	+
Phenols	Ferric chloride (5%) test	+
Tannins	Lead acetate test	+
Flavonoids	Sodium hydroxide test	+
Glycosides	Legal's test	--
Steroids	Libermann-Burchard test	+
Terpenoids	Salkowski test	+
Saponins	Foam test	--
Proteins	Xanthoproteic test	+
Diterpenes	Copper acetate test	+
Carbohydrates	Fehling's test	--
Aminoacid	Ninhydrin test	--

Figure 2. Dot-plot assay of ethanol extract of dried seeds of *Terminalia chebula*

level, antibacterial activity against *Staphylococcus* species (Molyneux et al., 1996; Gryglewski et al., 1987).

Determination of total phenols and flavonoids

The quantitative estimation of phenols and flavonoids were carried out in which phenol content was higher when compared to flavonoid. This proves these compounds might be responsible for potent antioxidant activity. Total phenolic content was found to be 48.52 ± 0.36 GAE/mg, flavonoid content was 13.59 ± 0.28 QE/mg. From the results, it is significant that due to presence of higher phenolic content, the antioxidant activities were found to be higher for ethanol extract of *Terminalia chebula*.

In vitro Antioxidant activities of ethanol extract of dried seeds of *Terminalia chebula*

(a) Free radical Scavenging activity

Antioxidant molecules can quench DPPH free radicals (i.e by providing hydrogen atoms or by electron donation, via a free radical attack on the DPPH molecule) and convert them to colourless. The percentage of DPPH[•] scavenging activity was

Table 2. Radical scavenging activity by DPPH[•] method of ethanol extract of dried seeds of *Terminalia chebula*

Concentration (µg/mL)	Percentage of Radical scavenging activity* (RSA)
5	33.19 ± 0.26
10	45.62 ± 0.41
15	48.19 ± 0.15
20	63.73 ± 0.37
25	69.25 ± 0.32
30	75.08 ± 0.19

*Average value of 3 replicates

$75.08 \pm 0.19\%$ in ethanol extract of dried seeds of *Terminalia chebula* at $30 \mu\text{g/mL}$ concentration (Table 2). The IC_{50} value was found to be $15.56 \mu\text{g/mL}$ concentration and was compared with standard (Ascorbic acid, IC_{50} value as $10.98 \mu\text{g/mL}$ concentration). The radical scavenging activity was well observed for ethanol extract in which 1,1-diphenyl-2-picryl hydrazyl was reduced to 1,1-diphenyl-2-picryl hydrazine (Awika et al., 2003).

(b) Phosphomolybdenum reduction activity

The total antioxidant activity of ethanol extract of dried seeds of *Terminalia chebula* was measured spectrophotometrically by phosphomolybdenum reduction method which is based on the reduction of Mo (VI) to Mo (V) by the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm (Yildirim et al., 2001). The maximum reducing ability for ethanol extract was higher as $78.93 \pm 0.18\%$ at $60 \mu\text{g/mL}$ concentration. The experiment demonstrated higher antioxidant activity the RC_{50} of $29.59 \mu\text{g/mL}$ concentration (Table 3) and was compared with standard Ascorbic acid ($\text{RC}_{50} = 20.28 \mu\text{g/mL}$ concentration).

Table 3. Phosphomolybdenum reduction of ethanol extract of dried seeds of *Terminalia chebula*

Concentration (µg/mL)	Percentage of Phosphomolybdenum reduction*
10	13.70 ± 0.37
20	32.27 ± 0.24
30	50.69 ± 0.26
40	57.02 ± 0.45
50	67.17 ± 0.40
60	78.93 ± 0.18

*Average value of 3 replicates

Table 4. Ferric reducing activity of ethanol extract of dried seeds of *Terminalia chebula*

Concentration (µg/mL)	Percentage of Fe^{3+} reduction*
20	48.32 ± 0.42
40	51.26 ± 0.33
60	53.89 ± 0.26
80	61.69 ± 0.37
100	64.75 ± 0.30
120	66.59 ± 0.26

*Average value of 3 replicates

(c) Ferric (Fe^{3+}) reducing power activity

The ethanol extract of dried seeds of *Terminalia chebula* react with Potassium ferricyanide and ferric chloride there by gets reduced to Potassium ferrocyanide and ferrous chloride (Fe^{3+} to Fe^{2+}) turning to various shades of green (Stadtman, 1990). The reducing power of Fe^{3+} to Fe^{2+} by ethanol extract of dried seeds of *Terminalia chebula* was studied and showed reduction ability in a dose dependent manner. The maximum reduction for ethanol extract of dried seeds of *Terminalia chebula* was $66.59 \pm 0.26\%$ at $120 \mu\text{g/mL}$ concentration (Table 4). The RC_{50} value for ethanol extract of *Terminalia chebula* was found to be $20.69 \mu\text{g/mL}$ concentration and was compared with standard ($15.11 \mu\text{g/mL}$ concentration) Ascorbic acid.

(d) Hydroxyl radical (OH) scavenging activity

Hydroxyl radical is the most reactive oxygen centered species and causes severe damage to adjacent biomolecule. Hydroxyl radical scavenging activity was estimated by generating the hydroxyl radicals using ascorbic acid-iron EDTA. The hydroxyl radicals were formed by the oxidation reaction with the Dimethyl

Sulphoxide (DMSO) to yield formaldehyde, which provides a convenient method to detect hydroxyl radicals by treatment with Nash reagent.

The hydroxyl radical is formed by the combination of Fe (II) and hydrogen peroxide, which is a Fenton reaction (Sanches-Moreno, 2002). This assay is used to quantify or measure the capacity of putative antioxidants to neutralize or stop the formation of precursor molecules (Magalhaes et al., 2008). These scavenging compounds would behave as antioxidant molecules in a preventive manner.

This particular scavenging activity is considered to be important since, the OH radical is highly reactive. The hydroxyl radical is a reactive free radical generated in biological/living systems. The maximum activity of hydroxyl radical scavenging potential was found to be $47.86 \pm 0.39\%$ at $300 \mu\text{g/mL}$ concentration (Table 5). The IC_{50} value was found to be $264.55 \mu\text{g/mL}$ concentration and was compared with standard ($20.87 \mu\text{g/mL}$ concentration) Ascorbic acid.

Table 5. Hydroxyl radical (OH) scavenging activity of ethanol extract of dried seeds of *Terminalia chebula*

Concentration ($\mu\text{g/mL}$)	Percentage of Hydroxyl radical (OH) scavenging activity*
50	16.46 ± 0.47
100	21.95 ± 0.40
150	29.26 ± 0.34
200	37.80 ± 0.25
250	43.29 ± 0.13
300	47.86 ± 0.39

*Average value of 3 replicates

Table 6. Alpha Amylase inhibition activity of ethanol extract of dried seeds of *Terminalia chebula*

Concentration ($\mu\text{g/mL}$)	Percentage of alpha amylase inhibition activity*
50	28.69 ± 0.18
100	33.04 ± 0.26
150	37.82 ± 0.47
200	43.46 ± 0.13
250	60.11 ± 0.34
300	62.53 ± 0.36

*Average value of 3 replicates

Table 7. Antibacterial activity of ethanol extract of dried seeds of *Terminalia chebula*

Test organisms	Zone of inhibition (mm)				
	Standard	250 $\mu\text{g/mL}$	500 $\mu\text{g/mL}$	750 $\mu\text{g/mL}$	1000 $\mu\text{g/mL}$
<i>S. aureus</i>	16	13	14	15	16
<i>M. luteus</i>	15	14	15	16	20
<i>E. coli</i>	23	13	13	14	19
<i>K. pneumoniae</i>	29	14	15	17	17
<i>P. vulgaris</i>	18	17	18	20	20
<i>B. subtilis</i>	16	12	14	14	15
<i>S. flexneri</i>	19	14	14	15	16

In vitro Anti-diabetic activity of ethanol extract of dried seeds of *Terminalia chebula*

The anti-diabetic activity was evaluated by alpha amylase inhibition method. Amylase enzyme hydrolyzes the alpha (α) bonds of multi-linked polysaccharide glycogen and starch as such to yield glucose and maltose. The anti-diabetic activity was well observed for the ethanol extract of dried seeds of *Terminalia chebula*, in which the percentage of alpha amylase inhibition was $62.53 \pm 0.36\%$ at $300 \mu\text{g/mL}$ concentration (Table 6). The IC_{50} value was found to be $43.46 \mu\text{g/mL}$ concentration and was compared with standard ($19.74 \mu\text{g/mL}$ concentration) Acarbose.

Thin Layer Chromatography analysis

Thin layer chromatography analysis was carried out in the optimized solvent system of Toluene (1 mL): Ethyl acetate (0.8 mL): Methanol (0.2 mL). The separated active compounds were visualized in UV light (short and long) and in iodine stain. The R_f values of the separated compounds in iodine stain were found to be 0.83, 0.75, 0.50 and 0.46.

Screening of crude extract for antibacterial activity

The maximum inhibition for ethanol extract of dried seeds of *Terminalia chebula* (Table 7 and Figure 3) was against *P. vulgaris* and *M. luteus* (20 mm), when compared to other

bacterial pathogens *E. coli* (19 mm), *K. pneumoniae* (17 mm), *S. flexneri* and *S. aureus* (16 mm), *B. subtilis* (15 mm). The ethanolic extract of *T. chebula* fruit was found effective against both gram-positive and gram-negative bacteria such as *Salmonella typhi* SSFP 4S, *Staphylococcus epidermidis* MTCC 3615, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* MTCC 441 and *Pseudomonas aeruginosa* ATCC 27853 suggesting its broad spectrum antimicrobial activity (Kannan et al., 2009). Present research work were similar to above findings in which ethanolic seed extract of *T. chebula* was effective against the tested bacterial pathogens proving to be an resistant antibiotic for treating several mode of bacterial infections.

Identification of bioactive compounds by Gas Chromatography-Mass Spectrometry analysis

The GC-MS analysis for ethanol extract of dried seeds of *Terminalia chebula* (Table 8 and figure 4) revealed the presence of phyto-active compounds such as Carnegine, Dodecahydropyrido (1,2-b) isoquinolin-6-one, Corynan-17-ol, 18, 19-didehydro-10-methoxy-acetate (ester), Estra-1,3,5 (10)-trien-17a-ol, Oleic acid, Phytol (Table 9) exhibiting biological activities.

Figure 3. Antibacterial activity by agar well diffusion method

Table 8. GC-MS analysis of ethanol extract of dried seeds of *Terminalia chebula*

S. No.	Compounds name	RT	Compounds structure	Molecular weight (g/mol)	Molecular formula
1	Furfural	4.95		96	C ₅ H ₄ O ₂
2	1,3-Octanediol	6.38		146	C ₈ H ₁₈ O ₂
3	d-Mannose	8.33		180	C ₆ H ₁₂ O ₆
4	2,4,5-(3H)-Pyrimidinetrione, dihydro-6-hydroxy-	9.07		144	C ₄ H ₅ N ₃ O ₃
5	Carnegine	9.8		221.87	C ₁₃ H ₁₉ NO ₂
6	2-(2-methyl-propenyl)-cyclohexanone oxime	11.85		167	C ₁₀ H ₁₇ NO
7	Dodecahydropyrido(1,2-b) isoquinolin-6-one	13.23		207	C ₁₃ H ₂₁ NO
8	Methyl Z-11-tetradecenoate	14.93		240.97	C ₁₅ H ₂₈ O ₂
9	2-Acetoxy-3,3-dimethyl-2-(3-oxo-but-1-enyl)-Cyclobutane carboxylic acid, methyl ester	15.77		226	C ₁₃ H ₁₆ O ₃
10	Cyclopentaneundecanoic acid, methyl ester	16.92		268.84	C ₁₇ H ₃₂ O ₂
11	Estra-1,3,5(10)-trien-17a-ol	17.58		256	C ₁₈ H ₂₄ O
12	Oleic acid	18.6		282	C ₁₈ H ₃₄ O ₂
13	Phytol	18.83		296.22	C ₂₀ H ₄₀ O
14	17-octadecen-1-ol acetate	19.43		309.60	C ₂₀ H ₃₈ O ₂
15	But-2-endiamide, N,N'-bis (4-methoxy phenyl)-	20.42		326	C ₁₄ H ₁₆ N ₂ O
16	Corynan-17-ol, 18,19-didehydro-10-methoxy-acetate (ester)	22.77		368	C ₂₂ H ₂₈ N ₂ O ₃
17	2,4-dimethoxy-10H-acridin-9-one	11.3		255	C ₁₆ H ₁₅ NO ₃

Figure 4. GC-MS Chromatogram of ethanol extract of dried seeds of *Terminalia chebula*

Table 9. Bio-activity of ethanol extract of dried seeds of *Terminalia chebula* from GC-MS analysis

S. No.	Compounds name	Pharmacological activity*
1	Furfural	Antiseptic agent and Flavouring agent
2	Dodecahydropyrido(1,2-b) isoquinolin-6-one	Antioxidant and Antimicrobial activities
3	Estra-1,3,5(10)-trien-17a-ol	Hair loss activity
4	Oleic acid	5- α reductase inhibitor, allergenic, anti-inflammatory, anti-androgenic, cancer preventive, anemiagenic, anti-alopecic, antileukotriene-D4, choleretic, dermatitigenic, hypocholesterolemic activities, insectifuge, perfumery, propeptic, flavouring agent
5	Phytol	Aromatic Ingredient, Antinociceptive Antioxidant, Anti-allergic, Anti-inflammatory, Antimicrobial, Immunostimulant, Hepato-protective activities
6	Corynan-17-ol, 18,19-didehydro-10-methoxy-acetate (ester)	Anti-diarrhoeal and Anti-inflammatory activities

(*Haider Mashkoor Hussein et al., 2016; Radhakrishnan et al., 2017; Gomathi Rajashyamala and Elango, 2015; Ryu et al., 2011; Lim et al., 2006)

Conclusion

The dose concentration effect of medicinal drugs is highly dependent on both efficacy and toxicity with specific and targeted mechanism. The present research investigation revealed that ethanolic seed extract of *Terminalia chebula* composed of secondary metabolites along with efficient therapeutic property. The pure compound fractionated from column chromatography with validated structure of the active drug should be studied along with molecular mechanism in genetic domain organization.

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