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COMPARATIVE STUDIES OF ANTIBACTERIAL EFFECT OF FRACTION AND CRUDE EXTRACT OF *TERMINALIA CHEBULA* ON TWO PATHOGENIC BACTERIA

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ABSTRACT

This study investigated the comparative effect of *Terminalia chebula* crude extract and fraction on two pathogenic bacteria. Generally, crude extracts and fractions were effective against both bacterial strain by disc diffusion method, respectively. However, ethanol mixes methanol fraction at 8:12 and 10:10 combinations were exhibited 5 mm and 7 mm zone of inhibition against *Streptococcus pneumoniae* and *Staphylococcus aureus*, which was greater anti-bacterial activity than the all individual crude extract and fractions. Compared to crude extract, the fraction elicited higher antibacterial properties. The major components of extracts tested were identified by gas chromatography coupled with mass spectrometry (GC/MS) analysis. Our result showed that ethanol with methanol fraction extracts of *Terminalia chebula* can be used alongside conventional antibiotics to fight agents of infections that are so prevalent in the hospitals.

INTRODUCTION

Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being (1). Now- a-day modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine (2), because of higher plants have the capacity to produce a large number of organic phytochemicals with complex structural diversity that is known as secondary metabolites. Several medicinal herbs practiced in traditional folk medicine in India were screened for the presence of antibacterial activity for thousands of year.

Terminalia chebula Retz, (Family *Combretaceae*) is a flowering evergreen tree attaining a height up to 30m, with is distributed in the sub-Himalayan tracks, and the eastern, western and southern parts of India (3). Different part of this plant has germinated substantial compounds to cure various diseases like cancer (4), bacteria (5), diabetic (6), and hepatoprotective (7) activity. Phytochemical investigations of *Terminalia chebula* have been reported on presence of tannins, carbohydrates, glycosides, phenols, alkaloids, terpenoids and flavonoids (8). In view of these reported medicinal values, the present work was carried out to examine the antibacterial potential of a different solvent crude extracts and fractions of *Terminalia chebula* against clinically important reference bacterial strains such as *Streptococcus pneumoniae* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Plant material

Young and mature leaves were collected separately from *Terminalia chebula* plants growing in University campus, Vels University. 2-4 days old leaves were collected during emergence time for anti-bacterial studies. The leaves were separated from stems, washed in clean water, and dried at room temperature. The shaded dried leaves were weighted and ground in a sterile mortar.

Extraction

The shade dried plant material was chopped into small pieces and finally pulverized into fine powder. 500g of powdered plant material was soaked and then extracted successively water, ethanol, methanol, acetone, hexane and butanol solvent in separate Soxhlet extractor for 48h. The extract was concentration to dryness in rotary vacuum evaporator and stored -30˚C until further use.

Fractionation

500g of powdered plant material was soaked and then extracts with ethanol in a soxhlet apparatus. After, collected extracts were evaporated to dryness in desiccators. After, some amount of these crude extract was fractionated with water, ethanol, methanol, acetone, hexane and butanol using column chromatography under reduced pressure over silica gel. These fractions were then stored in a refrigerator until used for the phytochemical and antimicrobial screening.

Micro organisms

The organisms used were clinical isolates of *Streptococcus pneumoniae* and *Staphylococcus aureus* (Dental clinics in and around Thanjavur and Chennai, Tamil Nadu, India) typed cultures.

Determination of antimicrobial activity

Culture supernatants with fractions and crude extracts of the plants were used in the disc-diffusion method separately. *Streptococcus pneumoniae* and *Staphylococcus aureus* were swabbed on the surface of the sabouraud agar plates and discs (Whatman No.1 filter paper with 9 mm diameter) impregnated with the 50 µl of each plant sample was place on the surface individually. To compare the anti-bacterial activities, Nystatin (20 µg/disc) used as standard antibiotic and negative control, a blank disc impregnated with solvent followed by drying was used. The plates (triplicates) were incubated 28°C for 72 h. The antimicrobial potency of the test samples was measured by determining the diameter of the zones of inhibition in millimeter.

GC-MS analysis

30 g powdered sample of *Terminalia chebula* were soaked and dissolved in 75 ml of methanol for 24 h. Then the filtrates were collected by evaporated under liquid nitrogen. The GC-MS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µm df capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised upto 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 ml/min. The ionization

voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

Table 1: Antimicrobial activity of *Terminalia chebula* individual solvent crude extract tested against *Streptococcus pneumoniae* and *Staphylococcus aureus* by disk diffusion method.

Plant sample / Solvent	Zone of inhibition (mm)					
	Water	Ethanol	Methanol	Acetone	Hexane	Butanol
Streptococcus pneumoniae	0.5	4	5	1	0.5	3
Staphylococcus aureus	1	6	4	0.5	1	2

Table 2: Antimicrobial activity of *Terminalia chebula* individual fraction tested against *Streptococcus pneumoniae* and *Staphylococcus aureus* by disk diffusion method.

Plant sample/ Fraction concentration	Zone of inhibition (mm)								
	18:2 (E/M)	16:4 (E/M)	14:6 (E/M)	12:8 (E/M)	10:10 (E/M)	8:12 (E/M)	6:14 (E/M)	4:16 (E/M)	2:18 (E/M)
Streptococcus pneumoniae	2	1	3	0.5	4	5	1	2	4
Staphylococcus aureus	3	2	1	2	7	3	2	0.5	6

Table 3: Antimicrobial activity of ethanol and methanol combined fractions of *Terminalia chebula* tested against *Streptococcus pneumoniae* and *Staphylococcus aureus* by disk diffusion method.

Plant sample / Solvent	Zone of inhibition (mm)					
	Water	Ethanol	Methanol	Acetone	Hexane	Butanol
Streptococcus pneumoniae	1	2	5	0.5	2	3
Staphylococcus aureus	0.5	3	4	2	0.5	1

Table 4: The main compounds identified by GC-MS in the extracts of *Terminalia chebula*

S.No.	Peak name	Retention time	Peak Area	%Peak Area
1.	Name: 2-Cyclopenten-1-one, 2-hydroxy- Formula: $C_5H_6O_2$ MW: 98	4.74	1610841	0.5755
2.	Name: 2-Furancarboxaldehyde, 5-methyl- Formula: $C_6H_6O_2$ MW: 110	5.32	2684362	0.9591
3.	Name: 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one Formula: $C_6H_8O_4$ MW: 144	5.52	256589	0.0917
4.	Name: Phenol Formula: C_6H_6O MW: 94	5.71	4753129	1.6982
5.	Name: 1,2-Cyclohexanedione Formula: $C_6H_8O_2$ MW: 112	5.96	1628614	0.5819
6.	Name: Cycloheptanone Formula: $C_7H_{12}O$ MW: 112	6.35	1561903	0.5580
7.	Name: 5H-1,4-Dioxepin, 2,3-dihydro-2,5-dimethyl-	6.71	1413768	0.5051

	Formula: $C_7H_{12}O_2$ MW: 128			
8.	Name: 6-Methoxytetrazolo(b)pyridazine Formula: $C_5H_5N_5O$ MW: 151	7.18	414774	0.1482
9.	Name: 1-Piperidineacetonitrile Formula: $C_7H_{12}N_2$ MW: 124	7.36	3172359	1.1334
10.	Name: Benzoic acid, hydrazide Formula: $C_7H_8N_2O$ MW: 136	7.59	354349	0.1266
11.	Name: 2,3-Dimethylfumaric acid Formula: $C_6H_8O_4$ MW: 144	7.76	3128878	1.1179
12.	Name: Levoglucosenone Formula: $C_6H_6O_3$ MW: 126	7.96	2071721	0.7402
13.	Name: Acetamide, 2,2,2-trifluoro-N-[2-(hexahydro-1(2H)-azocinyl)ethyl]- Formula: $C_{11}H_{19}F_3N_2O$ MW: 252	8.37	610798	0.2182
14.	Name: Piperazine, 1-(aminoacetyl)- Formula: $C_6H_{13}N_3O$ MW: 143	9.61	802018	0.2865
15.	Name: Resorcinol Formula: $C_6H_6O_2$ MW: 110	9.87	697368	0.2492
16.	Name: 2-Furancarboxaldehyde, 5-(hydroxymethyl)- Formula: $C_6H_6O_3$ MW: 126	10.12	5107018	1.8246
17.	Name: Ethanone, 1-(2-hydroxy-5-methylphenyl)- Formula: $C_9H_{10}O_2$ MW: 150	11.51	128554	0.0459
18.	Name: N-(5-Amino-4-cyano-1-pyrazolyl)phthalimide Formula: $C_{12}H_7N_5O_2$ MW: 253	11.69	554674	0.1982
19.	Name: 2-Butenoic acid, 4,4-dimethoxy-, methyl ester Formula: $C_7H_{12}O_4$ MW: 160	12.07	211709	0.0756

20.	Name: 2,2-Bis(2'-methoxyphenyl)propane Formula: $C_{17}H_{20}O_2$ MW: 256	12.92	9024535	3.2242
21.	Name: 1,2,3-Benzenetriol Formula: $C_6H_6O_3$ MW: 126	13.16	232141472	82.9379
22.	Name: D-Allose Formula: $C_6H_{12}O_6$ MW: 180	15.30	4999200	1.7861
23.	Name: Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, (R)- Formula: $C_{15}H_{22}O$ MW: 218	19.21	1451187	0.5185
24.	Name: Phenethylamine, 3,4,5-trimethoxy-à-methyl- Formula: $C_{12}H_{19}NO_3$ MW: 225	19.36	96635	0.0345
25.	Name: Tridecanoic acid, methyl ester Formula: $C_{14}H_{28}O_2$ MW: 228	21.19	776117	0.2773
26.	Name: Dodecanoic acid, 10-methyl-, methyl ester Formula: $C_{14}H_{28}O_2$ MW: 228	23.34	245332	0.0877

RESULT AND DISCUSSION

Antimicrobial activity of *Terminalia chebula* water, ethanol, methanol, acetone, hexane and butanol solvents fractions and crude extracts were examined and found to exhibit good anti-bacterial activity at disc diffusion method against both pathogenic organisms (Table 1 and 2). Among the test, all the soul fractions and extracts showed good anti-bacterial activity, the results were expressed in term of diameter of zone of inhibition in millimeter. However, moderate activity was observed ethanol and methanol fraction than extract, thus various combination of these fractions again treat fresh *Streptococcus pneumoniae* and *Staphylococcus aureus* strains (Table 3). The 8:12 and 10:10 combinations of ethanolic methanol fraction was showed 5 mm zone inhibition activity against *Streptococcus pneumoniae* and 7 mm zone inhibition activity against *Staphylococcus aureus* strain which was further comparable with that of standard antibiotic Nystatin. Whereas, the crude extract of different solvent did not show significant inhibition activity.

In addition, GC-MS analyses, totally 26 compounds identified from the methanol fractions of the Terminalia chebula are presented in Table 4. The plant samples revealed the synthesis of 2-Cyclopenten-1-one, 2-hydroxy-; 2-Furancarboxaldehyde, 5-methyl-; 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one; Phenol; 1,2-Cyclohexanedione; Cycloheptanone; 5H-1,4-Dioxepin, 2,3-dihydro-2,5-dimethyl-; 6-Methoxytetrazolo(b)pyridazine; 1-Piperidineacetonitrile; Benzoic acid, hydrazide; 2,3-Dimethylfumaric acid; Levoglucosenone; Acetamide, 2,2,2-trifluoro-N-[2-(hexahydro-1(2H)-azocinyl)ethyl]-; Piperazine, 1-(aminoacetyl)-; Resorcinol; 2-Furancarboxaldehyde, 5-(hydroxymethyl)-; Ethanone, 1-(2-hydroxy-5-methylphenyl)-; N-(5-Amino-4-cyano-1-pyrazolyl)phthalimide; 2-Butenoic acid, 4,4-dimethoxy-, methyl ester; 2,2-Bis(2'-methoxyphenyl)propane; 1,2,3-Benzenetriol; D-Allose; Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, (R)-; Phenethylamine, 3,4,5-trimethoxy-à-methyl-; Tridecanoic acid, methyl ester; Dodecanoic acid, 10-methyl-, methyl ester. All these compounds are of pharmacological importance as they possess the properties such as analgesic, anti-diabetic, antibacterial, and antifungal. Based on the results, we believe the plants used in this study have potential as sources for antibacterial drug, and we have experiments underway leading to the identification of the active molecules present in these plants.

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