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ANTIBACTERIAL EFFECT OF FRACTION AND CRUDE EXTRACT OF *TERMINALIA CHEBULA* ON *STREPTOCOCCUS PNEUMONIA* AND *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

This study investigated the effect of *Terminalia chebula* crude extract and fraction on two pathogenic bacteria. Generally, crude extracts and fractions were effective against both bacterial strains by disc diffusion method, respectively. Nevertheless, ethanol mixes methanol fraction at 8:12 and 10:10 combinations were showed 5 mm and 7 mm zone of inhibition against *Streptococcus pneumonia* and *Staphylococcus aureus*, which was better anti-bacterial activity than the all separate 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 contest agents of infections that are so predominant in the clinics.

KEYWORDS: *Terminalia chebula,* crude extracts, GC-MS analysis and anti-bacterial activity.

INTRODUCTION

Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and wellbeing.[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 pneumonia* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Plant material

Young and mature leaves of *Terminalia chebula* were poised distinctly from Thanjavur District, Tamilnadu. 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. 100g 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

100g of powdered plant material was soaked and then extracts with ethanol in a soxhlet's 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 pneumonia* and *Staphylococcus aureus* (Dental clinics in and around Thanjavur, 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 distinctly. *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 50µl of each plant sample was place on the surface separately. To relate the anti-bacterial activities, Nystatin (20µg/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 72h. The antimicrobial effectiveness of the test trials was measured by determining the diameter of the zones of inhibition in millimeter.

GC-MS analysis

30g pulverized sample of *Terminalia chebula* were soaked and dissolved in 75ml of methanol for 24h. Then the filtrates were collected by vaporized 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 µmdf capillary column. The apparatus 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 preserved 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.

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 antibacterial activity at disc diffusion technique 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.

Table 1: Antimicrobial activity of Terminaliachebulacrude extract tested against Streptococcus pneumoniaeand Staphylococcus aureusby disc 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 *Terminaliachebula*individual fraction tested against *Streptococcus pneumoniae*and *Staphylococcus aureus*by disc 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 3: Antimicrobial activity of ethanol and methanol pooled fractions of *Terminaliachebula*tested against *Streptococcus pneumoniae*and *Staphylococcus aureus*by disc 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

Though, 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.

This was supported by an earlier study on an alcoholic extract that exhibited greater activity than the aqueous and hexane extracts against bacteria, with no cellular toxicity.[9] The broad spectrum of antibacterial activity was reported for *T. chebula*[10] and *T. arjuna*[11] The ethanol extract at a concentration of 1 mg/disc showed maximum inhibition against *S. epidermidis*, followed by *B. subtilis*.

In addition, GC-MS analyses, totally 26 compounds identified from the methanol fractions of the Terminaliachebula 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.

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

S. No.	Peak name	Retention time	Peak Area	%Peak Area
1	Name: 2-Cyclopenten-1-one, 2-hydroxy- Formula: C5H6O2 MW: 98	4.74	1610841	0.5755
2	Name: 2-Furancarboxaldehyde, 5-methyl- Formula: C6H6O2 MW: 110	5.32	2684362	0.9591
3	Name: 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one Formula: C6H8O4 MW: 144	5.52	256589	0.0917
4	Name: Phenol Formula: C6H6O	5.71	4753129	1.6982

	MW: 94			
5	Name: 1,2-Cyclohexanedione Formula: C6H8O2 MW: 112	5.96	1628614	0.5819
6	Name: Cycloheptanone Formula: C7H12O MW: 112	6.35	1561903	0.5580
7	Name: 5H-1,4-Dioxepin, 2,3-dihydro-2,5-dimethyl-Formula: C7H12O2 MW: 128	6.71	1413768	0.5051
8	Name: 6-Methoxytetrazolo(b)pyridazine Formula: C5H5N5O MW: 151	7.18	414774	0.1482
9	Name: 1-Piperidineacetonitrile Formula: C7H12N2 MW: 124	7.36	3172359	1.1334
10	Name: Benzoic acid, hydrazide Formula: C7H8N2O MW: 136	7.59	354349	0.1266
11	Name: 2,3-Dimethylfumaric acid Formula: C6H8O4 MW: 144	7.76	3128878	1.1179
12	Name: Levoglucosenone Formula: C6H6O3 MW: 126	7.96	2071721	0.7402
13	Name: Acetamide, 2,2,2-trifluoro-N-[2-(hexahydro-1(2H)-azocinyl)ethyl]- Formula: C11H19F3N2O MW: 252	8.37	610798	0.2182
14	Name: Piperazine, 1-(aminoacetyl)- Formula: C6H13N3O MW: 143	9.61	802018	0.2865
15	Name: Resorcinol Formula: C6H6O2 MW: 110	9.87	697368	0.2492
16	Name: 2-Furancarboxaldehyde, 5-(hydroxymethyl)- Formula: C6H6O3 MW: 126	10.12	5107018	1.8246
17	Name: Ethanone, 1-(2-hydroxy-5-methylphenyl)- Formula: C9H10O2 MW: 150	11.51	128554	0.0459
18	Name: N-(5-Amino-4-cyano-1-pyrazolyl)phthalimide Formula: C12H7N5O2	11.69	554674	0.1982

	MW: 253			
19	Name: 2-Butenoic acid, 4,4-dimethoxy-, methyl ester Formula: C7H12O4 MW: 160	12.07	211709	0.0756
20	Name: 2,2-Bis(2'-methoxyphenyl)propane Formula: C17H20O2 MW: 256	12.92	9024535	3.2242
21	Name: 1,2,3-Benzenetriol Formula: C6H6O3 MW: 126	13.16	232141472	82.9379
22	Name: D-Allose Formula: C6H12O6 MW: 180	15.30	4999200	1.7861
23	Name: Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, (R)- Formula: C15H22O MW: 218	19.21	1451187	0.5185
24	Name: Phenethylamine, 3,4,5-trimethoxy-à-methyl- Formula: C12H19NO3 MW: 225	19.36	96635	0.0345
25	Name: Tridecanoic acid, methylester Formula: C14H28O2 MW: 228	21.19	776117	0.2773
26	Name: Dodecanoic acid, 10-methyl-, methyl ester Formula: C14H28O2 MW: 228	23.34	245332	0.0877

Based on the results, we trust the florae used in this study have prospective as sources for antibacterial drug, and we have experiments underway leading to the identification of the active fragments present in these plants.

CONCLUSION

Antimicrobial activity of *Terminalia chebula* were carried out in different types of solvent extracts *like* water, ethanol, methanol, acetone, hexane and butanol. Our result revealed that that ethanol with methanol fraction extracts of *Terminalia chebula* extracts showed more activity against *Streptococcus pneumonia* and *Staphylococcus aureus.* In future, can be used alongside conventional antibiotics to contest agents of infections and to produce antibiotics.

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