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GC-MS Profiling and Analysis of Bioprotective Properties of *Terminalia chebula* against Non-Fermenting Gram-Negative Bacteria Isolated from Tertiary Care Hospital

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ABSTRACT

Due to the extensive use of commercially available antibiotics, there is an emergence of NFGNBs as nosocomial pathogens. The major concern is the adoption of multi-drug resistance among them which limits their alternatives for treatment. Nowadays, great emphasis is on exploring the medicinal efficacy of traditional plants for combating the infections caused by human pathogens. *Terminalia chebula* possesses several significant phyto-ingredient such as tannins and chebulinic acid which have been well reported to exhibit high antimicrobial and antioxidant activities. The present study aims on combating NFGNB infections with the various solvent extracts of *Terminalia chebula*. Isolation and characterization of NFGNB pathogens were done. *T. chebula* extracts were prepared in a series of different organic and water solvents based on increasing polarity index {Ethyl acetate (4.4), Methanol (5.1), and Water (9.0)} using the Soxhlet extraction method. Antibacterial activity of the crude plant extracts was determined by DPPH assay. GC-MS and HPLC analysis revealed the presence of a significant bioactive compound. The results demonstrate the strong antimicrobial activity and effective MIC, MBC, and MIC index values of the S1, S2, and S3 extract of *T. Chebula* against isolated NFGNB pathogens. Inhibitory activities of extracts were compared with the standard antibiotic, colistin. The medicinal plants have been regarded as valuable and cheap sources of various phytoconstituents which are used extensively in the development of drugs against various diseases.

Keywords: *Terminalia chebula*, Antibiotics, NFGNB, antioxidant, Phytochemicals

INTRODUCTION

As a result of the multidrug resistance, non-fermenting gram-negative bacilli (NFGNB) has arisen as a major cause of healthcare-associated illnesses. The antibiogram and prevalence of NFGNB are required for efficient management of infection caused by them. Increased resistance to currently accessible antibiotics has become a global issue. With the rise in the number of MDR pathogens, an alternative and dependable means of therapy is required. Herbal medicine is becoming increasingly popular because it is cost-effective and has little side effects on patients. Medicinal plants contain all phytoconstituents, which serve as a source of bioactive molecules required for considerable pharmacological

benefits with no side effects. They can be utilised to create new classes of potentially safer pharmaceuticals or therapies to treat a wide range of illnesses. Because of their broad safety profile, herbal medicines have historically been utilised to treat a variety of ailments. Hydrolysable tannins (which might range from 20 to 50 percent) account for 33 percent of the total phytoconstituents in *Terminalia chebula*.

Gallic acid, ellagic acid, chebulic acid, and gallotallins such 1,6 di-O-galloyl-D-glucose, 3,4,6 tri-O-galloyl-D-glucose,2,3,4,6 tetra-O-galloyl-D-glucose,1,2,3,4,6 penta-Ogalloyl-D-glucose are Alkaloids, glycosides, resins, gums, mucilages and other bioactive substances contribute to the therapeutic properties of plant (Rathinamoorthy et al., 2014). Because herbal chemicals have proved to have a promising effect in therapeutics, the quest for plant-based products has radically altered the drug discovery program. The diversity of bacteria in disease-causing abilities has always presented complications in their treatment plan (Quiroga et al., 2001). Numerous plant-based substances show potential anti-cancer activity (Srigopalram et al., 2012).Antioxidant properties have been discovered in phytosignatures such as vitamins (A,C,E,K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes, and minerals (Kathiresan et al., 2006; Heber, 2004; Kaur et al., 2002). *T.chebula* is a medium-to-large tree found in tropical and subtropical Asia, including China and Tibet. Fever, cough, diarrhea, gastroenteritis, skin illnesses, candidiasis, urinary tract infection, and wound infection are all treated using *Terminalia chebula,* which is commonly referred to as "Kadukkaai" by tribal people in Tamil Nadu, India (Dash,1991). *Terminalia chebula* extracts have been found to have antibacterial action against many bacterial species (Bag et al., 2009). *Terminalia chebula* (Fruit) extracts have shown high antibacterial activity and antioxidant capability as majored by DPPH technique. The entire plant has a high medicinal value and has long been used to treat a variety of human ailments. This herb was used to treat sore throats, high coughs, asthma, ulcers, gout, heartburn, vomiting, diarrhea, dysentery, bleeding piles and bladder ailments by some rural people (Saleem et al., 2002; Kim et al., 2006; Lee et al., 2007). The plant has been found to have antidiabetic, antibacterial, antioxidant, antimutagenic, anti-proliferative,anti-inflammatory, cardioprotective, and wound healing properties (Rathinamoorthy et al., 2014).

Tannins, phenolic compounds, and other phytoconstituents identified in *Terminalia chebula* are the key phytoconstituents responsible for the herb's therapeutic action (Rathinamoorthy et al., 2014). *Terminalia chebula* (Fruit) tannins are pyrogallols, and other components contain phenolics including ellagic acid, chebulinic acid, anthraquinones, and polyphenols, such as galloylglucose, corilagin, terflavin A, punicalagin, and triterpenemaslinic acid.

The focus of the study was to analyse the efficacy of different *Terminalia chebula* (Fruit) extracts against infections caused by non-fermenting gram-negative bacteria. The active molecules responsible for the powerful anti-bacterial and anti-oxidant properties were investigated through phytochemical research.

MATERIALS AND METHODS

Isolation and characterization of NFGNB pathogens

Collection of clinical samples: The research was carried out in Dehradun's tertiary care hospital. Pus, urine, BAL, sputum, CSF, endotracheal, tubes, tip, blood, and other clinical samples were taken from patients of various ages.

Identification and antimicrobial susceptibility testing of NFGNB pathogens: Vitek2 compact system was used for antimicrobial susceptibility testing and identification (Sumana et al., 2017).

Isolated NFGNBs

The isolated NFGNBs were *Pseudomonas aeruginosa* (P1)*, Acinetobacter baumannii (P2), Achromobacter xylosoxidans* (P3)*, Stenotrophomonas maltophilia* (P4) *and Burkholderia cepcia* (P5).

Plant collection

The fruit of *Termanilia chebula* was obtained from a Dehradun local market.

Chemicals and Reagents

Analytical grade chemicals and reagents were employed throughout. Mueller Hinton agar, ethyl acetate, methanol, DMSO(1,1- Diphwnyl-2-picrylhydrazyl) were purchased from Hi media (Mumbai). To ensure that the water used for extraction and experimentation was free of contaminants, it was doubly distilled, deionized, and sterilized.

Crude plant extracts preparation

A thimble charge of 25g of powder was used to extract organic ethyl acetate (S1), methanol (S2), and water (S3) in order using the soxhlet extraction method (Vishnu et al., 2010). Using a rotary evaporator, all of the extracts were made solvent-free and concentrated, then stored at 4°C in an airtight bottle until further use.

Antimicrobial Susceptibility testing

Agar well diffusion method

Agar well diffusion was used to assess the antibacterial activity of all the selected extracts (ethyl acetate,methanol,

and aqueous) of *Terminalia chebula* (Fruit) in the reference (Chauhan et al., 2012; Sawhney et al., 2011). Concentrations of 0.5mg/100µl and 1mg/100µl of the extracts were prepared in DMSO (dimethylsulphoxide). Colistin (0.5mg/100µl,1mg/100µl) was used as a positive control, whereas DMSO was employed as a negative control.

Broth Dilution MIC test

Minimal inhibitory concentration (MIC) of plant extracts was measured using a macro broth dilution test (NCCLS 2000).Two-fold serial dilutions of all the extracts in well plates were prepared using Mueller- Hinton Broth(Hi-media, Mumbai, India) as diluents. In each dilution, 20µl of test microorganisms (NFGNB isolates) at the standard concentration (5 X 10^5Cfu/ml) were used. The experimental negative and positive controls were two-fold serial dilutions of DMSO and colistin and levofloxacin, respectively. The plates were incubated for 24 hours at 37°C. The MIC was determined for the lowest concentration at which the extract or standard antibiotics displayed no observable growth (turbidity).

Determination of Minimum Bactericidal Concentration

20µl of MIC test broth tube solutions were distributed over MHA plates and incubated at 37°C for 18-24 hours. The dilution was designated as MBC (Minimum Bactericidal Count) concentration of the extract, which is bactericidal in nature when the plates showed no bacterial growth. The MIC index values indicates bactericidal action (MIC/MBC < 4) or bacteriostatic action (MIC/MBC > 4) (Radhakrishnan et al., 2011).The test was repeated three times, and the average MIC and MBC values were calculated.

Antioxidant activity

TheDPPH test is used to assess antioxidant activity. The DPPH Assay(1,1-Diphenyl-2-picrylhydrazyl) is a purple-colored stable free radical(absorbed at 517nm). The test is based on the color change of DPPH to yellow, exhibiting the scavenging properties of the subjected extract.This feature is used to characterize the free radical scavenging capacity in medicinal plants(Crude extracts) (Farrukh et al., 2003).

Phytochemical analysis of the extracts

HPLC: HPLC analysis was performed with the use of HPLC (make-Shimadzu) and LC Software 2010. 20 µl of the respective samples were injected, and the programme was ran for 15 minutes (15min runtime). The conventional marker compound was gallic acid. The UV detector was used to detect the light at 260nm.

Gas Chromatography and Mass Spectroscopy (GC-MS):

The REX column was used to perform GC-MS analysis on ethyl acetate, methanol, and water extract. 2µl of respective samples were introduced using a split mode all-glass injector with helium as the carrier gas. Temperature programme: 70°C-300°C at 60°/minute for 10 minutes at 300°C. The components were identified by computer searches in a commercial library (Wiley 8 and NIST).

RESULTS

Isolation and characterization:

Different clinical samples (Pus, Urine, BAL, sputum, CSF, endotracheal tubes tip, Blood and other specimen) were inoculated on Macconkey agar and Blood agar media for isolation. Followed by subjection to Vitek-2 for identification and AST. Results obtained revealed the presence of *Pseudomonas aeruginosa, Acinetobacter baumannii, Achromobacter xylosoxidans, S. maltophila, B. cepcia as* NFGNB isolates.

Extraction of the plant samples

Using the Soxhlet extraction method, extracts were prepared in a series of various organic and aqueous solvents with increasing polarity index (ethyl acetate (4.4),methanol (5.1), and water (9.0)). The highest yield was obtained with ethyl acetate extract, followed by methanol, while the least yield was obtained with aqueous extract.(Table-1).

Table-1:The yield of *Terminalia chebula* in various solvents.

S. N.	Solvent	Yield (in g)	Yield %	Color	State
1	Ethyl acetate (S1)	48.53	9.706%	Brownish	Viscous
2	Methanol (S2)	42.78	8.556%	Brownish	Viscous
3	Aqueous (S3)	39.31	7.862%	Brownish	Solid

Antimicrobial Susceptibility testing

Distinct organic(ethyl acetate methanol) and aqueous extract of *T.chebula* fruit extracts had a substantial inhibitory impact of (S1, S2, and S3) extract on the isolated pathogens as the positive controls produced significantly larger inhibition zones against the tested pathogens (Table-2). S1 extract, out of all the extracts tested and evaluated, was shown to have the strongest inhibitory effect on all pathogens. The antibacterial activity of *Terminalia chebula* (Fruit) extracts was comparable to that of the conventional antibacterial drugs colistin and levofloxacin (positive control). When compared to the extracts, colistin and levofloxacin efficiently suppressed bacteria. The minimal inhibitory concentration necessary to suppress the growth of isolates in reference was found to be between 0.5 mg/ml and 0.0156mg/ml. (Table-3, 4, 5).

Table-2:The antimicrobial activity of Control and*T. chebula* fruit extract (S1,S2, S3) against NFGN Bisolates.

S. N.	Isolates	Control Colistin for P1,P2,P3 and levofloxacin for P4,P5		S1 extract		S2 extract		S3 extract	
		0.5mg/ml Concentration	1mg/ml Concentration	0.5mg/ml Concentration	1mg/ml Concentration	0.5mg/ml Concentration	1mg/ml Concentration	0.5mg/ml Concentration	1mg/ml Concentration
1	*Pseudomonas aeruginosa* (P1)	18mm	19mm	10mm	12mm	8mm	11mm	7mm	10mm
2	*Acinetobacter-baumanni* (P2)	18mm	19mm	10mm	11mm	9mm	11mm	9mm	12mm
3	*Achromobacter xylosoxidans* (P3)	18mm	19mm	10mm	11mm	7mm	9mm	8mm	8mm
4	*S. maltophila* (P4)	27mm	29mm	8mm	10mm	8mm	13mm	10mm	11mm
5	*Burkholderia cepia* (P5)	27mm	29mm	10mm	11mm	9mm	14mm	11mm	13mm

Table-3: The Minimum inhibitory concentration values of ethyl acetate extract against NFGNB isolates.

Microorganism	Range of MIC value (mg/ml)	MIC value (control) (mg/ml)	MBC value (Control) (mg/ml)	MIC value (extract) (mg/ml)	MBC value (extract) (mg/ml)	MIC Index value of Control	MIC Index value of Extract
P. aeruginosa (P1)	0.5- 0.0156	0.0156	0.0312	0.25	0.5	0.5	0.5
A.baumannii (P2)	0.5- 0.0156	0.0156	0.0312	0.25	0.5	0.5	0.5
Achromobacter xylosoxidans (P3)	0.5- 0.0156	0.0156	0.0312	0.25	0.5	0.5	0.5
S.maltophila (P4)	0.5- 0.0156	0.0156	0.0312	0.25	0.5	0.5	0.5
Burkholderia cepcia (P5)	0.5- 0.0156	0.0156	0.0312	0.125	0.25	0.5	0.5

Table-4: The Minimum Inhibitory Concentration values of methanol extract against NFGNB isolates.

Microorganism	Range of MIC value (mg/ml)	MIC value (control) (mg/ml)	MBC value (Control) (mg/ml)	MIC value (extract) (mg/ml)	MBC value (extract) (mg/ml)	MIC Index value of Control	MIC Index value of Extract
P. aeruginosa (P1)	0.5- 0.0156	0.0156	0.0312	0.125	0.25	0.5	0.5
A.baumanni (P2)	0.5- 0.0156	0.0156	0.0312	0.25	0.5	0.5	0.5
Achromobacter xylosoxidans (P3)	0.5- 0.0156	0.0156	0.0312	0.25	0.5	0.5	0.5
S.maltophila (P4)	0.5- 0.0156	0.0156	0.0312	0.25	0.5	0.5	0.5
Burkholderiacepcia (P5)	0.5- 0.0156	0.0156	0.0312	0.25	0.5	0.5	0.5

Table-5: The Minimum Inhibitory Concentration values of aqueous extract against NFGNB isolates.

Microorganism	Range of MIC value (mg/ml)	MIC value (control) (mg/ml)	MBC value (Control) (mg/ml)	MIC value (extract) (mg/ml)	MBC value (extract) (mg/ml)	MIC Index value of Control	MIC Index value of Extract
P. aeruginosa (P1)	0.5- 0.0156	0.0156	0.0312	0.25	0.5	0.5	0.5
A.baumanni (P2)	0.5- 0.0156	0.0156	0.0312	0.25	0.5	0.5	0.5
Achromobacter xylosoxidans (P3)	0.5- 0.0156	0.0156	0.0312	0.25	0.5	0.5	0.5
S.maltophila (P4)	0.5- 0.0156	0.0156	0.0312	0.25	0.5	0.5	0.5
Burkholderiacepcia(P5)	0.5- 0.0156	0.0156	0.0312	0.125	0.25	0.5	0.5

Antioxidant Power

In the present study, the antioxidant power is measured using the DPPH assay. The DPPH (1,1-Diphenyl-2picrylhytrazyl) is a stable purple-colored free radical (absorbed at 517nm). If free radicals are scavenged, the color DPPH changes to yellow. This feature is used in this experiment to demonstrate free radical scavenging capacity in medicinal plants (Crude extract). When oxidative stress levels rise, DNA is damaged, resulting in base damage, strand breakage, gene expression changes, and eventually mutagenesis occurs (Block et al., 1992; Byers et al., 1993; Bajpai et al., 2021). Parkinson's disease, Alzheimer's disease, Huntington's disease, Cardiovascular illness, Respiratory disease, Arthritis and other neurological disorder are caused by the overproduction of free radicals. The phytosignatures discovered in *Treminalia chebula* (Fruit) S3 extract were determined to be the most effective free radical scavenger. When the results were compared to the reference compound gallic acid, a well-known antioxidant, a powerful polyphenol that exhibited high antioxidant activity, the antioxidant capacity was found to be concentration dependent in all cases. The graph is displayed in (Fig.1). Polyphenols, flavonoids, and phenolic chemicals are active biosignatures in many plant-based therapeutics that protect cells from oxidated stress. (Acharya et al., 2010).

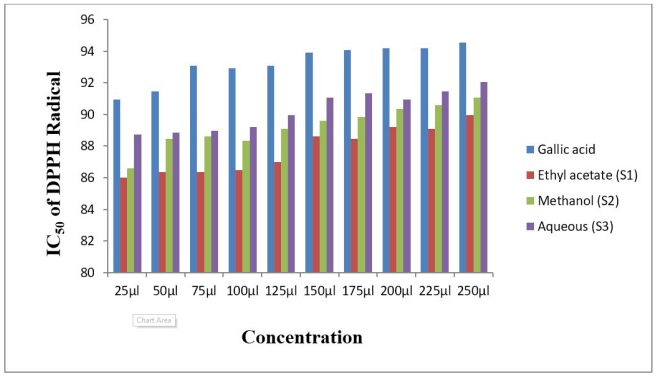

Fig. 1: Graphical representation of antioxidant capacity of various (S1, S2 and S3) extracts of *T.chebula* at increasing concentration with Gallic acid as reference compound.

Phytochemical Analysis

The phytosignature profiling was not harmed throughout the extraction process, as the chromatogram of the extract displayed multiple peaks of different phytochemicals when using HPLC with a UV detector at a wavelength of 260nm to identify chemicals, however gallic acid was the compound of interest in the study. The obtained results demonstrated the existence of gallic acid as the primary component of the methanolic extract of *Terminalia chebula* (Fruit),as well as the presence of other botanicals in lesser amounts(Fig. 2). The GC-MS analysis of the selected plant extracts has revealed the presence of several compounds. Traditionally water with highest polarity was the choice of solvent for the extraction of phytochemicals but other research showed that organic solvents had more consistent antibacterial actions than those extracted with water. The polarity of the solvent and the type of the bioactive chemicals recovered may be linked to the activities reported in organic solvents other than water. (Dey et al., 2010; Rautela et al., 2018; Sharma et al., 2015). Alkaloids, Tannins, Cynoanogenic, Saponins, Glycosides, Flavonoids, Phenolic compounds, and Lignins are only a few examples of phytoconstituents found in plants (Upadhyay et al.,2014; Rautela et al., 2018; Sharma et al., 2016) Phytoconstituents responsible for wider range biological activities were identified in *Terminalia chebula* (Fruit) extracts (S1,S2, and S3) (Table-6,7,8). Hexadecanoic acid, 1,2,3-Benzenetriol (pyrogallol), Thiositosterol disulfide, Ergost-5-en-3-ol, and other chemicals were found in the extracts(3. beta.,24R) Carbonic acid, 2-ethylhexyl nonyl ester,2-propanoic acid, tridecyl ester,etc. Hexadecanoic acid is used to treat rheumatic symptoms because of its anti-inflammatory properties. Propanoic acid has anti-fungal and anti-bacterial properties, and it is used to treat asthma in patients. This was also demonstrated in our investigations where the extracts were found to be highly antimicrobial in nature, possibly due to the chemicals present in diverse extract.1,2,3-Benzenetriol is a phenolic chemical that has antibacterial qualities and can be utilized in the coloring of suturing materials as well as for oxygen absorption in gas analysis. Ergost-5-en-3-ol assists in the treatment of liver disease, jaundice, and atherosclerosis.

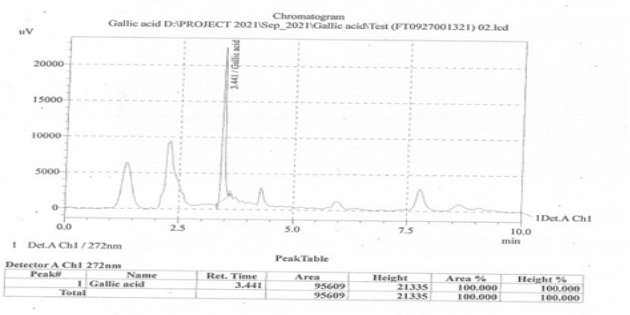

Fig. 2: HPLC analysis of *T.chebula* Methanolic extracts (S2) detecting Gallic acid as the Principle component.

Table-6: List of Phytochemicals in the S1 extract using GC-MS technique.

Peak	R.Time	Area	Area%	Name
1	7.393	103764	0.33	Benzene,1,3-bis(1,1-dimethylethyl)-
2	7.705	147336	0.47	TETRADECANE
3	8.366	70381	0.22	Nonane,5-(2-methylpropyl)-

4	9.632	320444	1.01	2-(4'-Methoxyphenyl)-2-(2'-methoxyphenyl)propane
5	10.206	9669301	30.57	1,2,3-Benzenetriol
6	10.584	1123800	3.55	1-Dodecanol
7	11.989	419919	1.33	DiethylPhthalate
8	13.081	1436563	4.54	Dodecylacrylate
9	13.144	186412	0.59	Propanoicacid, decylester
10	14.158	91400	0.29	Bromoaceticacid, pentadecylester
11	14.606	93300	0.29	Neophytadiene
12	15.059	87693	0.28	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
13	15.929	1098768	3.47	Dibutylphthalate
14	16.208	1854766	5.86	HEXADECANOICACID, ETHYLESTER
15	16.499	246795	0.78	Propanoicacid, 3-mercapto-, dodecylester
16	17.790	972275	3.07	Linoleicacidethylester
17	17.845	1383240	4.37	(E)-9-Octadecenoicacidethylester
18	18.076	454799	1.44	Octadecanoicacid,ethylester
19	19.798	185225	0.59	Docosanoicacid,ethylester
21	22.380	73262	0.23	OCTACOSANE
22	22.979	103523	0.33	Squalene
23	23.086	78694	0.25	Pentatriacontane
24	23.373	220121	0.70	8-Hexadecene, 8, 9-diheptyl-
25	23.572	169976	0.54	PENTACOSANE
26	23.650	233751	0.74	erythro-9,10-Dibromopentacosane
28	24.046	185145	0.59	HEXADECANOIC ACID, OCTADECYLESTER
29	24.352	779418	2.46	Triacontylacetate
30	24.456	159517	0.50	Tetracontane
31	24.992	380299	1.20	Tetratetracontane
32	25.163	1204989	3.81	Thiositosteroldisulfide
33	25.375	618008	1.95	2H-1-BENZOPYRAN-6-OL, 3, 4-DIHYDRO-2,5,7,8-TET
34	25.570	182986	0.58	11-Methylnonacosane
35	25.971	251356	0.79	Octacosylacetate
37	26.708	162344	0.51	ERGOSTA-7, 22-DIEN-3-OL, (3.BETA.,22E)-
39	27.371	4450231	14.07	ERGOST-5-EN-3-OL, (3.BETA.)
40	27.972	256377	0.81	8-Androsten-3-ol,17-(2-methylallyl)-4, 4, 14-trimethyl-
41	28.734	1098608	3.47	.beta.-Sitosterolacetate
42	30.539	419862	1.33	5,11,17,23 - TETRATERT-BUTYLPENTACYCLO [19.3.1.1

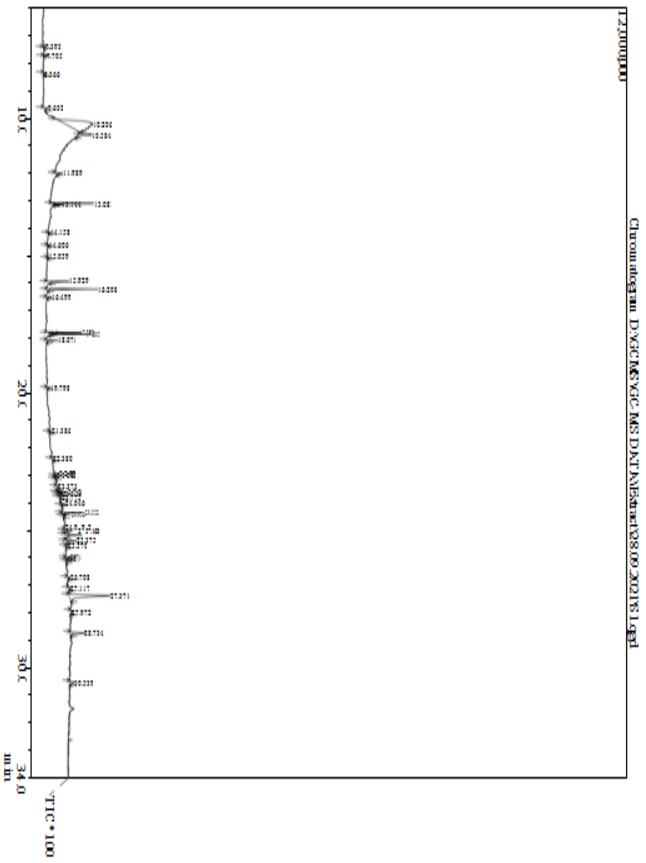

Fig. 3:Represents the GC chromatogram of S1 extract

Table-7: List of Phytochemicals in the S2 extract using GC-MS technique.

Peak	R.Time	Area	Area%	Name
1	6.648	54299	0.32	3, 5-Dimethyldodecane
2	6.838	70005	0.41	Undecane,2, 5-dimethyl-
3	6.965	86379	0.50	Decane,3, 7-dimethyl-
4	7.226	50789	0.29	HEPTADECANE
5	7.308	135377	0.79	Glutaricacid, monochloride, 2-methylpent - 3 - ylester
6	7.398	257719	1.50	Benzene, 1, 3-bis (1,1-dimethylethyl)-
7	7.534	206118	1.20	OCTADECANE
8	7.708	367788	2.13	Dodecane, 4, 6-dimethyl-
9	8.020	68372	0.40	1-Undecene, 4-methyl-
10	8.083	63724	0.37	OCTADECANE, 1-CHLORO-
11	8.367	162040	0.94	NONANE, 4, 5-DIMETHYL-
12	9.637	47943	0.28	2-Bromododecane

13	10.093	63544	0.37	HEPTANE, 3-(BROMOMETHYL)-
14	10.170	73814	0.43	Borane, diethyl (decyloxy)-
15	10.423	818631	4.75	L-Valine,N-(trifluoroacetyl)-, 1- methylpropylester
16	10.573	2869086	16.65	1-DODECANOL, 3, 7, 11-TRIMETHYL-
17	11.011	88977	0.52	1, 2, 3-Benzenetriol
18	11.137	79244	0.46	Dodecane, 2, 6, 11-trimethyl-
19	11.392	585748	3.40	.beta.-D-Glucopyranose, 1, 6-anhydro-
20	12.006	307699	1.79	1, 2-BENZENEDICARBOX-YLICACID, DIETHYLESTE
21	13.086	2614387	15.17	2-Propenoicacid, tridecylester
22	13.575	103142	0.60	2-Methyltetracosane
23	14.614	79298	0.46	Phytol, acetate
24	15.943	1268940	7.36	Dibutylphthalate
25	16.219	206158	1.20	HEXADECANOIC ACID, ETHYLESTER
26	16.512	255724	1.48	Propanoicacid, 3-mercapto-, dodecylester
27	17.267	111345	0.65	Cyclopropanebutanoicacid, 2-[[2-[[2-[(2-pentylcyclo-propy
28	18.088	97537	0.57	HEPTADECANOICACID, ETHYLESTER
29	20.663	218501	1.27	8-Amino-1, 3, 6-triazahomoadamantane
30	22.870	96979	0.56	1-Bromoeicosane
31	23.089	111138	0.64	Nonadecane, 9-methyl-
32	23.655	124899	0.72	Dodecane,1, 1'-oxybis-
33	23.771	211927	1.23	PENTACOSANE
34	24.042	181974	1.06	HEXADECANOICACID, OCTADECYLESTER
36	24.989	121314	0.70	Stigmasta-5, 22-dien-3-ol, acetate,(3.beta.)-
37	25.157	89323	0.52	Stigmastan-3-ol,5-chloro- ,acetate,(3.beta.,5.alpha.)-
38	25.210	111810	0.65	TETRACONTANE
39	25.567	171113	0.99	1-OCTADECANETHIOL
41	27.098	353602	2.05	HEXATRIACONTANE
42	27.363	1705942	9.90	.gamma.-Sitosterol
43	27.670	162030	0.94	5.alpha.-Cholestan-6.beta.-amine,N,N-dimethyl-

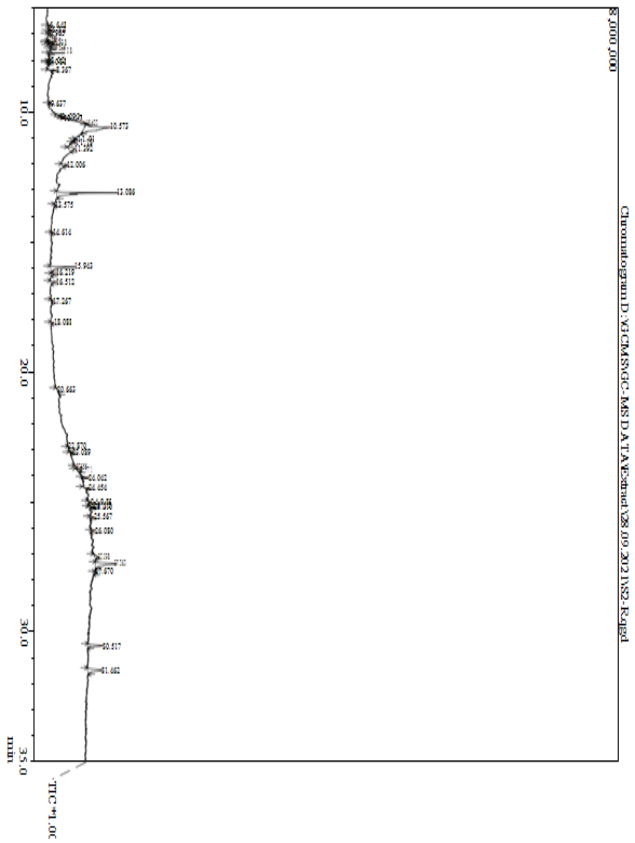

Fig. 4: Represents the GC chromatogram of S2 extract

Table-8:List of Phytochemicals in the S3 extract using GC-MS technique.

Peak#	R.Time	Area	Area%	Name
1	6.655	49085	0.67	PENTANE, 2, 2, 3, 3-TETRAMETHYL-
2	6.844	42299	0.57	Decane, 2, 5, 9-trimethyl-
3	7.227	37578	0.51	Decane, 2, 3, 5-trimethyl-
4	7.313	73466	1.00	HEXANE, 2, 2, 3, 3-TETRAMETHYL-
5	7.401	196589	2.67	Benzene, 1, 3-bis (1,1-dimethylethyl)-
6	7.544	129441	1.76	DECANE, 2, 3, 7-TRIMETHYL-
7	7.630	31987	0.43	Sulfurousacid, 2-ethylhexylisohexylester
8	7.711	344712	4.68	Dodecane, 4, 6-dimethyl-
9	7.830	40453	0.55	Nonane, 5-(2-methylpropyl)-
10	8.020	51893	0.70	Decane, 2, 3, 5, 8-tetramethyl-
11	8.372	128459	1.74	Nonane, 5-(2-methylpropyl)-
12	9.642	41981	0.57	PENTADECANE
14	10.523	44753	0.61	Heptane, 2, 2, 3, 3, 5, 6, 6-heptamethyl-

15	10.570	172876	2.35	Heptadecane, 2, 6, 10, 15-tetramethyl-
16	10.758	322252	4.38	1-Decanol,2-hexyl-
17	11.142	92519	1.26	OCTADECANE
18	12.081	181768	2.47	DiethylPhthalate
19	13.043	23265	0.32	3-PENTEN-2-ONE, 4-(METHYLAMINO)-
20	13.116	391916	5.32	Dodecylacrylate
21	13.576	96152	1.31	Heptadecane
22	15.969	971930	13.20	Dibutylphthalate
23	16.258	103616	1.41	HEXADECANOI-CACID,ETHYLESTER
24	16.554	149586	2.03	Propanoicacid,3-mercapto-,dodecylester
25	17.369	72193	0.98	2-Bromotetradecane
26	18.111	62609	0.85	Eicosanoicacid,ethylester
27	20.953	161269	2.19	Carbonicacid,2-ethylhexylnonylester
28	23.093	72738	0.99	2-Methyltetracosane
29	23.771	63218	0.86	erythro-9,10-Dibromopentacosane
30	24.040	162183	2.20	Hexadecanoicacid,tetradecylester
31	24.448	100674	1.37	TETRACONTANE
32	25.559	133042	1.81	Octadecanoicacid,heptylester
33	27.113	157859	2.14	9-Octadecenoicacid(Z)-,2-(octadecyloxy)ethylester
34	27.367	616657	8.37	gamma.-Sitosterol
35	27.863	75259	1.02	Phenol,2,4-bis(1,1-dimethylethyl)-,phosphite(3:1)
36	30.503	922143	12.52	5,11,17,23-TETRATERT-BUTYLPENTACYCLO[19.3.1.1
37	31.464	908455	12.33	Propanoicacid,3,3'-thiobis-,didodecylester

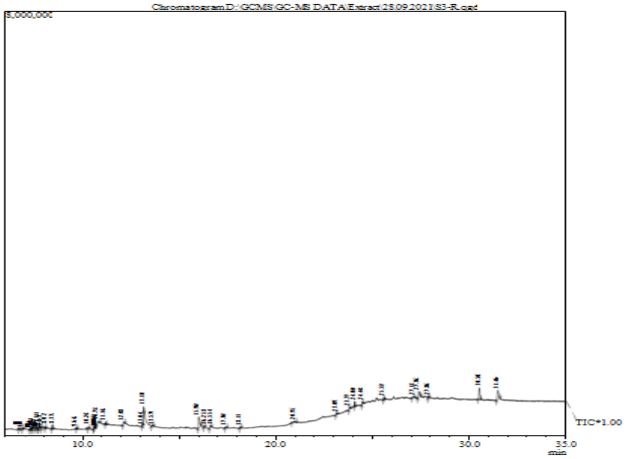

Fig. 5: Represents the GC chromatogram of S3 extract

DISCUSSION

Medicinal plants have been a source of novel antimicrobial drugs for their ability to treat a variety of illnesses as cited in the literature. Traditional medicinal plants are still being a part of various researches to see whether they may be used to treat diseases that have developed multidrug resistance to most antibiotics as a result of their overuse. Many *Terminalia* species have a history of being used to treat medical disorders caused by microbial infections, and multiple recent studies have found that they contain antibacterial characteristics. Antibacterial activities of *Terminalia* spp. used in traditional Indian medicine have been reported in several investigations (Cock,2015).For determining the therapeutic potential of medicinal plants as potent antimicrobial agents, they shall be explored extensively against MDR pathogens. Several studies have been published on the pharmacological screening of plant extract, which has been used to make a variety of medicines (Kumar et al., 2013). Our findings on the antibacterial activity of *Terminalia chebula* (Fruit) different extracts against pathogens that cause respiratory disorders in people show that they are effective, powerful, and comparable to other studies (Pandey et al., 2011; Thenmozhi et al., 2011; Vijayan et al., 2010). Antibiotic resistance and its associated toxicity had recently emerged as a problem that has limited the use of antimicrobial medicines (Eggleston et al., 2010), thereby encouraging the antibacterial role of plants against resistant bacterial strains due to safety and high efficacy (Alvianoet al., 2009). The rapid rise in the number of infections caused by NFGNB pathogens, as well as their multidrug resistance pattern, have made them a noteworthy and important pathogen. The extract's antimicrobial properties can be classified as follows: damage to the microbial genome, damage to the protein synthesis mechanism or structure, microbial membrane disintegration, or suppression of specific enzymes and metabolite production. *Terminalia chebula* has been shown to have broad-spectrum antibacterial activity against a variety of pathogenic gram-negative bacteria. The antibacterial activity of ethanolic extract of *T. chebula* fruits was tested against standard reference bacterial strains of clinical importance, and it was discovered that the extract was highly effective against *Bacillus subtilis, Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus typhi, and Pseudomonas aeruginosa* (Nigam et al., 2020; Kanna et al., 2015).

Terminalia chebula extracts have been found to have high antioxidant activities due to the presence of phenolic chemicals in the extract (Basha et al., 2017). The Antioxidant studies revealed that the different extracts of *Terminalia chebula* (Fruit) can neutralize the free

radicals that cause various ailments. It was discovered that antioxidant power is concentration-dependent. The scavenging activity of free radicals in the various extracts reduces in the following order: Methanol > Ethyl acetate > aqueous. The phytoconstituents detected in *Terminalia chebula* (Fruit) extract were shown to be antibacterial, antifungal, and antioxidant in nature, indicating that the plant could be employed in herbal medication formulation to treat bacterial pathogenicity-related diseases.

CONCLUSION

Non-fermenting gram-negative bacilli (NFGNB), due to the excessive use of antibiotics, have emerged as significant healthcare-related pathogens in recent years. The prevalence of multi-drug resistance among nosocomial pathogens has led to complications in their choice of treatment. Therefore, Herbal medicines are gaining significant importance as an alternative choice of therapy. It has been reported that phytochemicals and their chemical analogs have provided enormous amount of clinically useful therapeutic agents in the treatment of chronic and acute human infections. The increasing acceptance and popularity of herbal formulations have demonstrated that natural products are safe, less expensive, have the least side effects, and are within reach of those in underdeveloped countries. According to the previously given literature, herbal medications have always been utilized to treat a variety of diseases due to their broad safety profile. Our findings showed that the S2 (methanol) extract of *Terminalia chebula* (fruit) is a potent antibacterial and antioxidant agent. The phytochemicals discovered are important in establishing proof for their application in herbal medicines for the treatment of various infections. The research can be expanded to include in vivo experiments to determine the extract's precise mode of action.

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REFERENCES

Acharya, S., Sahu, A.R., Satyajit, and Mohanta, S.R. (2010). Free radical scavenging activity of thalamus of NymphaceastellataWilld. *Int J Pharmacy & Pharm Sci.***2**: 61-63.

Alviano, D.S. and Alviano, C.S. (2009). Plant extracts: search for new alternatives to treat microbial diseases. *Curr Pharm Biotechnol.***10(1)**: 106–121.

Bag, A., Bhattacharyya,S.K., Bharati, P., Pal, N.K. and Chattopadhyay, R.R. (2009). Evaluation of antibacterial properties of Chebulicmyrobalan (fruit of *Terminalia chebula* Retz.) extracts against methicillin-resistant *Staphylococcus aureus* and trimethoprim-sulphametho-xazole resistant uropathogenic*Escherichia coli.Afr. J. Plant Sci.***3(2)**:025-029.

Bajpai, A.B., Misra, P., Dhiman, M., Rautela, I., Rayal, R., Jeena, K. and Sharma, M.D. (2021). Study on the Chemical Composition and Antioxidant Activity of Extracts from Wild and in vitro Raised Endangered Medicinal Plant *Ephedra gerardiana*. *The Scientific Temper.***12(1-2):** 86-101.

Basha, S.J., Reddy, V.J., Rani, Y.S., Koshma, M., Hanumanthu, G. and Dadakhalandar, S. (2017).A review on *Terminalia chebula*. *Int. J.Pharm.Res.***7(10)**: 187-191.

Block, G., Patterson, B. and Subar, A. (1992). Fruit, vegetables and cancer prevention: a review of the epidemiological evidence. *Nutr. Cancer.***18**: 1-29.

Byers, T. and Bowman, B. (1993). Vitamin E supplements and coronary heart disease. *Nutr. Rev.***51**: 333-345.

Chauhan, N., Singh, D., Painuli, R.M. (2012). Screening of Bioprotective properties and Phytochemical analysis of various extracts of *E.alba* Whole plant. *Int.J.Pharm.Sci.***4(2):**554-560.

Cock, I.E. (2015). The medicinal properties and phytochemistry of plants of the genus Terminalia (Combretaceae).*Inflammopharmacology.***23(5)**: 203–229.

Dash, B. (1991).*MateriaMedica of Ayurveda*. *B. Jain Publishers,New Delhi.* 170-174.

Dey, S.K., Banerjee, D., Chattapadhyay, S. and Karmakar, K.B. (2010). Antimicrobial activities of some Medicinal Plants of West Bengal. *Int. J. Pharma Bio. Sci.***1(3)**: 1-10.

Eggleston, K., Zhang, R. and Zeckhauser, R.J. (2010). The global challenge of antimicrobial resistance: insights from economic analysis. *Int. J. Environ Res. Public Health.***7(8)**: 3141-3149.

Farrukh, A. and Iqabal, A. (2003). Broad-spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants. *World J.MicrobBiot.***19**: 653-657.

Heber, D. (2004). Vegetables, fruits and phytoestrogens in the prevention of diseases. *J Postgrad Med.***50**: 145–149.

Kanna, S., Hiremath, S.K. and Unger, B.S. (2015). Nephroprotective activity of Bilvadiagada in gentamicin induced nephrotoxicity in male Wistar rats. *Anc. Sci. Life.***34**: 126-129.

Kathiresan, K., Boopathy, N.S. and Kavitha, S.(2006). Coastal vegetation–an underexplored source of anticancer drugs. *Nat. Prod. Rad*. **5**: 115–119.

Kaur, C. and Kapoor, H.C. (2002). Antioxidants activity and total phenolic content of some Asian vegetables. *Int J Food Sci Tech*. **37**: 153–161.

Kim, H.G., Cho, J.H., Jeong, E.Y., Lim, J.H. and Lee, S.H. (2006). Growth inhibitory activity of active component of *Terminalia chebula* fruits against intestinal bacteria. *J. Food Prot.***69(9)**: 2205-2209.

Kumar, G., Pundir, R.K. and Rana, S. (2013).Evaluation of Antimicrobial Activity and Phytochemical Analysis of Antidiabetic Plant *Terminalia chebula*.*Inst. J.Tradit.Med*.**2(1)**: 9-20.

Lee, H.S., Jung, S.H., Yun, B.S. and Lee, K.W. (2007). Isolation of chebulic acid from *Terminalia chebula* Retz. And its antioxidant effect in isolated rat hepatocytes. *Arch Toxic.***81(3)**: 211-218.

National Committee for Clinical Laboratory Standards. (2000). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard. Fifth edition: M7-A5.NCCLS. Wayne, P.A.

Nigam, M., Mishra, A.P., Devkota, A.A., Dirar, I.A., Hassan, M., Adhikari, A., Belwal, T., Devkota, H.P. (2020). Fruits of *Terminalia chebula* Retz. A review on traditional uses, bioactive chemical constituents and pharmacological activities. *Phytother Res*. 1-16.

Pandey, M.K., Singh, G.N., Sharma, R.K. and Snehlata (2011). Antibacterial activity of *Ecliptaalba* (L.) Hassk. *J.Appl.pharm.***1(07)**: 104-107.

Quiroga, E.N., Sampietro, A.R. and Vattuone, M.A. (2001). Screening antifungal activities of selected medicinal plants. *J. Ethnopharmacol.***74(1)**:89-96.

Radhakrishnan, N., Gnanamani, A. and Mandal, A.B. (2011). A potential antibacterial agent Embelin, a natural benzoquinone extracted from Embeliaribes. *Biology and Medicine.***3(2)**: 1-7.

Rathinamoorthy, R. and Thilagavathi, G. (2014). Review on *Terminalia chebula* pharmacological and biochemical studies. *Int. J. Pharm. Tech. Res.***6(1)**: 97-116.

Rautela, I., Dheer,P., Thapliyal,P., Joshi,T., Sharma, N. and Sharma, M.D. (2018). GC-MS Analysis of Plant Leaf Extract of *DaturaStramonium* in Different Solvent System. *European J. Biomed. Pharm*. **5**: 236-245.

Rautela, I., Sharma, M.D., Sharma,N., Kishor, K., Singh, K. and Sharma, N. (2018). Comparative GC-MS Analysis of Leaf and Root Extract of Medicinal Plant *WithaniaSomnifera*. *World J. Pharm. Res.* **7**: 956-972.

Saleem, A., Husheem, M., Harkonen, P. and Pihlaja, K. (2002). Inhibition of cancer cell growth by crude extract and the phenolics of *Terminaliachebula* Retz. *Fruit. J Ethnopharmacol*.**81(3)**:327-336.

Sawhney, S.S., Painuli, R.M and Singh, D. (2011). Antioxidant and Antimicrobial activity of Phyllanthusemblica for its application in treatment of ophthalmic disorders. *Int. J. Pharm Sci*.**3(4)**: 129-132.

Sharma, M., Rautela, I., Sharma, N., Gahlot, M. and Koshy, E.P. (2015). GC-MS analysis of phytocomponents in juice sample of Indian cane: Saccharumbarberi. *Int. J. Pharm. Sci.Res.***6**: 5147-5153.

Sharma, N., Rautela, I. and Sharma,M.D. (2016). Mass Propagation and GC-MS Analysis of Critically Endangered Plant WithaniaCoagulans. *Int. J. Appl. Biol. Pharm. Technol*. **7**: 62-70.

Srigopalram, S. and Jayraaj, I.A. (2012). Effect of Terminalia Chebula Retz On Den Induced Hepatocellular Carcinogenesis in Experimental Rats*. Int. J. Pharm. Sci*.**4(2)**.

Sumana, P., Toleti, S. and Myneni, R.B. (2017). Prevalence of nonfermentinggram negative bacilli infections and their antimicrobial susceptibility pattern in a tertiary care. *Int.J.Curr.Res*. **9**: 63427-63431.

Thenmozhi, M. and Sivaraj, R. (2011). Phytochemical and Antimicrobial Evaluation of Ecliptaalba. *Int.J.Adv. Pharm*.**2(6)**: 246-251.

Upadhyay, A., Agrahari, P. and Singh, D.K. (2014). A Review on the Pharmacological Aspects of *Terminalia chebula. Int. J. Pharmacol.***10**: 289-298.

Vijayan, M.N., Barreto, I., Dessai, S., Dhuri, S., D' Silva, R. and Rodrigues, A. (2010). Antimicrobial activity of ten common herbs, commonly known as 'Dashapushpam' from Kerala, India. *Afr.J.Microbiol Res*. **4(22)**: 2357-2362.

Vishnu, K.A., Aadil, A.I. and Hameedullah, S.S. (2010). Antibacterial activity of the extracts obtained from *Ecliptaalba*Hassk and Andrographis lineate against human pathogens. *J.Pharm.Res*. **3(10)**: 2529-2532.