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Screening of Phytochemical and *In Vitro* Antimicrobial Activity of *Tinospora cordifolia* (Thunb.) and *Terminalia Chebula* Retz.

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A B S T R A C T

Medicinal plants are widely used in management of diseases all over the world. Large number of plants belonging to different families has been studied for their therapeutic properties. In the present study is aimed to investigate the phytochemical and antibacterial activity of methanolic leaves extract of *Tinospora cordifolia* and *Terminalia chebula.* The GC-MS analysis was carried out in methanol leaves extract of *T. chebula* and *T. cordifolia.* GC-MS analysis revealed the occurrence of 26 compound in *T. chebula* and 30 compounds in *T. cordifolia.* In the present study, antibacterial activity of methanol leaves extracts of *T. cordifolia* and *T. chebula* were tested against important human pathogens including Gram positive and Gram-negative bacteria. Among the plants, extract of *T. cordifolia* exhibited better results than *T. chebula.* From this study, methanolic leaves extract of *T. cordifolia* and *T. chebula* were showed promising DPPH scavenging activity. In the present study reported that the good sources of phytocompound from these experimental plants.

***Key words*:** *Tinospora cordifolia,* DPPH, GC-MS, Phytochemical, Antibacterial

Plants are the basic source of information of modern medicine. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from them, many based on their use in traditional medicine. Medicinal plants have the capacity to produce a large number of organic phytochemicals with complex structural diversity that is identified as secondary metabolites. Some of these secondary metabolites are produced for self-defence. Herbal medicines represent one of the most important fields of traditional medicine all over the world. To stimulate the use of herbal medicine and to determine their potential as a source for novel drugs, it is essential to study medicinal plants which have folklore reputation in a more intensified way [1].

Various medicinal properties have been attributed to natural herbs. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products [2]. The history of plants being used for medicinal purpose is probably as old as the history of mankind. Extraction and characterization of several active phytocompounds from these green factories have given birth to some high activity profile drugs. A growing body of evidence indicates that secondary plant metabolites play critical roles in human health and may be nutritionally important [3]. Phytochemical screening of plants has revealed the presence of numerous chemicals including alkaloids, tannins, flavonoids, steroids, glycosides, saponins etc. Many plant extracts and phytochemicals show antioxidant/free radical scavenging properties.

Plant based antimicrobials represent a vast untapped source of medicine. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Further continued exploration of plant derived antimicrobials is needed today. Historically, 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 [4]. The medicinal plants around the world contain many compounds with antibacterial activity [5]. Many efforts have been made to discover new antimicrobial compounds from various sources such as microorganisms, animals, and plants. Systematic screening of them may result in the discovery of novel effective antimicrobial compounds [6]. The use of botanical medicines is generally on the rise in many parts of the world. The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new anti-infective agents. Hence the present study is aimed to investigate the phytochemical and antibacterial activity of methanolic leaves extract of *Tinospora cordifolia* and *Terminalia chebula*. On the basis of the above facts and information, the present work has been designed and planned to evolve the strategy for the identification of bioactive

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compounds from the medicinal plants against oral bacterial infections causing chronic disease in human beings.

MATERIALS AND METHODS

Collection of experimental plants

Healthy and young leaves of *Terminalia chebula* Retz. and *Tinospora cordifolia* (Thunb.) were collected distinctly from Thanjavur District, Tamil Nadu, India. The collected leaves were identified and authenticated by Dr. S. John Brito, The director, Rapinat Herbarium and centre for molecular systematic, St. Joseph's college, Tiruchirappalli, Tamil Nadu, India. The leaves were separated from stems, washed in clean water, and dried at room temperature.

Preparation of plant extract

The collected leaves were cleaned and dried in shade for 7 days, then ground well to fine powder. About 500 g of each plants powder were extracted with methanol (80%) at 70°C by continuous hot percolation using Soxhlet apparatus separately. The extraction was continued for 24 hrs. The methanolic extract was then filtered and kept in hot air oven at 40°C for 24 hours to evaporate the methanol from it. A dark brown residue was obtained. The residue was kept separately in air tight containers and stored in a deep freezer [7].

Phytochemical analysis

Qualitative analysis

Phytochemical analysis of the plant extracts was under taken using standard qualitative methods as described by various authors [8-9]. The plant extracts were screened for the presence of biologically active compounds such as alkaloids, flavonoids, carbohydrates, phytosterols, proteins, phenolics, tannins and saponins.

GC-MS analysis

30 g powdered samples of *T. chebula* and *T. cordifolia* were soaked and dissolved in 75 ml of methanol for 24 hrs. Then the filtrates were collected by evaporated under liquid nitrogen. The GC-MS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto39System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbo mas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 mx 0.25 mm x 1µm 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 up to 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 [10].

Antibacterial activity

In the present study, the following bacterial pathogens like *Bacillus substils, Staphylococcus aureus*, *Klebsiella aerogenes* and *Pseudomonas aeruginosa* which were collected from Department of Microbiology, Marudupandiyar College, Thanjavur, Tamil Nadu, India. Culture supernatants with fractions and methanolic leaves extract of the experimental plants were used in the disc diffusion method separately. Bacterial cultures 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, Ampicillin (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 millimetre.

Antioxidant activity

DPPH radical scavenging activity

Antioxidant reducing activity on DPPH radical was estimated according to the method of Blois [11] with modification involving the use of high-throughput microplate system. Sample (50 μL of 1.0mg/mL) was added to 50 μL of DPPH (FG: 384.32) (1mM in ethanolic solution) and 150 μL of ethanol (absolute) in a 96-well microtiter plate in triplicates. The plate was shaken (15 seconds, 500 rpm) and left to stand at room temperature for 30 minutes. The absorbance of the resulting solution was measured spectrophotometrically at 520 nm. Ascorbic acid was used as positive control.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

Phytochemical screening was performed to respective medicinal plants *Terminalia chebula* and *Tinospora cordifolia*. The qualitative phytochemical analysis of methanolic extracts revealed the presence of alkaloids, carbohydrates, protein, saponins, phenols, terpenoids, phytosterols, flavonoids and tannins. However, phlobatannins was absent in *Tinospora cordifolia* (Table 1).

Table 1 Qualitative phytochemical analysis of selected medicinal plants

Phytochemicals	*T. cordifolia*	*T. chebula*
Alkaloids	+	+
Flavonoids	+	+
Carbohydrates	+	+
Protein	+	+
Phenols	+	+
Saponins	+	+
Tannins	+	+
Phytosterols	+	+
Terpenoids	+	+
Phlobatannins	-	+

+ Present, - Absent

GC-MS analysis of plants

The GC-MS analysis was carried out in methanol leaves extract of *T. chebula* and *T. cordifolia.* In addition, GC-MS analyses, totally 26 compounds identified from the methanol fractions of the *T. chebula* are presented in (Table 2, Fig 1). The plant samples revealed the synthesis of 2-Cyclopenten-1-one, 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, methylester and Dodecanoic acid, 10-methyl-, methyl ester. Among the compounds, the highest percentage of peak was observed in the 1,2,3-Benzenetriol (82.9379%) and followed by 2,2-Bis(2'-methoxyphenyl)propane (3.2242%). All these compounds are of pharmacological importance.

Table 2 Biologically active compounds of *Terminalia chebula* by GC-MS analysis

Name of the compounds	Molecular formula	Retention time	Percent peak area
2-Cyclopenten-1-one, 2-hydroxy-MW: 98	$C_5H_6O_2$	4.74	0.5755
2-Furancarboxaldehyde, 5-methyl-MW: 110	$C_6H_6O_2$	5.32	0.9591
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one MW: 144	$C_6H_8O_4$	5.52	0.0917
Phenol. MW: 94	C_6H_6O	5.71	1.6982
1,2-Cyclohexanedione MW: 112	$C_6H_8O_2$	5.96	0.5819
Cycloheptanone MW: 112	$C_7H_{12}O$	6.35	0.5580
5H-1,4-Dioxepin, 2,3-dihydro-2,5-dimethyl- MW: 128	$C_7H_{12}O_2$	6.71	0.5051
6-Methoxytetrazolo(b)pyridazine MW: 151	$C_5H_5N_5O$	7.18	0.1482
1-Piperidineacetonitrile MW: 124	$C_7H_{12}N_2$	7.36	1.1334
Benzoic acid, hydrazide MW: 136	$C_7H_8N_2O$	7.59	0.1266
2,3-Dimethylfumaric acid MW: 144	$C_6H_8O_4$	7.76	1.1179
Levoglucosenone MW: 126	$C_6H_6O_3$	7.96	0.7402
Acetamide, 2,2,2-trifluoro-N-[2-(hexahydro-1(2H)-azocinyl)ethyl]-MW: 252	$C_{11}H_{19}F_3N_2O$	8.37	0.2182
Piperazine, 1-(aminoacetyl)-MW: 143	$C_6H_{13}N_3O$	9.61	0.2865
Resorcinol MW: 110	$C_6H_6O_2$	9.87	0.2492
2-Furancarboxaldehyde, 5-(hydroxymethyl)-MW: 126	$C_6H_6O_3$	10.12	1.8246
Ethanone, 1-(2-hydroxy-5-methylphenyl)-MW: 150	$C_9H_{10}O_2$	11.51	0.0459
N-(5-Amino-4-cyano-1-pyrazolyl)phthalimide MW: 253	$C_{12}H_7N_5O_2$	11.69	0.1982
2-Butenoic acid, 4,4-dimethoxy-, methyl ester MW: 160	$C_7H_{12}O_4$	12.07	0.0756
2,2-Bis(2'-methoxyphenyl)propane. MW: 256	$C_{17}H_{20}O_2$	12.92	3.2242
1,2,3-Benzenetriol MW: 126	$C_6H_6O_3$	13.16	82.937
D-Allose MW: 180	$C_6H_{12}O_6$	15.30	1.7861
Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, (R)-MW: 218	$C_{15}H_{22}O$	19.21	0.5185
Phenethylamine, 3,4,5-trimethoxy-à-methyl- MW: 225	$C_{12}H_{19}NO_3$	19.36	0.0345
Tridecanoic acid, methylester MW: 228	$C_{14}H_{28}O_2$	21.19	0.2773
Dodecanoic acid, 10-methyl-, methyl ester. MW: 228	$C_{14}H_{28}O_2$	23.34	0.0877

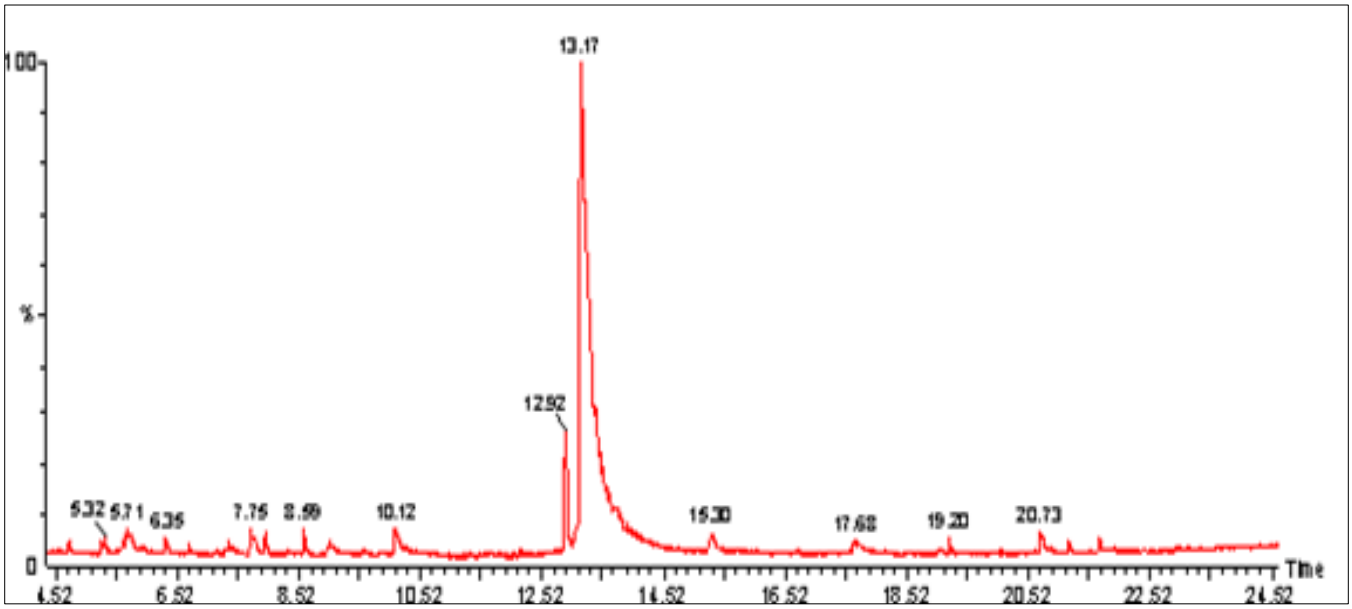

Fig 1 GC-MS analysis *Terminalia chebula*

However, totally 30 compounds were identified from the methanol fractions of the *T. cordifolia* by GC-MS analysis (Table 3). The *T. cordifolia* revealed the synthesis of biologically active compounds such as, Dl-Homoserine,2-Furanmethanol, 2-Cyclopentene-1,4-dione, Propanoic acid, 2-hydroxy-2-methyl, Butanoic acid, 4- hydroxy-4-Amino-4,5-dihydro-2(3H)-furanone, 1-Pyrrolidineethanamine amine 2, 4-Dihydroxy- 2,5-dimethyl-3(2H)-furan-3-one, Glycerin, 4(H)-Pyridine, N-acetyl, Aziridine, 2-isopropyl-1,3- dimethyl-, trans, Butane, 1-(ethenyloxy)-3-methyl, 2,3-Pentanedione, 4-methyl, 4H-Pyran-4-one, Butanamide, 1,3,5-Triazine-2,4,6-triamine, 4-(4-Methyl-piperazin-1-yl)-1,5,-dihydro-imidazol-2-one, Acetone, 1-[4-dimethylaminoethoxy)phenyl, 3-Amino-2-oxazolidinone, 4H-Pyran-4-one,dihydroxy-6-methyl, N-

Methylpyrrole-2- carboxylic acid, 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl, Proline, N-methyl-, butyl ester, L-Proline, 1-methyl-5-oxo-, methyl ester, 2-Methoxy-4-vinylphenol, Phenol, 2,6-dimethoxy, Phenol, 2-methoxy-3-(2- propenyl), 1,5- Diazabicyclo[4.4.0]dec-5-en-2-one, 4-Isopropenyl-4,7-dimethyloxaspiro[2.5]octane, 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a- trimethyl- and Ethanone, 1-(3,4-dimethoxyphenyl)- (Fig 2). among this compound, the highest peak percentage was recorded in the 4H-Pyran-4-one, dihydroxy-6-methyl- (5.0637%), Propanoic acid, 2-hydroxy- 2-methyl (2.6803%) and 1,3,5-Triazine-2,4,6- triamine (2.0638%). The highest retention time was noted in the 1,3,5-Triazine-2,4,6- triamine (16.22).

Table 3 Biologically active compounds of *Terminalia cordifolia* by GC-MS analysis

Name of the compounds	Molecular formula	Retention time	Percent peak area
dl-Homoserine MW: 119	$C_4H_9NO_3$	3.36	0.1589
2-Furanmethanol MW: 98	$C_5H_6O_2$	3.73	0.2979
2-Cyclopentene-1,4-dione MW: 96	$C_5H_4O_2$	4.14	0.6345
Propanoic acid, 2-hydroxy- 2-methyl-MW: 104	$C_4H_8O_3$	4.43	2.6803
Butanoic acid, 4-hydroxy- MW: 104	$C_4H_8O_3$	4.56	1.6129
(±)-4-Amino-4,5-dihydro-furanone MW: 101	$C_4H_7NO_2$	4.85	0.2036
1-Pyrrolidineethanamine MW: 114	$C_6H_{14}N_2$	5.00	0.5918
2,4-Dihydroxy-2,5- dimethyl-furan-3-MW: 144	$C_6H_8O_4$	5.52	0.4571
Glycerin MW: 92	$C_3H_8O_3$	5.60	1.4658
4(H)-Pyridine, N-acetyl- MW: 123	C_7H_9NO	6.22	0.1516
Aziridine, 2-isopropyl-1,3-, trans-MW: 113	$C_7H_{15}N$	6.34	0.4721
Butane, 1-(ethenyloxy)-3- methyl-MW: 114	$C_7H_{14}O$	6.50	0.1535
2,3-Pentanedione, 4- methyl-MW: 114	$C_6H_{10}O_2$	6.72	0.8311
Butanamide MW: 87	C_4H_9NO	7.13	0.0629
1,3,5-Triazine-2,4,6- triamine MW: 126	$C_3H_6N_6$	7.36	2.0638
4-(4-Methyl-piperazin-1- yl)-1,5,-dihydro-imidazol-2-one MW: 182	$C_8H_{14}N_4O$	7.93	1.4753
Acetone, 1-[(dimethylaminoethoxy)- MW: 221	$C_{13}H_{19}NO_2$	8.29	0.0583
3-Amino-2-oxazolidinone MW: 102	$C_3H_6N_2O_2$	8.47	0.4537
4H-Pyran-4-one, dihydroxy-6-methyl- MW: 144	$C_6H_8O_4$	8.59	5.0637
N-Methylpyrrole-2- carboxylic acid MW: 125	$C_6H_7NO_2$	9.13	0.4791
4H-Pyran-4-one, 3,5- dihydroxy-2-methyl-MW: 142	$C_6H_6O_4$	9.30	0.2474
Proline, N-methyl-, butyl ester MW: 185	$C_{10}H_{19}NO_2$	9.50	0.3525
L-Proline, 1-methyl-5-oxo-, methyl ester MW: 157	$C_7H_{11}NO_3$	10.90	0.0945
2-Methoxy-4-vinylphenol MW: 150	$C_9H_{10}O_2$	11.50	1.5741
Phenol, 2,6-dimethoxy- MW: 154	$C_8H_{10}O_3$	12.09	0.4274
Phenol, 2-methoxy-3-(2- propenyl)-MW: 164	$C_{10}H_{12}O_2$	12.16	0.6467
1,5- Diazabicyclo[4.4.0]dec-5-en-2-one MW: 152	$C_8H_{12}N_2O$	13.10	0.0791
4-Isopropenyl-4,7- dimethyloxaspiro[2.5]octane MW: 180	$C_{12}H_{20}O$	15.78	0.2506
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a- trimethyl-MW: 180	$C_{11}H_{16}O_2$	15.86	0.2709
1,3,5-Triazine-2,4,6- triamine MW: 180	$C_{10}H_{12}O_3$	16.22	0.2399

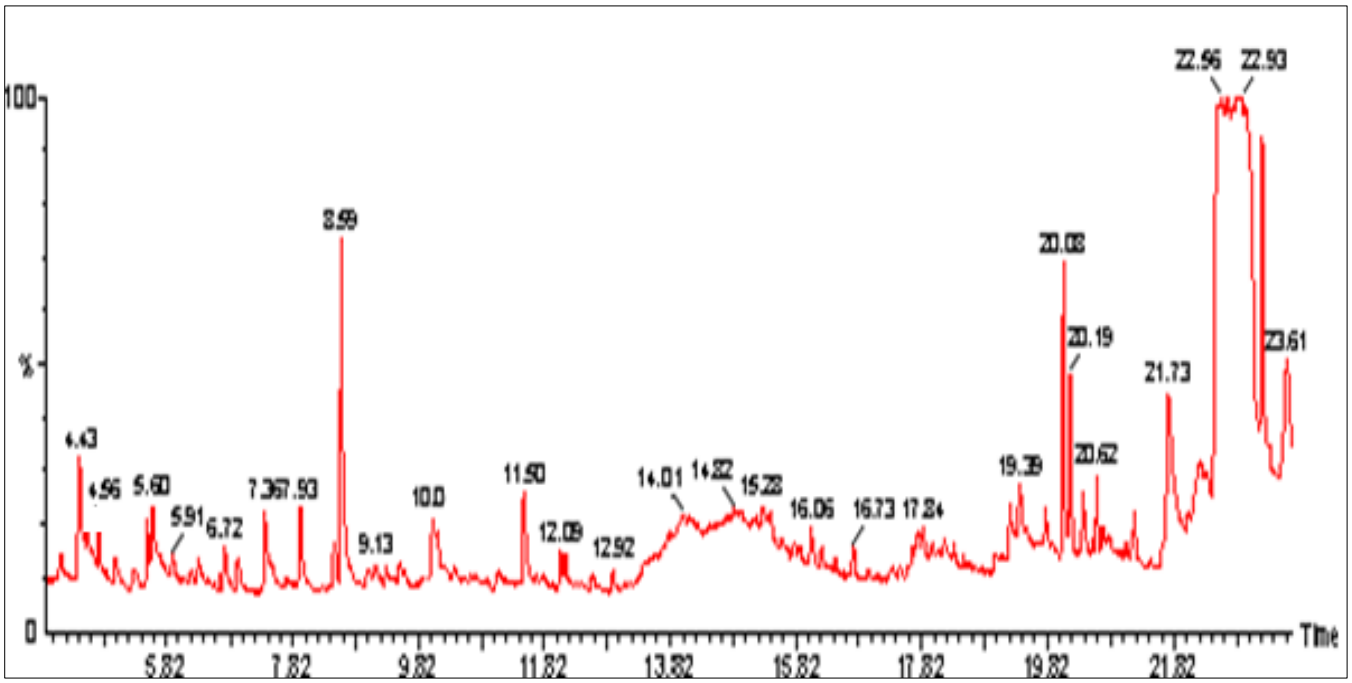

Fig 2 GC-MS analysis *Tinospora cordifolia*

Table 4 Antibacterial activity of methanolic extracts of selected plants against human pathogens

Test organisms	Diameter of zone of inhibition (mm)	
	T. cordifolia	*T. chebula*
B. substilis	10.7±2.3	8.7±1.7
S. aureus	11.3±2.5	7.3±1.4
K. aerogenes	14.1±3.1	12.5±2.1
P. aeruginosa	9.6±1.5	7.8±1.5

Antibacterial activity

Antibacterial activity of methanol leaves extracts of *T. cordifolia* and *T. chebula* were tested against important human pathogens including Gram positive and Gram-negative bacteria by agar disc diffusion method (Table 4). Maximum antibacterial activity (inhibition zone in mm) was recorded against *K. aerogenes* (14.1±3.1mm) followed by *S. aureus* (11.3±2.5mm) *B. subtilis* (10.7±2.3mm) and *P. aeruginosa* (9.6±1.5mm) with the methanol extract of *T. cordifolia*. The moderate activity was noted against *S. aureus* (7.3±1.4mm). *T. chebula* also showed the maximum inhibition was observed against *K. aerogenes* (12.5±2.1mm) followed by *B. subtilis* (8.7±1.7mm). The moderate activity was observed against *P. aeruginosa* (7.8±1.5mm) and *S. aureus* (7.3±1.4mm). Among the plant extracts, *T. cordifolia* showed maximum activity against all oral bacterial strain compared with other plant extracts. Among the pathogens, *K. aerogenes* was more susceptible all the plants followed by *S. aureus*, *B. subtilis*, and *P. aeruginosa*.

Antioxidant activity

Antioxidants are transfer, an electron or a hydrogen atom to DPPH, thus neutralizing its free radical character. Free radicals are known to be a major factor in biological damages and DPPH was used to evaluate the free radical scavenging activity of natural antioxidants. The antioxidant activity of DPPH free radical scavenging assay showed the different plants extract. The scavenging activity of DPPH was increased at a concentration of 500 µg/ml, the scavenging ability on DPPH was 85.22±5.3% in methanolic leaves extract of *V. Cinerea* followed by 82.34±1.5% in methanolic leaves extract of *T. cordifolia*. The methanolic leaves extract of *C. sinensis* and *T. chebula* were showed the highest activity in 78.72±6.2% and 75.38±5.1% respectively at 500 µg/ml concentrations (Table 5).

Table 5 DPPH radical scavenging activity of methanolic leaves extracts of selected plants

Methanolic plants extracts (µg/ml)	DPPH Scavenging activity (%)				
	100	200	300	400	500
T. chebula	18.25±1.2	43.32±2.3	64.71±3.5	68.04±4.2	75.38±5.1
T. cordifolia	32.13±1.7	46.22±1.7	63.78±2.4	67.89±1.2	82.34±1.5

Large numbers of plants belonging to different families have been studied for their therapeutic properties [12]. In the present study *T. chebula* and *T. cordifolia*, reported the phytochemical constituents and pharmacological properties. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non-polar components and volatile essential oil, fatty acids, lipids [13] and alkaloids [14]. In the present study, the quantitative GC/MS Phytochemical investigations of the two different plants *T. chebula* and *T. cordifolia* have been reported on presence of tannins, carbohydrates, glycosides, phenols, alkaloids, terpenoids and flavonoids. The result of the GC-MS analysis of 30 compound of *T. cordifolia* and 26 compound of *T. chebula* were identified. The plant samples revealed the synthesis of these compounds are of pharmacological importance. All these compounds are of pharmacological status 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 tests under way leading to the identification of the active molecules present in these plants.

The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic pro types [15]. The demand on plant-based therapeutics is increasing in both developing and developed countries due to growing recognition that they are natural products, non-narcotic, easily biodegradable, pose minimum environmental hazards, have no adverse side-effects and are easily available at affordable prices. The present investigation involving *T. chebula* and *T. cordifolia* also lends credence to the above observations.

Antioxidant compounds in natural products play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases. Most of the antioxidant compounds are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. DPPH assay is widely used to determine the antioxidant activity of plant extract. This assay is based on the ability of antioxidant compound to decolourise the purple colour of DPPH free radical in alcoholic solution to yellow colour [16]. In the present study, the scavenging activity of DPPH was increased at a concentration of 500 µg/ml, the scavenging ability on DPPH was 82.34±1.5% in methanolic leaves extract of *T. cordifolia*.

CONCLUSION

Herbal medicines are in of great demand in the developed as well as developing countries for primary health care because of their wide biological and medicinal activities, high safety margins and less cost. In the present study reported that the good sources of phytocompound from these experimental plants. 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 compounds present in these plants. In future, stability studies are required to ensure the usage of this plant for formulations and *in vivo* pharmacokinetic evaluation is also required to assess the bioavailability.

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