

RESEARCH ARTICLE

CHARACTERIZATION OF THE BIOACTIVE CONSTITUENTS PRESENT IN THE HEXANE EXTRACT OF *TERMINALIA CHEBULA* FRUITSP. Radha^{1*}, S. Vennila² and K. Kumaran³¹Biochemistry, Forest College and Research Institute, MettupalayamEmail: radhubctnau@gmail.com²Forestry, Agricultural College and Research Institute, Tiruvannamalai³Forestry, Forest College and Research Institute, Mettupalayam

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Abstract: A study was conducted to assess the biochemical constituents present in the fruits of *Terminalia chebula*. The hexane extract of *Terminalia chebula* fruits was prepared by using soxhlet apparatus and the extract was subjected to GC-MS and FTIR analysis. The hexane extract of *T. chebula* fruits showed the presence of large number of phytoconstituents that possess antioxidant, antibacterial and antifungal activities. FTIR analysis revealed the presence of functional groups such as OH, CH, C=O, NH, C=C, CF, =CH that represents the presence of polyphenols, tannins, terpenoids, flavonoids, carbohydrate and protein compounds in *T. chebula*.

Keywords: *Terminalia chebula*, phytoconstituents, GCMS, FTIR

INTRODUCTION

About 80% of world population depend on plant based traditional medicine for their primary healthcare needs (WHO, 2002). Medicinal plants fight against human diseases from the beginning of evolution (Bag *et al.*, 2013). *Terminalia chebula* (*T. chebula*) Retz. (Combretaceae), possess a number of medicinal activities which are attributed to the large number of different phytoconstituents that are present in it. The fruits of *T. chebula* possess diverse health benefits and have been used since time immemorial as a traditional medicine against various human diseases (Khare, 2007). It is an important Indian herb used extensively in the indigenous system of medicine (ayurvedic) for its homeostatic, antitussive, laxative, diuretic, and cardiogenic activities (Barthakur and Arnold, 1991). It has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. The extract of *T. chebula* has been reported to exhibit a variety of biological activities, including anticancer, antidiabetic, antibacterial and anticaries effects (Balaji *et al.*, 2015). The present study was carried out to study the phytochemical constituents present in the hexane extract of *T. chebula* fruits.

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MATERIALS AND METHODS

Collection of *T. chebula* fruits

The fruits of *T. chebula* was collected from tribal community in Dharmapuri district, Tamil Nadu. The collected sample was dried, powdered and used for the analysis.

Preparation of hexane extract of *T. chebula* for GC-MS analysis

Exactly 5 g of the crushed fruit sample of *T. chebula* was extracted with 250 ml of hexane in a soxhlet apparatus. The extraction was performed at 60°C for 6 hours.

Gas Chromatograph and mass spectrometer (GC-MS) analysis

The chemical composition of the hexane extract of *T. chebula* fruits was analysed using Shimadzu Gas Chromatography and Mass Spectrophotometer - QP 2020 with a SH-Rxi-5 Sil MS Cross Band capillary mid-polar column. Sample size of 1.0µl hexane extract was injected for analysis and Helium was used as a carrier gas at 1.2 mL/minute. The column oven temperature was programmed from 80°C to 285°C (80°C for 5 min, 4°C rate 260°C, and 2°C rate 285°C hold for 10 minutes). The MS was set to scan from 45-650Da. The MS also had inbuilt pre-filter which reduces the neutral particles. The data system has two inbuilt libraries for searching and matching the spectrum viz., NIST4 and WILEY9 containing more than a million references.

Fourier transform infrared spectroscopy (FTIR) analysis

FTIR was performed with a FT/IR-6800 typeA ATR PRO ONE. The analysis range was selected between 4000 and 400 cm^{-1} using a resolution of 4 cm^{-1} . The generated IR spectroscopy was tentatively assigned based on the wavelength.

RESULTS AND DISCUSSION**Characterization of the bioactive compound in the hexane extract of *T. chebula* fruits by GC- MS Analysis**

The chromatogram and spectrum of the peaks were obtained and visualized. The particular compounds present in the samples were identified by matching their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored in CAS library. *Terminalia chebula* fruit showed 40 peaks from the chromatogram of the extract. The results are presented in Table 1. The major phytochemicals identified in the hexane extract of *Terminalia chebula* fruit by GC-MS and their molecular formulae, percentage compositions, molecular masses of the compounds and their reported activity are shown in Table 2.

Table 1. Phytochemical profiling of hexane extract of *T. chebula* fruits

Peak No.	RT	Area%	Compound Name
1.	3.023	0.292	Hexadecane, 1,1-bis(dodecyloxy)-
2.	3.073	0.568	3-Hexene, 2-methyl-, (E)-
3.	3.259	1.835	Benzene, 1,3-dimethyl-
4.	3.349	1.845	p-Xylene
5.	3.639	5.388	o-Xylene
6.	5.009	0.676	Benzene, 1,2,3-trimethyl-
7.	5.449	0.476	Benzene, 1,2,4-trimethyl
8.	5.669	33.032	2-Pyrrolidinone, 1-methyl-
9.	5.890	0.717	Undecane, 5-methyl-
10.	5.965	0.527	Decane, 2,4,6-trimethyl-
11.	6.250	0.368	Benzene, 2-ethyl-1,4-dimethyl-
12.	6.375	0.498	Benzene, 1-ethyl-2,4-dimethyl-
13.	6.550	0.470	Undecane, 4,7-dimethyl-
14.	6.915	0.416	Benzene, 1,2,4,5-tetramethyl-
15.	7.400	0.357	Benzene, 1,2,3,4-tetramethyl-
16.	9.136	0.400	Decane, 2,4,6-trimethyl-
17.	9.771	0.289	Decane, 2,3,5,8-tetramethyl-
18.	10.957	0.305	Dodecane, 2-methyl-
19.	15.083	1.287	Phthalic acid, bis(7-methyloctyl) ester
20.	16.619	0.396	aR-Turmerone
21.	19.275	0.836	Octadecane
22.	20.720	0.312	1,2-Benzenedicarboxylic acid, butyl octyl ester
23.	22.456	2.711	n-Hexadecanoic acid
24.	22.556	5.024	Dibutyl phthalate
25.	22.726	0.384	Acetamide, 2-[6-cyclopropyl-3-cyano-4-(2-furyl)-2-pyridylthio]-
26.	23.186	0.856	Heptacosane
27.	25.072	0.518	à-D-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-(trimethylsilyl)-, cyclic methylboronate
28.	25.652	1.176	Linoelaidic acid
29.	25.747	7.485	trans-13-Octadecenoic acid
30.	26.043	0.303	Dasycarpidan-1-methanol, acetate (ester)
31.	26.093	1.493	3,6,9,12,15-Pentaoxonadecan-1-ol
32.	26.168	1.291	Hexa-t-butylselenatrisiletane
33.	26.283	0.310	Erucic acid
34.	26.308	0.775	1-O-Retinoyl á-D-methyl-2',3'-4'-tri-O-acetylglucopyranuronate
35.	26.413	0.297	Clocortolonepivalate
36.	26.913	0.648	Tetratetracontane
37.	27.668	0.308	Triamcinolone Acetonide
38.	28.709	1.384	Octadecane, 3-ethyl-5-(2-ethylbutyl)-
39.	29.594	0.392	.psi...psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-
40.	29.819	0.582	Heptadecane, 9-hexyl-

Table 2. Major phytochemicals identified in the hexane extract of *T. chebula* fruits and their reported activity

Peak No.	RT	Area%	Compound Name	Mol. formula	Mol. wt g/mol	Reported activity
1.	3.259	1.835	Benzene, 1,3-dimethyl-	C ₈ H ₁₀	106.16	Antibacterial
2.	5.669	33.032	2-Pyrrolidinone, 1-methyl-	C ₅ H ₉ NO	99.13	Antibacterial
3.	15.083	1.287	Phthalic acid, bis(7-methyloctyl) ester	C ₂₆ H ₄₂ O ₄	418.6	Antibacterial
4.	22.456	2.711	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42	Antioxidant, pesticide
5.	22.556	5.024	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278.34	Antibacterial
6.	25.652	1.176	Linoelaidic acid	C ₁₈ H ₃₂ O ₂	280.4	Antimicrobial antifungal
7.	25.747	7.485	trans-13-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	282.5	Antibacterial
8.	26.093	1.493	3,6,9,12,15-Pentaoxonadecan-1-ol	C ₁₄ H ₃₀ O ₆	294.38	Antibacterial
9.	26.168	1.291	Hexa-t-butylselenatrisiletane	C ₂₄ H ₅₄ SeSi ₃	505.9	Antibacterial
10.	28.709	1.384	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C ₂₆ H ₅₄	366.7	Antimicrobial, antifungal

Various methyl and ethyl esters of phenolic compounds obtained in many cases are reported to be the artefacts generated during the extraction and isolation procedures (Lee *et al.*, 2017). Various pharmacological activities have been reported for the

extracts or isolated individual compounds from the fruits of *T. chebula* (Nigam *et al.*, 2020).

Fourier transform infrared spectroscopy (FTIR) analysis

The results of FTIR analysis of *T.chebula* fruits is presented in Figure 1.

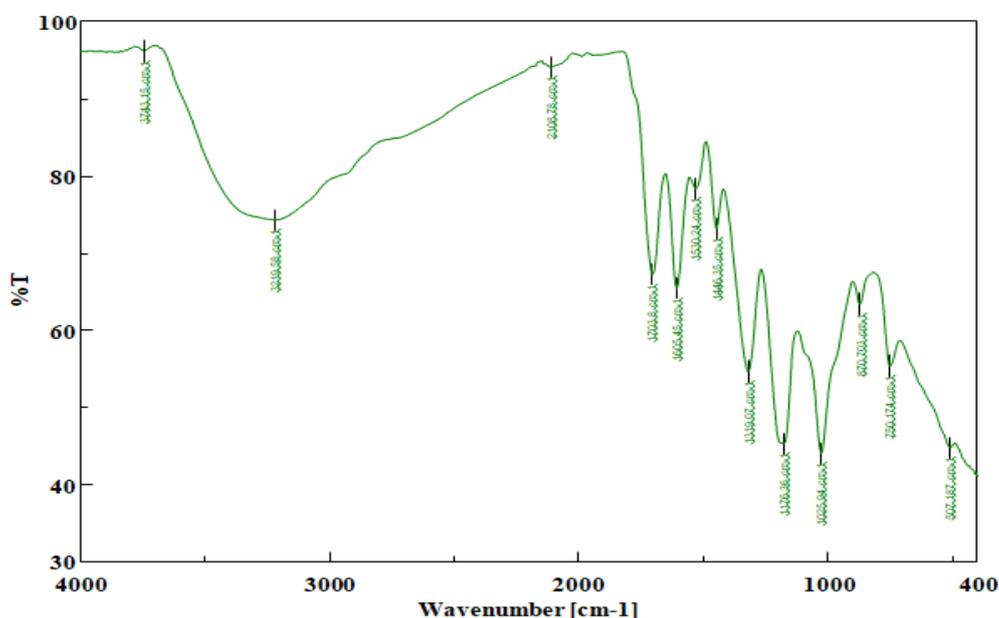


Fig. 1. FTIR spectrum of *T. chebula* fruit

FTIR study carried out for *T. chebula* fruit extract (Fig.1.) showed strong bands at 507, 750, 870, 1025, 1319, 1446, 1530, 1605, 1703, 2108, 3219 and 3743 cm⁻¹. The FTIR spectra of *Terminalia chebula* fruit extract at 3743 and 3219cm⁻¹ represented O-H stretching of polyols. The absorption peak at 2108, 1703 and 1605cm⁻¹ represented C=O stretching vibrations. Whereas absorbance peak at 1446 cm⁻¹

attributed to C-H stretching. Band at 1319 cm⁻¹ and 1025 cm⁻¹ corresponds to C-F and C-O stretching vibration. The functional groups such as OH, CH, C=O, NH, C=C, CF, =CH represents the presence of polyphenols, tannins, terpenoids, flavonoids, carbohydrate and protein compounds in *T. chebula*.

CONCLUSION

GCMS analysis of *T. chebula* fruits showed a maximum of 40 peaks most of which are found to possess antioxidant, antibacterial and antifungal properties. FTIR spectral analysis of *T. chebula* showed the presence of functional groups such as OH, CH, C=O, NH, C=C, CF, =CH represents the presence of polyphenols, tannins, terpenoids, flavonoids, carbohydrate and protein compounds in *T.chebula*.

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