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Research Article

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Evaluation of *in vitro* anticancer activity of *Terminalia chebula* and Identification of Phytocompounds by GC MS analysis

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ABSTRACT

The present study was aimed to evaluate the potential of the ethanolic extract of Terminalia chebula in mitigating breast cancer, which is crippling several women around the world. We have identified the phytochemicals and confirmed by GC-MS studies. Further investigations were carried out to determine the anticancer activity of the extract invitro by MTT assay and antioxidant activity by Reducing Power assay. The results obtained were found to be effective in authenticating the pharmacological nature of T. chebula. In this effort, we have surfaced with a promising potential therapeutic agent as a part of our research work. Our observations are suggestive of the fact that this extract could ably serve as a drug candidate for further research, being a harbinger of hope to cancer patients.

Keywords: Breast cancer, Terminalia chebula, MTT assay, GC-MS, Antioxidant

INTRODUCTION

Cancer is a multifactorial disease that involves modulation of multiple pathways and targets. In India, breast cancer is the second leading cause of cancer deaths in women and the risk of its incidence is increasing every year **[1]**. There are various treatment approaches that have been aimed towards the treatment of the disease but the success rate of the chemotherapeutic drugs are reported to be low with high rate of recurrence and various side effects. Although several drugs are generally well tolerated and effective, they have been found to cause many adverse side effects. This necessitates the need to evolve alternative therapies, such as herbal therapy.

Herbal medicine derives its properties mainly from plants or extracts to provide cure and treat illness **[2].** It is also known as Phytotherapy or Phytomedcine. There is not widespread clinical data to support the proven effect of plant remedies to cure cancer. So, an approach was made to test the effectiveness of the ethanolic extract of the fruits of *Terminalia chebula* for its potential to develop as an anticancer drug in future. Inorder to identify the compounds present in the extract we relied upon GC-MS studies which would help proceed further with our research work providing deeper understanding and greater insights into the possible anticancer constituent present in the plant. The extract was screened for its phytochemical, cytotoxicity and antioxidant activity to provide supportive evidence to the findings observed from GC-MS.

The secondary metabolites of plants are grouped according to their chemical structures and properties as alkaloids, flavonoids, anthroquinones, phenolic compounds, cortico steroids, essential oils etc., [**3**]. Some medicinal plants contain a wide variety of natural antioxidants, such as phenolic acids, flavonoids and tannins, which possess more potent antioxidant activity than dietary plants. Many investigations indicate that these compounds are of great value in preventing the onset and/or progression of many human diseases. [**4**].

Terminalia chebula has been extensively used in ayurveda, unani and homeopathic medicines and has become a cynosure of modern medicine. The fruit possess diverse health benefits due to the presence of various phytochemicals like polyphenols, flavonoids, and alkaloids **[5].** The present study proves the traditional claims of the plant against cancer. This paper will possibly help to bridge between traditional claims and modern therapy on *T.chebula.*

EXPERIMENTAL SECTION

Collection of Plant Materials

The dried fruits of *Terminalia chebula* were collected from markets in Trichy district. The fruits were identified by the Department of Botany, St. Joseph College, Trichy and the specimen voucher was deposited there.

Preparation of Plant Extract

The coarse powder (500gm) of the given sample was extracted with 2.5 litres of Ethanol 95% by continuous hot percolation using Soxhlet apparatus until the extraction was completed. After the completion of the extraction, the extract was filtered and the solvent was removed by distillation under reduced pressure. Greenish black colored residue was obtained.

Phytochemical Screening

The extracts were screened for the presence of various phytochemicals as alkaloids, tannins, saponins, phenols, steroids, cardiac glycosides, carbohydrates, amino acids and monosaccharide's **[6]**

GC-MS Analysis

GC-MS analysis is a non-volatile rapid simultaneous determination of various metabolites in a sample. Gas chromatography is used for separation and mass spectrometry is used for the detection of the compounds. By this way, hundreds of metabolites in a sample can be identified in a single analysis. For the purpose of analysis, a Gas chromatograph (Agilent, USA), hyphenated to High Resolution Mass spectrometer was employed. Helium was used as carrier gas at the rate of 1 mL/min. A capillary column of internal diameter 0.25 mm was used. The inlet temperature and detector temperature were 1000 C and 2800 C respectively. The temperature was programmed at 1000 C at the start, and then changed to 2000 C (100 C/min) for 2 min. It was raised to 2400 C (100C/min), kept for 3 min and finally raised to 2800C (300C/min) for 3 min. Total GC running time was 27 minutes. The peak area, that is, the % amount of every component was calculated by comparing its average peak area to the total areas. HPCHEM software was used to handle mass spectra and chromatogram. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight and structure of the components of the test materials were ascertained.

Identification of compounds

Compounds were identified by comparing their linear retention indexes (LRIs) and matching their mass spectra with those of reference compounds in the data system of the Wiley library and NIST Mass Spectral Search program (Chem. SW. Inc; NIST 98 version database) connected to a Varian Saturn 2000R MS. Identifications were also determined by comparing the retention time of each compound with that of known compounds. Components were identified by comparison of their mass spectra and retention indices with those published in and contained in the NIST '98 MS computer library.

Antioxidant Activity

Reducing power assay

Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide ($Fe3^{+}$) to form potassium ferrocyanide ($Fe2^{+}$), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700nm.

Method

A spectrophotometric method **[7]** was used for the measurement of reducing power. For this 2.5ml of each extracts(200,100,50,25,12.5 and 6.25 mg/ml) was mixed with 2.5ml phosphate buffer (0.2M,pH 6.6) and 2.5ml of 1% potassium ferricyanide [10mg/ml].The mixture was incubated at 50^{0}c for 20 minutes, then rapidly cooled, mixed with 2.5ml of 10% Trichloroacetic acid and centrifuged at 6500rpm for 10 minutes. An aliquot [2.5ml] of the

supernatant was diluted with distilled water [2.5ml] and ferric chloride [0.5ml, 0.1%] was added and allowed to stand for 10 minutes. The absorbance was read spectrophotometrically at 700nm.Ascorbic acid used as standard for construction of the calibration curve and the reducing power was reported as Ascorbic acid equivalent per 100gm of sample.

RESULTS AND DISCUSSION

Phytochemical analysis revealed the ethanolic extract of fruit of *Terminalia chebula* to contain tannins, saponins, terpenoides and phenols in greater proportions, while cardiac glycosides, flavonoids in smaller concentrations.

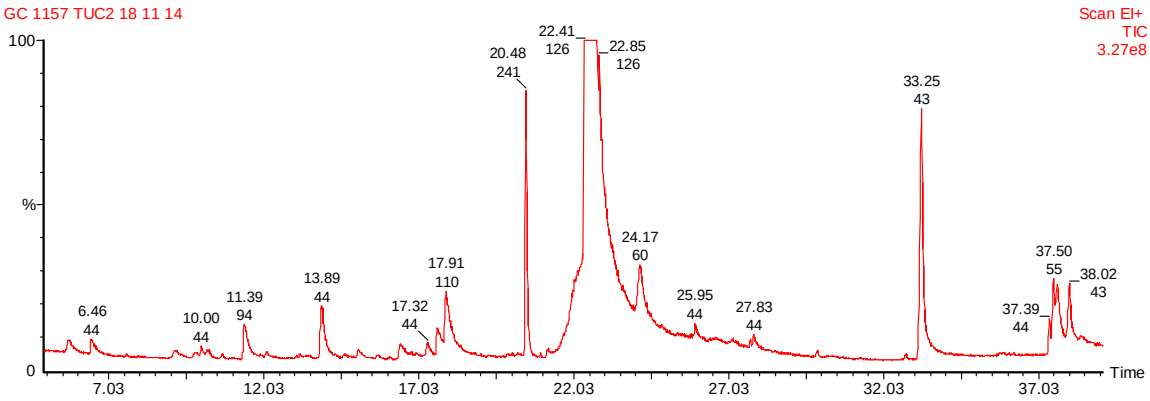

Fig 1 chromatogram

Chromatogram is indicative of some vital compounds that help the extract to liberate potential anticancer activity. A few compounds have already been reported in crude extract and fractions of ethanolic extract of *T. chebula* [**24] & [25**]. These traditional claims contribute to the additional evidence that the plant is best suited to find a place in the pharmacological market if explored further with effective methodology and sensitive techniques.

Screening of ethanolic extract of *Terminalia chebula* demonstrated remarkable anticancer activity against MCF-7 cells. The percentage cell inhibition profile was found to be concentration dependent. The maximum concentration used in the study was 400µg/ml. Responses of MCF-7 cells toward increase concentrations of ethanolic extract were exponential. From the analysis, results indicate a 64% MCF-7 cell line inhibition at the maximum dose. The *in vitro* dosage- and time-dependent effects of extract against cultured MCF-7 cells are shown in Table 1 and 2. Following incubation of these cells with 400µg, approximately 50%, 60% and 64% decreases of cell growth were observed after 24, 48and 72 hours of incubation, respectively, as compared to the untreated control cells. Under the same conditions, incubation of these cells with 25µg of ethanolic extract resulted in 14%, 22% and 23% decreases of cell growth at these same time points. A bar graph had been plotted to demonstrate a pictorial representation of the percentage of cell viability of test extract (ethanolic extract of *Terminalia chebula* (25-400 µg/ml) on MCF-7 cells after 24, 48 and 72 hours of treatment in MTT assay. Our results are in accordance with the finding of Bharath Reddy **[26]**. However, the effects of the extracts at higher concentration have to be performed to determine the exact dosage. Nevertheless, the underlying mechanism by which the plant exhibits anticancer property has to be studied and further work in this regard has been undertaken and is in progress, so that a novel and a safer drug could be designed.

Table 1: List of compounds identified by GC-MS

S.No.	Peak Name	Retention Time(min)	% Peak area	Biological activity	Nature of the compound	Reference
1.	Name: 1H-Pyrazole, 1,3-dimethyl- Formula: $C_5H_8N_2$ MW: 96 (PYRAZOLE DERIVATIVES)	6.46	0.4021	Antimicrobial Anticancer Potent against MCF-7 Antioxidant Anti inflammatory	Alkaloid Aromatic	**[8]**
2.	**Name: Phenol** **Formula: C_6H_6O** **MW: 94**	**11.39**	**1.1058**	**Antimicrobial** **Anticancer** **Antioxidant** **Anti inflammatory**	Phenol	**[9]** **[10]** **[11]**
3.	Name: 2-Octanone Formula: $C_8H_{16}O$ MW: 128	12.13	0.0865	Antimicrobial		**[12]**
4.	**Name: 2,5-Dimethyl-4-hydroxy-3(2H)-furanone** **Formula: $C_6H_8O_3$** **MW: 128**	13.08	0.0318	Antimicrobial		**[13]**
5.	**Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-** **Formula: $C_6H_8O_4$** **MW: 144**	15.08	0.1975	Antimicrobial Anticancer Anti inflammatory	Flavanoid	**[14]** **[13]** **[15]** **[11]**
6.	Name: 2,3-Anhydro-d-galactosan Formula: $C_6H_8O_4$ MW: 144	17.32	0.2833	Antimicrobial Anticancer	Sugar moiety	**[16]**
7.	**Name: 2-Furancarboxaldehyde, 5-(hydroxymethyl)-** **Formula: $C_6H_6O_3$** **MW: 126**	17.64	0.7136	Antimicrobial	Aldehyde	**[14]** **[13]** **[12]** **[11]**
8.	Name: 3-(4-Methylpent-3-enyl)thiophene Formula: $C_{10}H_{14}S$ MW: 166	17.90	1.1909			
9.	Name: Benzene, 1,1'-(1-methylethylidene)bis [4-methoxy- Formula: $C_{17}H_{20}O_2$ MW: 256	20.48	2.7679	**Anticancer**		**[17]**
10.	Name: Benzaldehyde, 3-hydroxy-4-methoxy- Formula: $C_8H_8O_3$ MW: 152 **ISOVANILLIN**	21.21	0.0826	Antimicrobial		**[18]**
11.	**Name: 1,2,3-Benzenetriol** **Formula: $C_6H_6O_3$** **MW: 126** **Pyrogallol**	**22.41**	**82.7986**	**Antimicrobial** **Anticancer** **Antioxidant**	Poly phenolic	**[18]** **[13]** **[19]**
12.	**Name: D-Allose** **Formula: $C_6H_{12}O_6$** **MW: 180**	**24.17**	**1.5040**	**Antimicrobial** **Anticancer**	Sugar	**[17]**
13.	Name: Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, (R)- Formula: $C_{15}H_{22}O$ MW: 218 **XANTHORRHIZOL**	27.83	0.0717	Antimicrobial esp G+ & G- Anticancer Antioxidant		**[20]**
14.	**Name: n-Hexadecanoic acid** **Formula: $C_{16}H_{32}O_2$** **MW: 256**	**33.25**	**4.9938**	**Antimicrobial** **Anticancer** **Antioxidant**	Palmitic acid	**[21]** **[15][11]** **[17]** **[22]**
15.	Name: 9,17-Octadecadienal, (Z)- Formula: $C_{18}H_{32}O$ MW: 264	37.39	0.1581	Antimicrobial Anti inflammatory	Aldehyde	**[23]**

Table 2: Average absorbance for various treatments of MCF-7 cells by the ethanolic extract of *Terminalia chebula* (25-400 µg/ml) after 24, 48 and 72 hours in MTT assay

Sample	Average Absorbance (570 nm)		
	After 24 hrs	After 48 hrs	After 72 hrs
Control	2.45	2.45	2.45
Test extract (25 µg/ml)	2.110	1.913	1.888
Test extract (50 µg/ml)	1.943	1.642	1.632
Test extract (100 µg/ml)	1.763	1.431	1.333
Test extract (200 µg/ml)	1.592	1.210	1.055
Test extract (400 µg/ml)	1.241	0.992	0.887

Table 3: Effects of the ethanolic extract of *Terminalia chebula* (25-400 µg/ml) on viability of MCF-7 cells after 24, 48 and 72 hours in MTT assay

Sample	Cell Viability (Percentage)		
	After 24 hrs	After 48 hrs	After 72 hrs
Control	100	100	100
Test extract (25 µg/ml)	86.12	78.08	77.06
Test extract (50 µg/ml)	79.31	67.02	66.61
Test extract (100 µg/ml)	71.96	58.41	54.41
Test extract (200 µg/ml)	64.98	49.39	43.06
Test extract (400 µg/ml)	50.65	40.49	36.20

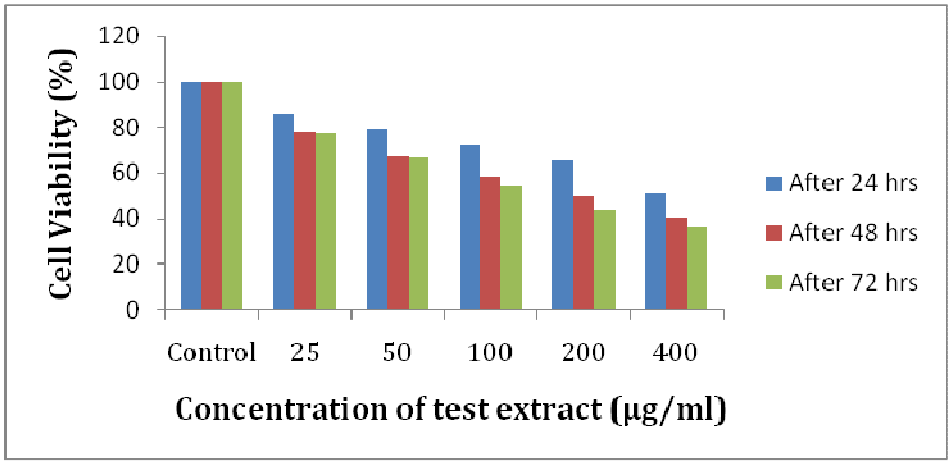

Fig 2 Percentage of cell viability of the ethanolic extract of *Terminalia chebula* (25-400 µg/ml) on MCF-7 cells after 24, 48 and 72 hours of treatment in MTT assay

Table 4 :Antioxidant Activity by Reducing Power assay

Sample	Concentration mg/ml					
	6.25	12.5	25	50	100	200
Ethanol	0.393	0.423	0.579	0.699	0.874	0.953
Standard (Ascorbic acid)	0.301	0.435	0.556	0.734	0.896	0.982

It is well known that there is a strong relationship between total phenol content and antioxidant activity, as phenols possess strong scavenging ability for free radicals due to their hydroxyl groups. Therefore, the phenolic content of plants directly contributes to their antioxidant action. [27; 28; 29].

Antioxidant based drug for formulations are used for the prevention and treatment of complex diseases like arthrosclerosis, stroke, diabetes, Alzheimer's disease and cancer. **[30].**

CONCLUSION

This paper reveals the significant relationship between laboratory findings and literature survey that serves to support our ideology of proving the medicinal property of the ethanolic extract of the fruits of *Terminalia chebula*. In spite of the well-known fact of chebula being the king of medicines much more knowledge and effort is needed to

tap the potential of this plant so as to formulate and develop a novel drug in order to treat humans suffering from complex medical conditions. Our aim is to find a single drug to cure multiple problems associated with the sickness and our predominant strategy is to focus on woman without whom the society is baseless.

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