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Full Length Article

Profiling of phytochemical constituents of *terminalia chebula* fruit extract by different solvent effects and synchronized analysis of FTIR and GCMSMohamed Farouk Elsadek<sup>a,\*</sup>, Khalid S. Al-Numair<sup>b</sup><sup>a</sup> Department of Biochemistry, College of Sciences, King Saud University, P.O.Box 2455, Riyadh 11451, Saudi Arabia<sup>b</sup> Department of Community Health Sciences, College of Applied Medical Sciences, King Saud University, P.O.Box 10219, Riyadh 11433, Saudi Arabia

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## ABSTRACT

**Background:** Detecting the *Terminalia chebula* best extract-enriching bioactive compounds using four different solvents; Ethanol, water, chloroform and petroleum ether for medicinal applications.**Methods:** Qualitative analysis of phytochemicals of *T. chebula* (such as Tannins, saponins, flavonoids, alkaloids, terpenoids); Quantitative Determinations (Condensed tannins; total flavonoids, and phenolics), also, determination of enzymatic and non-enzymatic antioxidants. GC-MS and FT-IR analysis of ethanolic extract were investigated.**Results:** The assays for phenolics, tannins and total flavonoids indicated an extensive difference in the total phenolic content ranging from 26 to 104, 13 to 106 and 106 µg/g of extract. It was found that, the highest phenolics, tannins and total flavonoids content were found in the ethanolic extract and the lowest by petroleum ether extract. In examining the amount of total protein content also, high amount of total proteins observed in the ethanolic extract as 67 µg/g whereas, in contrast, the high amount of total carbohydrates observed in the aqueous extract with 103 µg/g. Further, the level of the different enzymatic antioxidant markers (SOD, Catalase and Gpx) and non-enzymatic antioxidant markers (vitamin C and vitamin E) produced by the ethanolic *T. chebula* fruit extract were 0.46 µg, 97 µg and 127 µg and 39.08 µg and 18 mg/liter. The DPPH and reducing power assay radical scavenging activities of different solvent extracts of *T. Chebula* exhibited free radical scavenging properties up to 96 % of inhibition. The functional groups of the components were separated based on its peaks through FTIR analysis showed the existence of alcohol, alkanes, carboxylic acid, alkenes, alkanes, ethers, halogen respectively. The active compounds identified based on GCMS analysis showed the presence of furaldehyde, 2,5-furandicarboxaldehyde Dodecanoic acid, ethylester n-pentadecanol, 1,2,3-Benzenetriol pyrogallol, 3,4,5-trihydroxy benzoic acid, Octadecanoic acid, ethyl ester and Hentriacontane.**Conclusion:** Among four solvent screened, the ethanol extract of *T. chebula* revealed variety of bioactive phytochemicals. In addition to higher antioxidant activities and highest level of radical scavenging activity. Accordingly, it can be inferred that *T. chebula* may serve as an antibacterial, antifungal, anti-inflammatory, and anti-diabetic agent in addition to being a plant-based antioxidant.

## 1. Introduction

Photochemicals are naturally occurring chemicals found mostly in plants, especially vibrant ones. They are abundant in medicinal plants and herbs and function as primary and secondary plant metabolites with anti-inflammatory, anti-microbial, anti-diabetic, and anti-hyperglycemic properties (Saiful Yazan and Armania, 2014). Natural antioxidants mostly comprise plant phenolic components such flavonoids, tocopherols, and phenolic acids. The potential natural source for disease prevention and treatment provided by medicinal plants is mostly

due to their secondary metabolites. In order to access these natural chemicals from plant extracts vital for their therapeutic characteristics, many researchers have been drawn to plants (Ali et al., 2008).

Therefore, emphasis has been put on using natural antioxidants, such as bioactive flavonoids, which are crucial because of their native origin and potent capacity to scavenge and trap free radicals. *T. chebula* has been used extensively in Ayurveda to treat diabetes. In addition, according to Bag et al. (2013), it has been used to treat vomiting, sore throats, coughs, dysentery, diarrhoea, ulcers, bleeding piles, heart, gout, asthma and bladder issues. Vitamin K and calcium, which are essential

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for blood homeostasis and bone health, are abundant in *T. chebula* leaves, claimed by Malik et al. (2020). *T. chebula* fruits are immature, black, ovoid and contain about 20–40 % of tannins, anthraquinones,  $\beta$ -sitosterol, palmitic, oleic and linoleic acids. Bajpai et al. 2005 examined the antioxidant properties of *T. chebula* and its bioactive component-rich fractions and reported When compared to the leaves and fruits of *T. chebula* (80.1 0.9 % and 79.8 0.5 %, respectively), the bark exhibits exceptional antioxidant activity (85.2 1.10 %). The phenolic particles in phenolic compounds, which stifle free radicals to prevent oxidative stress, were said to be responsible for this antioxidant effect.

Numerous research teams have thoroughly investigated the antimicrobial, antioxidant, anticarcinogenic, hypocholesterolemic, diuretic, antimicrobial and anti-carcinogenic properties of *T. chebula* (Cheng et al., 2003; Saleem et al., 2002) These effects of *T. chebula* include diuretic, antitussive and wound healing. They can also be used as a dentifrice to treat loose gums, ulcers, and gum bleeding, as well as a tonic for hepatic issues, spleen enlargements, and skin conditions (Pouly and Larue, 2007). *T. chebula* fruit pulp extracts in water ethanolic form demonstrated notable antibacterial activity, antiviral activity at minimum inhibitory concentrations (MIC), and minimum bactericidal effects (Bag et al., 2013). Also, *T. chebula* has been reported to be effective against *Alternaria brassicicola*, *A. alternata*, *Aspergillus niger*, *Helminthosporium*, *Fusarium oxysporum*, *F. solani* and *Phytophthora capsica* (Mehmood et al., 1999; Shinde et al., 2011). Tayal et al. (2012) showed that *T. chebula* extract had cyto-protective effects on the MDCK and NRK-52E renal epithelial cells by lowering LDH leak and boosting cell survival. Also, they reported it inhibited calcium oxalate crystal formation in vitro and it is a strong candidate for additional pharmacological studies.

Therefore, the objective of the current study is to assess the phytochemical contents of four extracts from *T. chebula* with different solvents (Ethanol, Water, Chloroform and Petroleum ether) and their antioxidant potential by DPPH and reducing power assay. Also, the level of the different enzymatic antioxidant markers (SOD, Gpx and Catalase) and non-enzymatic antioxidant markers (Vitamin E and C) were screened. Further, these extracts were then Characterized using FTIR and GCMS analysis for identifying the best extract with maximum bioactive compounds could be used in different diseases management.

## 2. Materials and methods

### 2.1. Plant material collection

Healthy *T. chebula* fruit was collected from Kanchipuram, Tamil Nadu, India. The plant material was washed and rinsed with 70 % alcohol and sterilized distilled water respectively, dried in air, powdered and the extracts were prepared.

### 2.2. Preparation of extracts

The plant material was ground into a powder, and the powdered components are utilized to create extracts for usage with water, ethanol, petroleum ether, and chloroform. The extraction of each extract was carried out independently using 150 ml of ethanol, chloroform, petroleum ether, and water for 4 h in the sohxlet apparatus using 10 gm of carefully weighed powdered ingredients. The extract was collected and allowed to dry entirely while evaporating. The dried extract was gathered and used for other analysis.

### 2.3. Qualitative analysis of phytochemicals of *t. Chebula*

In order to establish the presence (+) and absence (–) of phytochemicals like tannins, terpenoids, flavonoids, saponins, alkaloids, glycosides, phenol, steroids, amino acids, and proteins, the various solvent extracts of *T. chebula* fruit underwent a variety of standard qualitative

assays. Tannins, saponins, flavonoids, alkaloids, terpenoids, glycosides, proteins, and steroids are among the substances that have been tested qualitatively (Abdullahi, 2013, Banso and Adeyemo, 2006, Roopashree et al., 2008, Joshi et al., 2013, Kancherla et al., 2019).

### 2.4. Quantitative determination of *T.chebula*

Condensed tannins were assessed using the butanol-HCl method (Terrill et al., 1992), total flavonoids were assessed using Aiyegoro and Okoh's (2010) methodology, and total phenolics were quantified using Graham's (1992) Prussian blue method in *T. chebula* fruit extract. Further, the estimate of proteins using Lowry's method (Lowry et al. 1951) and the estimation of carbohydrates using the Anthrone method as per Yemm and Willis (1954) in *T. chebula* fruit extract.

### 2.5. Antioxidant analysis of *t. Chebula*

#### 2.5.1. Determination of enzymatic and non-enzymatic antioxidants

*T. chebula* fruit extract was tested for catalase (CAT) activity using the Aebi (1984) method using Spectrophotometer. Glutathione peroxidase (Gpx) activity was evaluated using the method provided by Hafemann et al. (1974) and superoxide dismutase (SOD) activity was tested using the method described by Das et al. (2000). The method of Baker (1988) was used to calculate the concentration of tocopherol (vitamin E-tocopherol), and the method of Omaye et al. (1979) was used to calculate the concentration of ascorbic acid.

#### 2.5.2. DPPH scavenging activity of *t. Chebula*

Using the methodology described by Shimada et al. (1992), the scavenging capacity of polyphenolic extracts from *T. chebula* against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was evaluated. Reduced absorbance was seen at 517 nm when 0.1 mM DPPH solution was added to plant extract/ascorbic acid at various doses (20, 40, 60, 80, and 100 g/mL) in the presence of Tris-HCl buffer (50 mM, pH 7.4). The IC50 values of polyphenolic extract were determined and compared with the accepted reference chemical ascorbic acid, using a mixture of methanol and extract as the blank, as indicated by the equation below:

$$\text{Percent of inhibition} = \frac{[(\text{Absorbance of control} - \text{Absorbance of test solution}) / \text{Absorbance of control}] \times 100}{100}$$

#### 2.5.3. Ferric reducing antioxidant power of *t. Chebula*

Spectrophotometric calculations were used to determine the antioxidant content of the *T. chebula* fruit extract (Ferreira et al., 2007). 1 mL extract of *T. chebula* fruits with potassium ferricyanide and phosphate buffer (pH 6.6) and incubating it at 50 °C for 20 min at various concentrations (20, 40, 60, 80, and 100  $\mu\text{g/mL}$ ). The mixture was centrifuged at 3000 rpm for 10 min after being added TCA (10 %). The supernatant was mixed with  $\text{FeCl}_3$  (0.1 %), and the absorbance was measured at 700 nm.

### 2.6. FT-IR analysis of ethanolic extract of *t. Chebula* fruit

The ethanolic fruit extract of *T. chebula* was dried at 600 °C in an oven and then crushed into a fine powder. A salt disc (3 mm in diameter) was made by compressing 2 mg of the sample with 100 mg of KBr (FT-IR grade). The disc was retained in the sample holder right away, and FT-IR spectra were taken in the 400 – 4000  $\text{cm}^{-1}$  absorption range using Shimadzu FT-IR spectrometer.

### 2.7. GC-MS analysis of ethanolic extract of *t. Chebula* fruit

Analysis was conducted using Gas Chromatography (Agilent, USA) hyphenated to a high resolution mass spectrometer. Helium was used as the carrier gas at a rate of 1 mL/min. The capillary column used has a 0.25 mm internal diameter. The input temperature was 1000 °C, while the detector was at 2800 °C. The temperature was raised from 1000 °C to

2000 °C (100 °C/min) for two minutes. It was heated to 2800 °C (300 °C/min) for an additional 3 min after being maintained at 2400 °C (100 °C/min) for 3 min. The GC lasted 27 min in total. The % amount of peak area was calculated by comparing the average peak area for each component to the total areas. The mass spectrum from the GC-MS was deciphered, and the chromatogram and mass spectra were evaluated using HPCHEM software and compared with the National Institute of Standards and Technology (NIST) database (contains more than 62,000 patterns).

### 3. Results

#### 3.1. Phytochemical analysis of *t. Chebula*

The results obtained from *T. chebula* fruit extracts using a variety of solvents, including ethanol, chloroform, petroleum ether, and water, showed that the ethanolic and aqueous extract of *T. chebula* fruit had a good extraction yield of all compounds examined as shown in Table 1. In contrast to the petroleum ether extract, which only revealed the presence of tannins, alkaloids, terpenoids, and phenols whereas the chloroform extract revealed the existence of phenols, flavonoids, alkaloids, glycosides, amino acids and proteins.

#### 3.2. Total phenolics, tannins and flavonoids content determination

Fig. 1 illustrates the sample concentration of phenolic compounds ( $\mu\text{g/g}$  of extract) in various solvent extracts of *T. chebula* fruit. The phenolic assays revealed a wide range in the total phenolic content of the extracts, ranging from 26 to 104  $\mu\text{g/g}$ . The ethanolic extract has highest phenolic content at 104  $\mu\text{g/g}$ , followed by the aqueous extract at 87  $\mu\text{g/g}$ , the chloroform extract at 48  $\mu\text{g/g}$ , and the petroleum ether extract at 26  $\mu\text{g/g}$ . Tannin concentrations in *T. chebula* fruit extracts were measured using a variety of solvent types, and the results ranged from 13 to 106  $\mu\text{g/g}$  of extract (Fig. 1). It was found that the ethanolic extract had the highest concentration of tannins (106  $\mu\text{g/g}$  of extract), followed by the aqueous extract (75  $\mu\text{g/g}$ ), the chloroform extract (42  $\mu\text{g/g}$ ), and the petroleum ether extract (13  $\mu\text{g/g}$ ) (Fig. 1). Between 19 and 98  $\mu\text{g/g}$  of extract contained total flavonoids extracted from *T. chebula* fruit extracts using various solvents. The ethanolic extract had the highest concentration of total flavonoids (98  $\mu\text{g/g}$ ), followed by the aqueous extract (50  $\mu\text{g/g}$ ), the chloroform extract (38  $\mu\text{g/g}$ ), and the petroleum ether extract (19  $\mu\text{g/g}$ ).

#### 3.3. Total protein and carbohydrates content in fruits extract of *t. Chebula*

Using different solvents, the total protein concentration in *T. chebula* fruit extracts ranged from 25 to 67  $\mu\text{g/g}$  of extract. The ethanolic extract had the highest concentration of total proteins (67  $\mu\text{g/g}$ ), followed by the aqueous extract (52  $\mu\text{g/g}$ ), the chloroform extract (33  $\mu\text{g/g}$ ), and the petroleum ether extract (25  $\mu\text{g/g}$ ). Utilizing different solvents, the total

amount of carbohydrates in *T. chebula* fruit extracts ranged from 18 to 103  $\mu\text{g/g}$  of extract. Aqueous extract had the highest concentration of total carbohydrates (103  $\mu\text{g/g}$ ) followed by ethanolic extract (87  $\mu\text{g/g}$ ), chloroform extract, (39  $\mu\text{g/g}$ ) and petroleum ether extract (18  $\mu\text{g/g}$ ) [Fig. 2]. (Fig. 3.).

#### 3.4. Measurement of different enzymatic and non-enzymatic antioxidant markers

The levels of the various enzymatic (SOD, Catalase and Gpx) and non-enzymatic (Vitamin C and Vitamin E) antioxidant indicators were shown in Table 2. It revealed that the levels of enzymatic antioxidant such as SOD, Catalase, and Gpx were of 0.46  $\mu\text{g}$  of pyrogallol auto-oxidation inhibition/min, 97  $\mu\text{g}$  of hydrogen peroxide utilized/min, and 127  $\mu\text{g}$  of reduced glutathione oxidized/min respectively. The non-enzymatic antioxidant indicators such as vitamins C and E were detected and found to be 39.08  $\mu\text{g/L}$  and 18  $\mu\text{g/L}$  respectively.

#### 3.5. DPPH radical scavenging assay

The DPPH radical scavenging ability of various solvent extracts of *T. chebula* studied at dosages of 20, 40, 60, 80, and 100  $\text{g/ml}$  is shown in Fig. 2. All of the *T. chebula* solvent extracts evaluated demonstrated free radical scavenging activities, with inhibition ranging from 41 to 96 %. The highest levels of scavenging action were demonstrated by the ethanolic extract of *T. chebula* at concentrations of 20, 40, 60, 80, and 96 %, respectively. The aqueous extract of *T. chebula* suppresses free radicals by 41, 50, 66, 75, and 91 % at concentrations of 20, 40, 60, 80, and 100  $\mu\text{g/ml}$  respectively. Furthermore, at doses of 20, 40, 60, 80, and 100  $\mu\text{g/ml}$ , the chloroform fruit extract of *T. chebula* suppresses free radicals by 43, 52, 50, 63, 68 and 76 %, respectively. The standard reference, ascorbic acid, on the other hand, showed inhibition of 14, 30, 63, 76, and 92 % at various concentrations.

#### 3.6. Ferric reducing power (FRAP) assay of *t. Chebula*

The results of the reducing power assay for the various solvent extracts of *T. chebula* investigated in this work (20, 40, 60, 80, and 100  $\mu\text{g/ml}$ ) are displayed in Fig. 4. All of the solvent extracts of *T. chebula* that were tested showed antioxidant activity, with inhibition levels ranging from 12 to 96 %. The ethanolic extract of *T. chebula* at 20, 40, 60, 80, and 100  $\mu\text{g/ml}$  exhibited the highest antioxidant activity of 20, 46, 63, 86, and 96 % respectively. The concentrations of 20, 40, 60, 80, and 100  $\mu\text{g/ml}$ , the aqueous extract of *T. chebula* inhibits free radicals by 16, 34, 48, 72, and 86 %, respectively. Furthermore, at concentrations of 20, 40, 60, 80, and 100  $\mu\text{g/ml}$ , the *T. chebula*, chloroform extract inhibits free radicals by 12, 23, 42, 51 and 62 %, respectively. Ascorbic acid served as the standard control and showed 14, 40, 63, 76 and 92 % inhibition.

#### 3.7. FTIR analysis

Further, the FTIR analysis was performed on the ethanolic extract that was discovered to be abundant in phytochemicals and to have the highest antioxidant activity. According to their peaks, the functional groups of the constituents were divided (Fig. 5), and Table 3 provides an explanation of the chemical linkages. The distinctive absorption band at 3351.08, 2977.05, 2885.80, 1647.80, 1381.99, 1082.17, 1045.28, 879.37 and 684.86  $\text{cm}^{-1}$  revealed the existence of the following functional groups, namely alcohol, alkanes, carboxylic acid, alkenes, alkanes, ethers, ethers, halogen, and halogen, respectively (Table 3). The respective chemical bonds identified in the study comprise alcohol, alkanes, carboxylic acid, alkenes, ethers, and halogens. They also included C=C stretching, O-H stretching, C-H stretching, C-O stretching, and C-Cl stretching.

**Table 1**  
Preliminary phytochemical analysis of *T. chebula*.

S. No.	Phytochemicals	Ethanol extract	Petroleum ether extract	Chloroform extract	Aqueous extract
1	Tannins	++	-	-	++
2	Flavonoids	++	-	+	++
3	Alkaloids	++	+	+	+
4	Terpenoids	++	+	-	+
5	Saponins	++	+	-	++
6	Glycosides	++	-	+	++
7	Phenols	++	+	++	+++
8	Steroids	+	-	-	+
9	Amino acid	+	-	+	++
10	Proteins	+	-	+	+

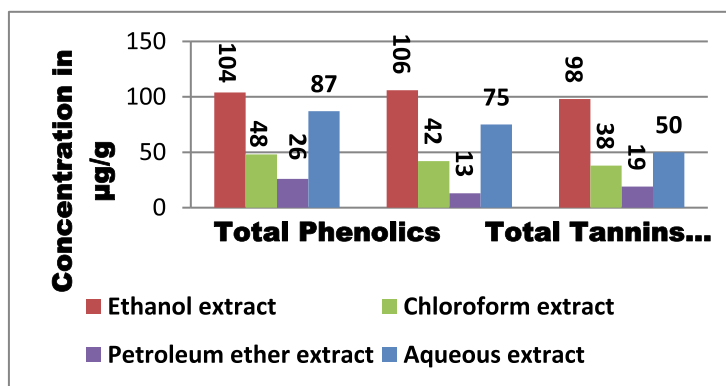


Fig. 1. Total Phenolics, Tannins and Flavonoid content in the fruit extract of *T.chebula* extracted using different solvents.

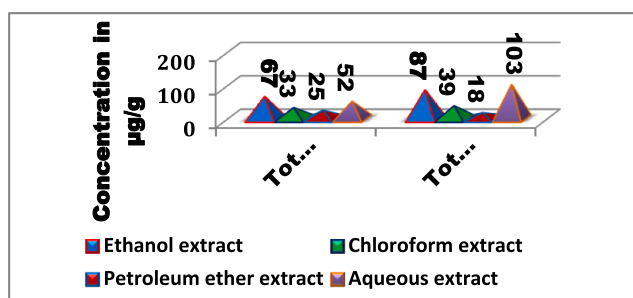


Fig. 2. Total proteins and carbohydrates content in *T. chebula* fruit extract.

### 3.8. GCMS analysis

The active principles are outlined in Table 4 and Fig. 5, which demonstrated the presence of nine bioactive phytochemical compounds in the ethanolic fruit extract of *T. chebula* by using gas chromatography (Fig. 6). The active principles are listed along with their retention time (RT), molecular formula, peak name, nature of the compound, and activity of the compound. Furaldehyde, 2,5-Furandicarboxaldehyde Dodecanoic acid, ethylester n-pentadecanol, 1,2,3-Benzenetriol pyrogallol, 3,4,5-trihydroxy benzoic acid, Octadecanoicacid, ethyl ester, and hentriacontane were the substances identified based on the relative amounts. Aldehydes, phenols, palmitic acid, alcohols, polyphenols, gallic acid, and alkanes are found in *T. chebula* by GCMS (Table 4).

### 4. Discussion

In the current study, the fruit extract from the medicinal plant *T. chebula* was produced using quite a few solvent extraction techniques

and its phytochemical content was tested. The *T. chebula* fruit extract was subjected to preliminary qualitative phytochemical analysis utilizing a variety of solvents, including ethanol, chloroform, petroleum ether, and water. This analysis identified the presence of tannins, flavonoids, alkaloids, terpenoids, saponins, glycosides, phenol, steroids, amino acids, and proteins. Additionally, it was determined that the ethanolic extract of *T. chebula* fruit had the highest extraction of all the phytochemicals screened that has been examined followed by the aqueous extract. Congestive heart failure and cardiac arrhythmia have both been treated with phytochemicals known as cardiac glycosides, also, alkaloids exhibit cytotoxicity against leukemia and *HeLa* cell lines as well as bioactivity against Gram-positive bacteria (Vladimir and Ludmila, 2001).

In our investigation, in general, the total phenolic tannins, and total flavonoids contents ranged from 26 to 104, 13 to 106, and 19 to 98 µg/g of extract respectively. According to the data, the order of efficiency for extracting phenolics, tannins, and total flavonoids is ethanol > aqueous

Table 2  
Determination of enzymatic and non-enzymatic antioxidants activity of *T. chebula*.

S. No.	Parameters	Concentration of antioxidants
Enzymatic antioxidants		
1.	Superoxide dismutase	0.46 µg of pyrogallol auto-oxidation inhibition/minute
2.	Catalase	97 µg of hydrogen peroxide utilized/minute
3.	Glutathione peroxidase (Gpx)	127 µg of reduced glutathione oxidized/minute
Non-enzymatic antioxidants		
4.	Vitamin C	39.08 µg/litre
5.	Vitamin E	18 mg/litre

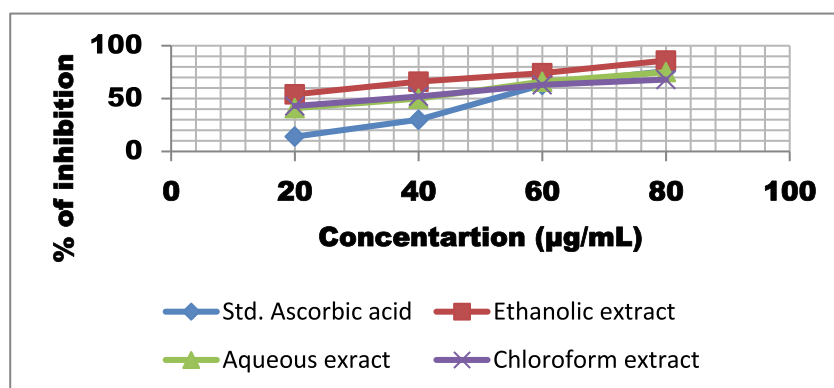


Fig. 3. DPPH scavenging activity of *T. chebula* fruit extract using different solvents.

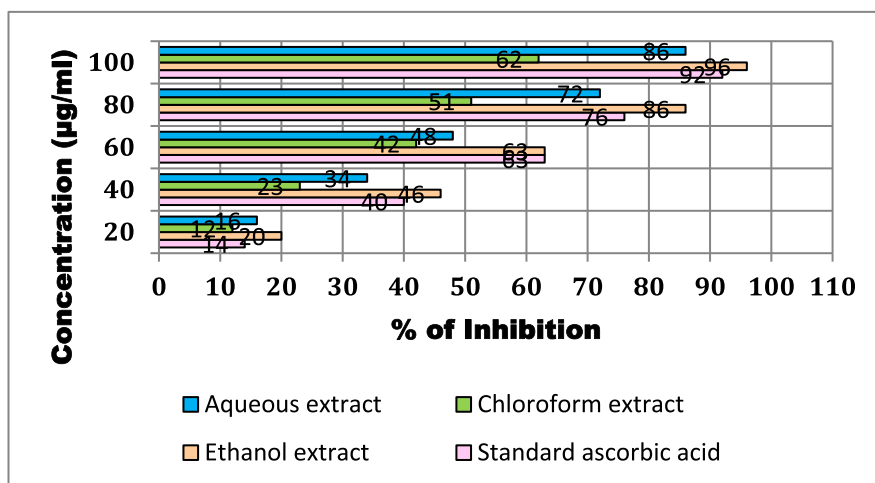


Fig. 4. FRAP assay of *T. chebula* fruit extract using different solvents.

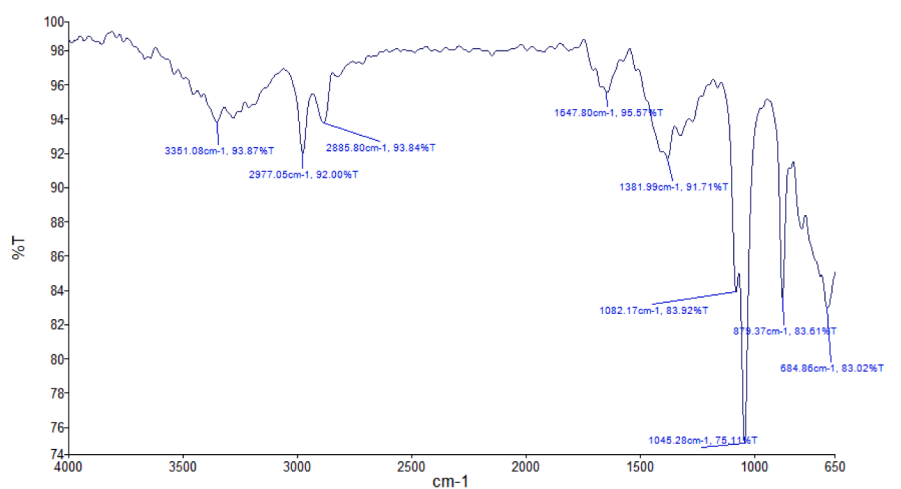


Fig. 5. FTIR analysis of ethanolic fruit extract of *T. chebula*.

Table 3

FTIR analysis of ethanolic fruit extract of *T. chebula*.

Peak value	Functional group	Compound
3351.08	–OH group	Alcohol
2977.05	C–H group	Alkanes
2885.80	O–H Stretching	Carboxylic acid
1647.80	C=C group	Alkenes
1381.99	C–H Stretching	Alkanes
1082.17	C–O Stretching	Ethers
1045.28	C–O Stretching	Ethers
879.37	C–Cl Stretching	Halogen
684.86	C–Cl Stretching	Halogen

> chloroform > petroleum ether. It has been shown that the polarity of the extraction medium and the solute to solvent ratio appeared to affect a component’s ability to be extracted. This study showed that ethanol has greater extractability power than the other solvents utilized, because the range of phenolics, tannins, and total flavonoids are highest in ethanolic extract and lowest in petroleum ether extract. The findings are in line with those of [Ao et al. \(2008\)](#) who found that, when compared to other solvents, methanol extract had the greatest total phenolic concentration in *Ficus microcarpa*. Plant phenolic compounds play a significant role in scavenging because of their hydroxyl groups ([Nunes et al., 2012](#)). Research has shown that tannins have antibacterial, anticancer,

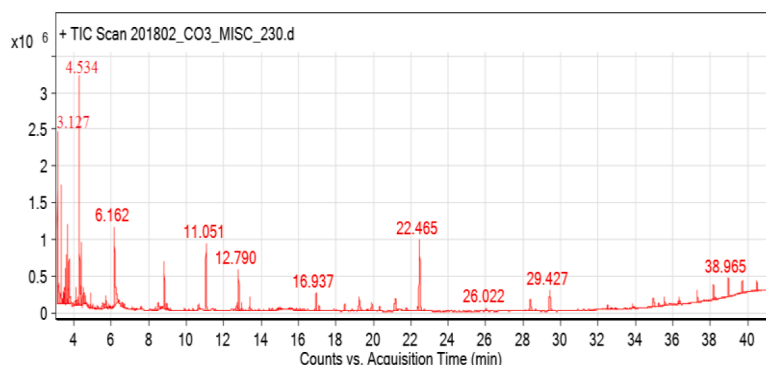
and antiviral properties. Studies on flavonoidic derivatives have revealed numerous anti-inflammatory, antiviral, antibacterial, anti-allergic, and anti-cancer properties as reported by [Montoro et al. \(2005\)](#).

In our study, the highest amount of total proteins are present in the ethanolic extract of and in contrast the highest amount of total Carbohydrates observed in the aqueous extract of *T. chebula* fruit extracts. The total carbohydrate and protein contents has been apparently varied in the plants such as Zingiber officinale, Camellia sinensis and Annona muricata as reported by [Serge Cyrille et al. \(2021\)](#). In our study, the examination on the level of the different enzymatic antioxidant markers (SOD, catalase and Gpx) were found to be 0.46 µg, 97 µg and 127 µg respectively in the ethanolic fruit extract of *T. chebula*. [Batinic-Haberle et al. \(2015\)](#) reported that to counteract the harmful effects of ROS, the CAT and peroxidase must work in concert with SOD to remove O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub>. SOD usually comes into contact with singlet oxygen form ROS in its early phases, as well as free radicals that are successively eliminated with the aid of GPx and CAT. Also, the high level of presence of non-enzymatic antioxidant markers (Vitamin C and Vitamin E) in ethanolic fruit extract of *T. chebula* were proved in the current study. It was reported that a powerful chain-breaking antioxidant, vitamin E prevents the formation of reactive oxygen species molecules during the oxidation of fat and the spread of free radical reactions, an earlier study indicated that a 70 % ethanol extract of *T. chebula* fruits had good efficacy in terms of its capacity to scavenge free radicals ([Hazra et al. 2010](#)).



**Table 4**GC-MS analysis of phyto-compounds in the ethanolic extract of *T. chebula* fruit.

Retention Time	Peak Name	Formula	Nature of the compound	Activity
3.127	Furaldehyde	C5H4O2	Aldehyde	Antimicrobial
4.534	2,5- Furandicarboxaldehyde	C6H4O5	Aldehyde	Antimicrobial
11.051	2,4 Di-t-butylphenyl	C5H8O4	Phenol	Antineoplastic
12.790	Dodecanoic acid, ethylester	C12H24O2	Palmitic acid	Antimalarial, Antioxidant
16.937	n-Pentadecanol	C15H30O2	Alcohol	Antioxidant
22.465	1,2,3-Benzenetriol pyrogallol	C6H6O3	Poly phenol	Antimicrobial, Anticancer, Antioxidant
26.022	3,4,5 Trihydroxy benzoic acid	C7H6O5	Gallic acid	Antioxidant, Anticancer, Antimicrobial
29.427	Octadecanoicacid, ethyl ester	C18H32O	Aldehyde	Antimicrobial, Anti inflammatory
38.965	Hentriacontane	C31H64	Alkane	Anticancer

**Fig. 6.** GC-MS analysis of phyto-compounds in the ethanolic extract of *T. chebula* fruit.

According to [Sultana et al. \(2007\)](#), ethanolic extract of *T. arjuna* has the greatest scavenging potential. In contrast, ethyl acetate, a solvent that is much less polar, has the lowest IC 50 value, according to [Jegadeesware et al. \(2014\)](#). So, in current study, the DPPH and Reducing power assay was done only for ethanolic, aqueous and chloroform and petroleum ether extract was avoided as the solvent was less polar and low yield of phytochemicals. It was also found that, the ethanolic extracts of *T. chebula* fruits studied exhibited free radical scavenging properties in highest range of 96 % of inhibition in DPPH radical scavenging activity and reducing power assay. These findings agreed with those of [Iloki-Assanga et al. \(2015\)](#), who found that the order of acetone > methanol > aqueous > ethanol was found varied effectiveness at scavenging free radicals when comparing the extracts of *Bucida buceras* (Oak) and *Phoradendron californicum* (mesquite).

The ethanolic extract found rich in phytochemicals and recorded highest antioxidant activity were further analyzed using FTIR indicated the presence of following functional groups viz., alcohol, alkanes, Carboxylic acid, alkenes, Alkanes, Ethers and Halogen respectively. The functional groups of carboxylic acids and halogens in *T. chebula* has many therapeutic benefits. These are in line with [Starlin et al. \(2012\)](#), showed the ethanolic extracts of *T. chebula* indicated functional group components of organic hydrocarbons, halogens, and carboxylic acids through FTIR. The transition metal carbonyl compounds and aliphatic fluoro compounds were exclusively found ethanolic fruit extract of *T. chebula* ([Parag and Pednekar, 2013](#)).

Furthermore, the GCMS analysis of ethanolic fruit extract of *T. chebula* revealed the presence of 9 bioactive phytochemical compounds and it is identified that *T. chebula* contains Aldehydes, Phenols, Palmitic acid, Alcohols, Polyphenols, Gallic acid and alkanes. The furaldehyde and 2,5-furandicarboxaldehyde present in *T. chebula* has antimicrobial action, 2,4,di-t-butylphenyl are Antineoplastic and Dodecanoic acid, ethylester has antimalarial and antioxidant effect. Further, n-pentadecanol antioxidant, 1,2,3-Benzenetriol pyrogallol and 3,4,5 trihydroxy benzoic acid proved the antimicrobial, anticancer and antioxidant action. The compound Octadecanoicacid, ethyl ester has antimicrobial and anti-inflammatory properties and finally Hentriacontane has anticancer

effect. Gallic acid (3, 4, 5-trihydroxybenzoic acid) has been reported to have antioxidant, antimutagenic, antitumor, anticancer, anti-inflammatory and apoptotic properties ([Kahkeshani et al. 2019](#)). When cells are signaling, the essential fatty acids linoleic and linolenic (9, 12 octadecanoic acid) play a critical role in the synthesis of lipid rafts.

## 5. Conclusion

The results of the current investigation showed that, among four solvent screened, the *T. chebula* fruits (ethanolic extract) showed variety of phytochemicals, including phenolic compounds, carotenes, alkaloids flavonoids, saponins, and amino acids etc. among others. Further, in vitro antioxidant activity of the ethanolic fruit extract of *T. chebula* was found to be higher and also proved highest level of radical scavenging activity. Accordingly, it can be inferred from the aforementioned study that this *T. chebula* fruits have not only medicinal properties and also be used as an antioxidant source. The study also suggests that *T. chebula* fruit may be a source of bioactive phytochemicals that may serve as an antibacterial, antifungal, anti-inflammatory, and anti-diabetic agent in addition to being a plant-based antioxidant. It is important to use this information to promote additional research so that it may be used in the pharmaceutical and food industries.

## CRedit authorship contribution statement

**Mohamed Farouk Elsadek:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Khalid S. Al-Numair:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Data curation.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

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