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Evaluation of phytochemical profile and *in vitro* antioxidant properties of *Terminalia chebula* fruits

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Abstract

The fruit of *Terminalia chebula* have wide applications in folk medicine since ancient times due to the presence of several bioactive compounds. This study aims at the anti-oxidant capacity and the screening of phytochemicals from the fruits of the *T. chebula*. Preliminary phytochemical screening of leaf showed the presence of Carbohydrates, protein, steroids, glycosides, tannins, flavonoids, alkaloids, saponin, starch, terpenoids, anthrocyanin, coumarin, phytosterol, glycosides and chalcogens in the different extracts. The ethanol, aqueous and chloroform extracts of the fruit were tested for antioxidant activity using scavenging activities of DPPH radical, Nitric oxide, reducing power, hydrogen peroxide and superoxide methods. Among the three different extracts tested, the ethanol extract has registered noticeable effect in all scavenging assays than other extracts tested. The finding suggests that the three different extracts of *T. chebula* fruit showed different level of antioxidant activity and it is potential source of natural antioxidants.

Keywords: *Terminalia chebula*, fruit, phyto-constituents, anti-oxidant

Introduction

Free radicals and oxidants are formed either from internally produced sources of mitochondria, xanthine oxidase, peroxisomes, inflammation, phagocytosis, arachidonate pathways, exercise, ischemia and reperfusion injury or from external sources of cigarette smoke, environmental pollutants, radiation, certain drugs, pesticides, Industrial solvents and Ozone. When an excess of free radicals produced in the body and they cannot be easily destroyed, their accumulation in the body produces an oxidative stress. This process creates a main part in the progress of chronic and degenerative diseases such as rheumatoid arthritis, aging, cataract, cancer, autoimmune disorders, neurodegenerative and cardiovascular diseases. The human body produces various mechanisms to neutralize the oxidative stress by antioxidants, which are either naturally generated *in situ*, or externally taken through foods and/or supplements (Mukherjee *et al.*, 2011).

Antioxidants are familiarly concerned in the obstacles of cellular damage the common pathway for aging, cancer and a range of diseases. The scientific society has begun to reveal some of the ambiguity neighboring this topic and the media have begun kindles our hunger for knowledge (Freeman *et al.*, 1982). In sight of rising risk factors of human to different deadly diseases, there has been a universal trend toward the use of natural substance present in medicinal plants and nutritional plants as therapeutic antioxidants (Citra *et al*., 2018). There are many reports showing antioxidant potential of fruits (Wang, 1996). Strong antioxidants activities have been found in citrus, cherries, prunes, berries and olives. Black and green teas have been widely investigated in the antioxidant properties since they contain up to 30% of the dry weight as phenolic compounds (Lin *et al.*, 1998).

Terminalia chebula is commonly known as harrar, harad and haritaki and is important multi-purpose tree species (Warrier, 1993), which belongs to Combretaceae family. This species is indigenous to India and Asia (Dymock *et al*., 1976). *T. chebula* is mainly used in traditional folk medicine. It is widely used in homeopathy, unani, andayurvedic medicines. The fruit is widely used because of broad spectrum of pharmacological activities. It is used as a household remedy throughout sub-continent for treating stomach ache, colic of sucking infants and as a laxative for many years now (Singh *et al*., 2003). Taking into consideration the medicinal value and utility of *T. chebula* fruits, the present study has been initiated to evaluate the phytochemical screening and antioxidant activity through different free radical scavenging assays.

Materials and Methods

The fruit of *Terminalia chebula* was collected locally and washed thoroughly using running tap water, rinsed in distilled water and shade dried in open air and grounded in to powder. The powdered fruit was extracted with petroleum ether, chloroform, ethyl acetate, ethanol and water, by using soxhlet apparatus. The fruit extracts were concentrated using rotary flash vaporator and stored in desicator.

Phytochemical studies

Carbohydrates- Molisch's test

1 g extract powder with 10 mL ethanol for 15 minutes in a boiling water bath and filtered. Appearance of purple color in addition of napthol and concentrated sulphuric acid (H_2S0_4) to the filtrate indicates the presence of carbohydrates (Trease and Evans, 2002).

Proteins-Millon’s test (Mishra, 2009)

A small amount of extract is separately dissolved in about 5 ml of distilled water and filtered. To 2 ml of the filtrate, 5-6 drops of Millon’s reagent (solution of mercury nitrate and nitrous acid) are added and observed in the formation of red precipitates as an indication of the presence of proteins.

Alkaloids

1 g of powder is extracted with 20 mL alcohol by refluxing for 15 minutes, filtered and the filtrate is evaporated to dryness. The residue is dissolved in 15 mL of H_2S0_4 (2N) and filtered. After making it alkaline, the filtrate is extracted with chloroform. The residue left after evaporation is tested for the presence of alkaloids with Dragondroff's reagent. Development of colored precipitate indicates the presence of alkaloid (Sim, 1969).

Flavonoids -Shinoda test (Geissman, 1954)

1 g of powdered fruit are extracted with 10 mL of solvents for 15 minutes in a boiling water bath and filtered. To the filtrate is added a small piece of magnesium ribbon and 3 to 4 drops of concentrated H_2S0_4. Red coloration formation indicated the presence of flavonoids.

Sterols and triterpenoids -Salkowski test (Robinson, 1964)

To the 2 mL of extract, 2 mL chloroform and 2 mL concentrated H_2S0_4 are added and shake well. The positive result of sterol(s) and triterpenoid(s) indicated if chloroform layer appeared red and acid layer showed greenish yellow fluorescence.

Tannins (Robinson, 1964)

The extracts is prepared by refluxing 10 g of fruit powder with 50 ml of solvents for about 1 hour on a water bath and is used for the following test: To the extracts of the plant, add 2 mL of a10% w/w solution of lead acetate. Precipitate indicated the presence of tannin(s).

Phenolic compounds -Ferric chloride test

The small amount of extract is separately shaken with water and warmed. Now about 2 ml of 5% ferric chloride ($FeCl_3$) solution is added and observed in the formation of green or blue color (Ashutosh Mishra *et al*., 2009).

Anthraquinone glycoside - Borntrager's test (Mukherjee, 2002)

The powdered extracts is taken and extracted with solvents. To the filtered extract, ammonia is added. The pink, red or violet color appearance after shaking indicated the anthraquinone glycoside(s) presence.

Saponins - Froth test (Fishcer, 1952)

0.1 g of powdered extracts is vigorously shaken with 5 mL of solvents for 30 seconds and is left undisturbed for 20 minutes. Persistent froth indicated the presence of saponin(s).

Coumarins (Trease and Evans, 2002)

Take a drop of ammonia on a filter paper, a drop of extracts of the leaf and fruit is added. Fluorescence indicated the presence of coumarin(s).

Cardiac Glycosides

Test solution was treated with few drops of ferric chloride solution and mixed with H_2SO_4 containing $FeCl_3$ solution forms two layers, lower layer reddish Brown, upper layer bluish green indicated the presence of deoxy sugar (Trease and Evans, 2002).

Glycosides

For detection of glycosides, about 50 mg of extract was hydrolyzed with concentrated HCl for 2 hrs on a water bath, filtered and the hydrolyzed. A greenish ring may form just gradually throughout thin layer which shows the presence of glycosides.

GC-MS analysis

GC-MS analysis was performed on Shimadzu (2010) plus consist of auto sampler of AOC-20i and gas chromatograph interfaced to a mass spectrometer instrument performing the following conditions: column RTX 5Ms (Column diameter is 0.32mm, column length is 30m, column thickness 0.50µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml/mins and the volume of injection 0.5 µl was injected (split ratio of 10:1), injector temperature 270 ºC; ion-source temperature 200 ºC. The oven temperature was adjusted to 40ºC (isothermal for 2 minutes), with an increase of 8ºC/ mins, to 150ºC, then 8ºC/mins to 250ºC, ending with a 20 mins isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 51.25 minutes. The amount of relative percentage of each component was measured by relating its average peak area to the total areas. Software employed to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2 (Srinivasan *et al*., 2013).

In vitro antioxidant activity

Total DPPH assay

The methanolic solution of DPPH (90.25 mM), an equal volume of ethanol, chloroform and aqueous fruit extract of *T. chebula* (25-250 µg) was added and made up to 1.0 mL with methanolic DPPH. For control tube containing an equal amount of methanol was added to the extracts. After 20 minutes, the absorbance was recorded at 517 nm in a Systronics UV-visible Spectrophotometer. Ascorbic acid was used as standard for comparison. The inhibition of free radicals by DPPH in percentage terms (%) was calculated by using the following equation,

$$\% \text{ Scavenging} = \frac{\text{A Control OD - A sample}}{\text{A blank}} \text{ X100}$$

Nitric oxide radical scavenging assay

Two ml of 10 mM sodium nitroprusside was prepared in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of fruit extract, at various concentrations (25 - 250µg). The mixture was incubated at 25°C. After 150 minutes, 0.5 ml of incubation solution was withdrawn and mixed with 0.5 ml of Griess reagent. The mixture was incubated at room temperature for 30 minute, followed by the measurement of absorbance at 540 nm. The amount of NO radical inhibited by the extract was calculated using the following equation:

$$\% \frac{\text{A Control}}{\text{A blank}} \text{ X100}$$

Reducing Power

Varying concentrations of ethanol, chloroform, aqueous fruit extract of *T. chebula*. (25, 50, 100, 150, 200 and 250µg) in double distilled water was mixed with 2.5 mL of phosphate buffer and 2.5 mL of KCN. The mixture was incubated at 50ºC for 20 minutes after which, 1.5 mL of TCA was added and centrifuged at 3000xg for 10 minutes. After centrifuge, 0.5 mL of supernatant was mixed with 1.0 mL of distilled water and 0.5 mL of $FeCl_3$ to all tubes. The absorbance was measured at 700 nm in a UV-spectrophotometer. Elevated absorbance level of the reaction mixture indicated increasing reducing power. The distilled water mixed with additives was used as the blank.

$$\% \text{ Scavenging} = \frac{\text{A Control OD - A sample}}{\text{A blank}} \text{ X100}$$

Hydrogen peroxide radical scavenging assay

The H_2O_2 (40 mM) solution was prepared in phosphate buffer (pH 7.4). H_2O_2 concentration was determined spectrophotometrically from absorption at 230 nm in a UV-spectrophotometer. Extracts (25, 50, 100, 150, 200 and 250 µg) in distilled water were added to a H_2O_2 solution (0.6 mL, 40 mM) and kept for 10 mins. After 10minutes, the absorbance of H_2O_2 at 230 nm was determined against a blank solution containing phosphate buffer without H_2O_2. The percentage of scavenging of H_2O_2 of ethanol, chloroform, and aqueous fruit extract of *T. chebula* standard was calculated using the following equation:

$$\% \text{ Scavenging} = \frac{\text{A Control OD - A}}{\text{A blank}} \text{ X100}$$

Superoxide anion scavenging activity

1 mL of NBT, 1 mL of NADH solution and varying volumes of all the extracts ethanol, chloroform, aqueous fruit extract of *T. chebula* (25, 50, 100, 150, 200 and 250 µg) were mixed well. The reaction was started by the addition of 100 µM of PMS and it was incubated at 30ºC for 15 minutes. After incubation, the absorbance was measured at 560 nm in a UV- spectrophotometer. Incubation without Ethanol, chloroform, aqueous *T. chebula* fruit was used as blank. The reduced absorbance of the reaction mixture showed elevated superoxide anion scavenging. The % of inhibition was calculated as shown below:

$$\% \text{ Scavenging} = \frac{\text{A Control OD - A sample}}{\text{A blank}}$$

Results and Discussion

Medicinal plants are rich source of novel drugs, in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceuticals intermediates, and bioactive principles and as important part of lead compounds in synthetic drugs. One of the important medicinal plant, which is widely used in the traditional system of medicine, is *Terminalia chebula*. The screenin g of phytoconstituents from medicinal plants is very important in identifying new sources of therapeutics and industrial importance (Yogananth *et al*., 2019).

In this study evaluation for qualitative estimation of the phytochemical constituents of fruit extracts of *T. chebula* showed the presence of various secondary metabolites (Table 1) including Carbohydrates, protein, steroids, glycosides, tannins, flavonoids, alkaloids, saponin, starch, terpenoids, anthrocyanin, coumarin, phytosterol, glycosides, chalcogens are present in the ethanolic extract of *T. chebula* fruits. Aqueous extract of *T. chebula* fruits contains, carbohydrates, glycosides, tannin, flavonoids, alkaloids, tannins, terpenoids and coumarin. Carbohydrates, proteins, terpenoids, coumarin and phytosterol are present in chloroform extracts of *T. chebula* fruits . Various studies revealed that natural and synthetic derivates of alkaloids have possessed medicinal importance includes; analgesic, antisplasmodic and bactericidal activities, antioxidant and are useful in renal disorder (Okwu, 2004). Flavonoids are polyphenolic compounds, it has antioxidant and antimicrobial properties while saponins are glycosides possessed antimicrobial and inhibit Na+ efflux, by blockage of the entrance of the sodium^{+} out of the cell, falling congestive heart failure (Abou-Donia *et al.*, 2008). Tannin possessed spasmolytic activity in smooth muscles cells, free radical scavenger and antioxidant (Tona *et al.*, 1999). Saponin acts as an antimicrobial agent (Sheikh *et al.*, 2013).

Compound identification by GC-MS Method

GC-MS is one of the finest procedures to discover the constituents of hydrocarbons, alcohols acids, esters volatile matter, long chain, branched chain, etc. It can provide meaningful information for components that are volatile, non-ionic, and thermally stable and have relatively low molecular weight.

In the present study, Fig 1 showed nearly 20 compounds were identified by GC-MS analysis in *T. chebula* fruits. The compounds are 1,2-benzene dicarboxylic acid, 1,3-dipheny 1,3,5,5-tetramethyl cyclotrisibxane, 2-Benzofuranone, 2,6,10-trimethyl, 1,4-ethylene-14-pentadecane, hexadecanoic acid, 1,2-benzene dicarboxylic acid, 9,12-octa decadienoic acid, phytol, uridine, 9,12,15-octadecatrienoic acid, squalene etc. Among these various compounds some phytoconstituents has possess highly medicinal activities. The compound of 1, 2-benzene dicarboxylic acid diethyl ester which have the anti-microbial (Karthika *et al*., 2013) and anti-fungal activity of the compound 3, 7, 11, 15 tetra methyl 2 hexadecane have an antimicrobial and anti-inflammatory activity (Rajeswari *et al.*, 2012) and and cancer preventive activity (Dr. Duke's phytochemical and ethnobotanical databases). The compound of hexadecanoic acid, squalene, phytol have an anti-oxidant and anti-cancer (Senthilkumar *et al*., 2006) activity. Phytol and octadecatrienoic acid have an anti-inflammatory and cancer preventive activity (Sermakkani and Thangapandian, 2012). Squalene has an anti-bacterial, anti-oxidant, anti-tumour, anti-cancer, immune stimulant, chemopreventive activities (Senthilkumar *et al*., 2006).

Table 1: Phytochemical analylsis of *T. chebula* fruits of various extracts

S.No.	Characteristics	*T. chebula*		
		Ethanol	Aqueous	Chloroform
1.	**Carbohydrat**	+	+	+
2.	**Protein**	+	-	+
3.	**Steroid**	+	-	-
4.	**Glycoside**	+	+	-
5.	**Tannin**	+	+	-
6.	**Flavonoids**	+	+	-
7.	**Alkaloids**	+	+	-
8.	**Saponin**	+	+	-
9.	**Starch**	+	+	-
10.	**Terpenoids**	+	+	+
11.	**Anthocyanin**	+	-	-
12.	**Coumarin**	+	+	+
13.	**Emodins**	+	+	-
14.	**Phytosterol**	+	+	+
15.	**Phlobatannin**	+	-	-
16.	**Cardial**	+	+	-
17.	**Chalcones**	+	+	-

a) + sign indicates positive test (presence of compound);
b) – sign indicates negative test (absence of compound)

Fig 1: GC-MS chromatogram of *T. chebula* fruits extract

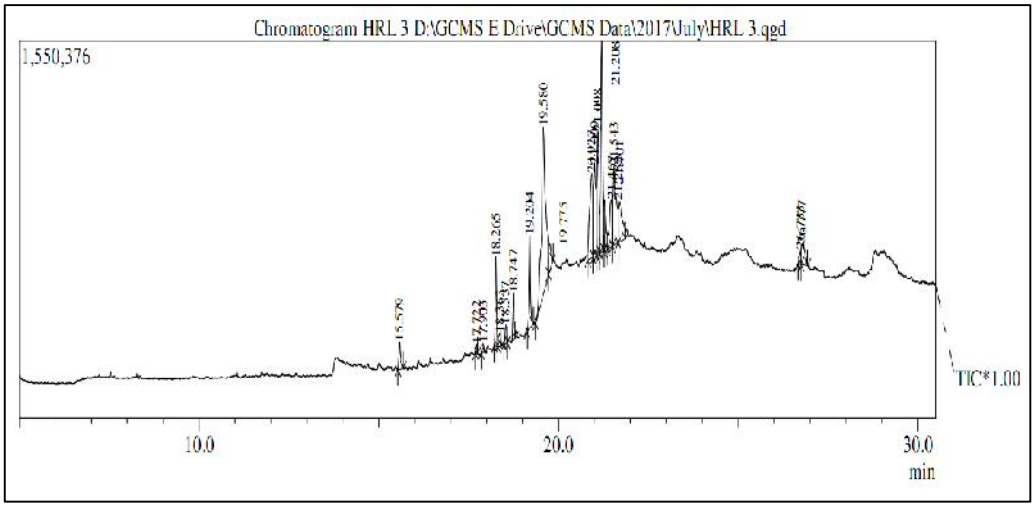


Table 2: GC-MS analysis revealed the presence of phytochemical components in *Terminalia chebula* extract

Peak#	R.Time	Area%	Molecular formula	Molecular Weight	Name of the Compounds
1	15.579	1.51	$C_{12}H_{14}O_4$	222	1,2 Benzenedicarboxylic Acid, Diethyl Ester
2	17.722	0.27	$C_{16}H_{22}O_3Si_3$	346	1,3-Diphenyl-1,3,5,5-Tetramethyl-Cyclotrisiloxane
3	17.903	0.44	$C_{11}H_{16}O_3$	196	2(4h)-Benzofuranone, 5,6,7,7a-Tetrahydro-6-Hydroxy-4,4,7a-Trimethyl-, (6s-Cis)-
4	18.265	3.28	$C_{20}H_{38}$	278	2,6,10-Trimethyl,14-Ethylene-14-
5	18.363	0.85	$C_{18}H_{36}O$	268	2-Pentadecanone, 6,10,14-Trimethyl
6	18.537	0.90	$C_{20}H_{40}O$	296	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol
7	18.747	1.93	$C_{20}H_{40}O$	296	2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl
8	19.204	4.81	$C_{17}H_{34}O_2$	270	Hexadecanoic Acid, Methyl Ester
9	19.580	22.78	$C_{16}H_{32}O_2$	256	N-Hexadecanoic Acid
10	19.775	1.82	$C_{16}H_{22}O_4$	278	1,2-Benzenedicarboxylic Acid
11	20.927	9.01	$C_{11}H_{10}N_2O_2$	202	1h-Pyrrolo[2,1-B]Quinazolin-9-One, 3-Hydroxy-2,3-Dihydro
12	21.009	7.23	$C_{19}H_{34}O_2$	294	9,12-Octadecadienoic Acid (Z,Z)-Methyl Ester
13	21.098	8.05	$C_{19}H_{32}O_2$	292	9,12,15-Octadecatrienoic Acid- Methyl Ester, (Z,Z,Z)-
14	21.208	12.34	$C_{22}H_{42}O_2$	338	Phytol, Acetate
15	21.282	2.82	$C_{19}H_{38}O_2$	298	Octadecanoic Acid,
16	21.467	4.57	$C_{16}H_{30}O_2$	254	Z-8-Tetradecen-1-Yl Acetate
17	21.543	9.06	$C_{18}H_{30}O_2$	278	9,12,15-Octadecatrienoic Acid, (Z,Z,Z)
18	21.701	5.13	$C_{18}H_{36}O_2$	284	Octadecanoic Acid
19	26.733	0.88	$C_{12}H_{16}N_2O_6$	284	Uridine, 2',3'-O-(1-Methylethylidene
20	26.777	2.32	$C_{30}H_{50}$	410	Squalene

In-vitro antioxidant activity

DPPH scavenging assay

The ability of electron donation of natural products are calculated by 2, 2′-diphenyl-1-picrylhydrazyl radical (DPPH) purple-coloured solution bleaching (Nunes *et al.,* 2012). Scavenging of DPPH radical has been used as a regular test for found the antioxidant properties of plant extracts (Atawodi, 2005) and their components. In the present investigation, Fig 2 shows the anti-oxidant potential of *T. chebula* fruits on DPPH radical. The percentage of inhibition were found to be 89.41%, 88.9% and 87.02% of inhibition was observed in 250 µg/ml of *T. chebula* fruits extracts of ethanol, aqueous and chloroform respectively. Similarly, the lowest percentage of inhibition 20.32%, 19.12% and 18.75% were observed in *T. chebula* fruits in lower concentrations (25 µg/ml) of different extracts. The higher inhibitory activity, which is similar to the standard ascorbic acid treated values. The IC_{50} values of *T. chebula* fruits have 90 µg/ml, 92.5 µg/ml and 92.5 µg/ml of IC_{50} values of the ethanolic, aqueous and chloroform extracts respectively. The IC_{50} values standard ascorbic acid is 77.5 µg/ml. Like that of our study, Loganayaki *et al.*, 2010 registered that the high DPPH radical scavenging activity registered in 100 µg/ml extract methanolic extracts of *S. torvum* and *S. nigrum* fruits and leaves.

Nitric oxide assay

NO is a reactive radical, it easily reacts with superoxide anion, to produce a stronger oxidant of peroxynitrite, it involves the damage of DNA and nitration of proteins and nucleic acids. The inhibition of NO accumulation is an important therapeutic strategy of natural antioxidants. Fig 3 shows the NO scavenging effect of *T. chebula* fruits with standard ascorbic acid. The maximum dose of plants extracts were highest percentage of scavenging activity. The lower doses of the plants extract were lower percentage of scavenging activity. *T. chebula* fruits show the inhibition at 250 µg/ml were 89.01%, 87.60% and 86.13% and the inhibition at 25 µg/ml were 19.86%, 17.11% and 16.6%. The std drug shows the inhibition of 94.02% of inhibition at 250 µg/ml and IC_{50} is 115 µg/ml. The IC_{50} value of ethanol extract of *T. chebula* is 125 µg/ml respectively. Divya and Vijaya Anand (2015) prove the NO inhibition activity of *T. catappa* leaves which favours the anti-oxidant nature in an *in vitro* model.

Reducing power

The anti-oxidant compound reduces the ferric ion into ferrous ion, this effect reduces the free radical generations as well as the reductants, which break the free radical chains. This reducing power of the plant extracts mainly depends on the presence of phenolic content. A higher absorbance value indicated stronger reducing power by the samples. The reductive potential of *T. chebula* fruits with standard was shown in Fig 4. The reduction percentage was increased in increasing concentration and decreased in decreasing concentration of the plants extracts and also in standard α- tocopherol. Different extracts of *T. chebula* fruits shows 82.21%, 81.11% and 79.17% of inhibition at 250 µg/ml and 29.31%, 27.22% and 26.13% of inhibition at 25 µg/ml. The IC_{50} values of the *T. chebula* fruits are 130 µg/ml, 95 µg/ml and 97.5 µg/ml in the ethanolic, aqueous and chloroform extracts respectively. The IC_{50} value of α-tocopherol is 100 µg/ml. In the present study, fruit extracts of all the solvents have a reducing potential. Among these the ethanolic extracts have a high ability of the both of the *T. chebula* fruits extracts. The similar effect is noted in the *Meconopsis integrifolia* (Zhou *et al.,* 2013).

Hydrogen peroxide assay

H_2O_2 is a substance, it undergoes some reactions and it produces the hydroxyl radicals in the cells, it may damages the cell membranes. Hence, the inhibition of H_2O_2 is an important work for the antioxidants. These antioxidants donate the electrons to H_2O_2 and prevent the hydroxyl radical formation. In this study, Fig 5 shows the H_2O_2 radical scavenging effect of *T. chebula* fruits with standard α-tocopherol. The maximum dose of the plants extracts were highest

percentage of scavenging activity. The lower doses of the plants extract were lower percentage of scavenging activity. Ethanolic, aqueous and chloroform extracts of *T. chebula* fruits shows the inhibition at 250 µg/ml were 90.11%, 90.13% and 88.01% and the inhibition at 25 µg/ml were 18.10%, 16.15% and 14.18% respectively. The IC_{50} value of *T. chebula* fruits extract on H_2O_2 radical was 120 µg/ml, 110 µg/ml and 120 µg/ml in the ethanolic, aqueous and chloroform extracts respectively. The IC_{50} value of standard drug α-tocopherol is 120 µg/ml. The result of H_2O_2 scavenging activity of this study is similar to the results of the *in vitro* anti-oxidant activity of *Cressacretica* by Priyanka *et al*. (2005).

Super oxide radical assay

The enzymatic antioxidants are involved in the direct elimination of ROS (Halliwell and Gutteridge, 1985). SOD is an important anti-oxidant enzyme, it catalyzes the dismutation process. SOD plays a defensive role against the potential oxygen toxicity. It protect the cells from oxidative stress. The percentage of inhibition of this enzyme is directly proportional to the anti-oxidant ability of the plant extracts. In this study, Fig 6 shows the superoxide radical scavenging effect of *T. chebula* fruits with standard BHT. The maximum dose of the both plants extracts were highest percentage of scavenging activity. The lower doses of the both plants extract were lower percentage of scavenging activity. Ethanolic, aqueous and chloroform extracts of *T. chebula* fruits shows the inhibition at 250 µg/ml were 92.14%, 92.17% and 90.01% and the inhibition at 25 µg/ml were 18.01%, 17.12% and 14.02% respectively. In the present study, *T. chebula* fruits extracts have an effective inhibiting of SOD The IC_{50} value of standard is 117.5 µg/ml and the IC_{50} values of *T. chebula* fruits is 120 µg/ml, 100 µg/ml, 115 µg/ml in the ethanolic, aqueous and chloroform extracts respectively.

Fig 2: DPPH radical scavenging activity

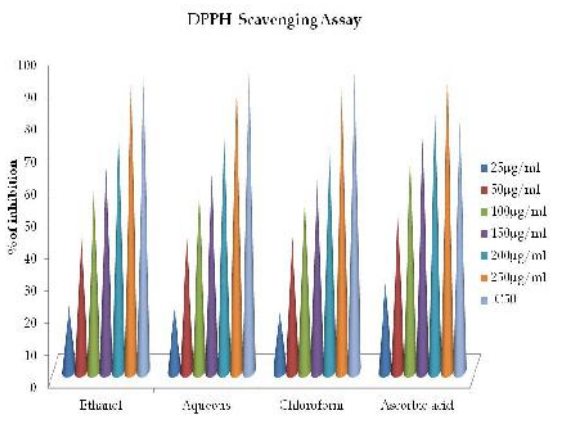


Fig 3: NO scavenging activity

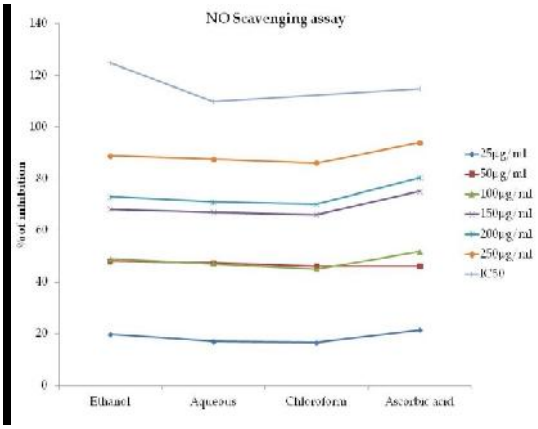


Fig 4: Reducing power assay

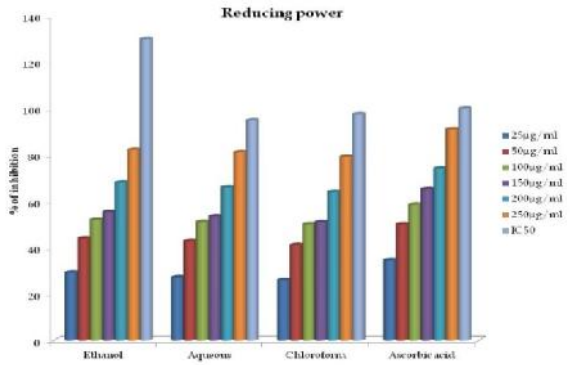


Fig 5: H_2O_2 radical scavenging activity

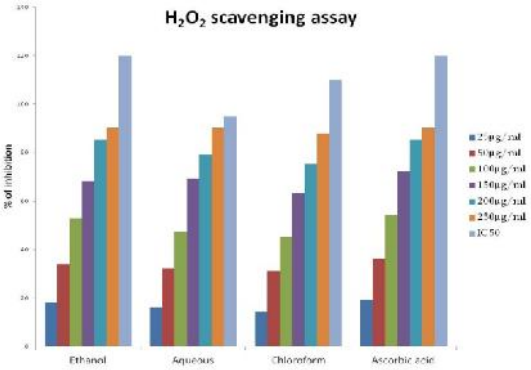
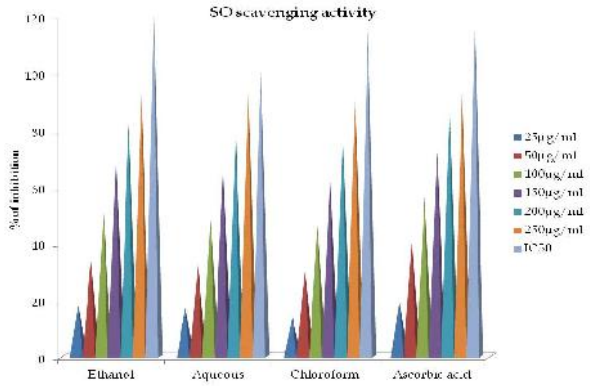


Fig 5: SO radical scavenging activity



Conclusion

From the results obtained in the present study, it is concluded that a ethanol, aqueous and chloroform extracts of *T. chebula* fruits, which contains great volume of phytochemical constituents shown the best antioxidant and free radical scavenging activities. The *in vitro* assay of present study represents that this fruit extracts is a considerable source of natural antioxidant, which might be useful in arresting the improvement of different oxidative stresses. Hence, further research need to be carried out to isolate and identify the antioxidant metabolites present in the fruit extract.

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