Available online on 15.01.2021 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Research Article

Study on correlation of Antioxidant activities with presence of phenolic and Flavanoid contents in *Emblica officinalis* and Terminalia chebula

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Article Info:

Article History:
Received 09 Nov 2020;
Review Completed 23 Dec 2020
Accepted 30 Dec 2020;
Available online 15 Jan 2021

Cite this article as:
Singh R, Kumar S, Study on correlation of Antioxidant activities with presence of phenolic and Flavanoid contents in Emblica *officinalis and Terminalia chebula*, Journal of Drug Delivery and Therapeutics. 2021; 11(1):32-35
DOI: http://dx.doi.org/10.22270/jddt.v11i1.4667

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Abstract

Background: Reactive oxygen species have been known to cause cellular damages that have been implicated to be a causal of major diseases; therefore natural antioxidants have shown a significant impact on human robustness. The present study was carried out to appraise the anti-oxidant activities (In- vitro) and their correlation with presence of Flavanoid and Phenolic content in fruits of *Terminalia chebula* and *Emblica officinalis* fruit extracts which is common in herbal Kitchen of India.

Methods: The 70% extracts of fruits from *Terminalia chebula* and *Emblica officialis* were applied for the study of Anti-oxidant activity. Scavenging radical ability of extracts of these extracts were judged by radical like DPPH.

Results: The capability of the extracts of Fruits in exhibiting Antioxidative properties follow the sequence of *Terminalia chebula* > *Emblica officinalis.* Since the antioxidant activities were studied in comparison with the standards of Flavanoid, Phenolic and Ascorbic acid. The Flavanoid and Phenolic quantity / amount along with subsequent dilution of Ascorbic acid as in case of DPPH radical assay were assessed as 127.60 ± 0.001 mg/ml, 133.00 ± 0.003 mg/ml for Phenolic Content as Gallic acid equivalent per 100 mg of the Fruit extract.

Keywords: Antioxidant, Phenol, Flavanoid, *Emblica*, *Terminalia*.

1. INTRODUCTION:

Oxidative stress plays an important role in the pathogenesis of various diseases such as atherosclerosis, alcoholic liver cirrhosis and cancer etc. Oxidative stress is initiated by reactive oxygen species (ROS), such as superoxide anion ($O^{\cdot}_2$), perhydroxy radical (HOO-) and hydroxyl radical (HO·). These radicals are formed by a one electron reduction process of molecular oxygen (O_2). ROS can easily initiate the lipid peroxidation of the membrane lipids, causing damage of the cell membrane of phospholipids, lipoprotein by propagating a chain reaction cycle[1,2]. Thus, antioxidants defense systems have coevolved with aerobic metabolism to counteract oxidative damage from ROS. Most living species have efficient defense systems to prevent themselves against oxidative stress induced by ROS[3]. Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases and aging process[4]. In this respect flavonoids and other polyphenolic compounds have received the greatest attention[18,19,20].

The fruits of *Terminalia chebula* and *Emblica officinalis* are widely used in the Indian traditional system of medicine. The half ripe fruit of the pericarp of *T. chebula* fruit were reported to be purgative[5]. The fruit of *T. chebula* was traditionally used to cure asthma, urinary disorders, heart disease and it has cardiotonic activity[6,7]. In Ayurveda, the fruit of *E. officinalis* is used as a cardiotonic, cerebral and intestinal tonic[8], and it is also reported to have anticancer properties[9]. The fruit of *E. officinalis* is a rich source of vitamin C, a well-known antioxidant[10]. The crude extract of *E. officinalis* was reported to counteract the hepatotoxic and renotoxic effects of metals[11] due to antioxidant properties. *Emblica officinalis* popularly known as amla, is a deciduous tree having average height of 5.5 meters. The fruit is drupe, fleshy globose, 1.5-2.5 cm. in diameter, smooth, shiny with light colored specks. It is distinctly marked in six lobes. The fruit is green when tender but the color changes to light yellow or brick red on maturity. The taste is sour and astringent giving feeling of sweetness afterwards [5]. The plant is found in the mixed deciduous forests of India, Sri Lanka, China, Bangladesh and Malaya ascending to 1,500 meters on the hills.

Dried fruit is useful in haemorrhage, diarrhea, diabetes and dysentery. The fruit has antibacterial, antifungal, and antiviral activities[12,13,14]. These two plants have been chosen for the present investigation because of its availability and wider indication in various diseases[15,16, 17]. Therefore, in present work a humble attempt was made to detect the presence of antifungal activities of this plant's fruits by a simple and commonly used agar disk diffusion method for investigation and their Phenolic and Flavanoid content as well as their antioxidant activities.

2. MATERIALS AND METHODS:

2.1 Procurement of Plant material and development of extract:

The fruit samples of *Terminalia chebula* and *Emblica officinalis* was taken from Ayodhya region of Uttar Pradesh in India. The development of plant extract was performed by Soxhlet extraction method 100 mg of dried fruit powder of *Terminalia chebula* and *Emblica officinalis*

2.2 Determination of Total Phenolic and Flavonoids Content

2.2.1 Reagents and Chemicals

Folin-Ciocalteu reagent, gallic acid, and quercetin standards were obtained from Sigma-Aldrich Co. Aluminum chloride hexahydrate, methanol, and sodium carbonate were obtained from Fisher Scientific. Water was purified using a Milli-Q system (Millipore).

2.2.1 Sample Preparation

About 10–50 mg of the extract was dissolved in 5 mL methanol and sonicated for 45 minutes at 40°C followed by centrifugation at 1,000 ×g for 10 min. The clear supernatant was collected and stored in an amber bottle for analysis.

2.2.2 Total Phenolic Content

The total phenolic of the extracts were determined using the Folin and Ciocalteu reagent, following the method described by Singleton and Rossi with slight modifications. Sample and standard readings were made using a spectrophotometer (Cary 50 Bio UV-Vis Spectrophotometer, Varian) at 765 nm against the reagent blank. Test sample (0.2 mL) was mixed with 0.6 mL of water and 0.2 mL of Folin-Ciocalteu's phenol reagent (1: 1). After 5 min, 1 mL of saturated sodium carbonate solution (8% w/v in water) was added to the mixture and the volume was made up to 3 mL with distilled water. The reaction was kept in the dark for 30 min and after centrifuging the absorbance of blue color from different samples was measured at 765 nm. The phenolic content was calculated as gallic acid equivalents GAE/g of dry plant material on the basis of a standard curve of gallic acid (5–500 mg/l). All determinations were carried out in triplicate.

2.2.3. Total Flavonoids Content

The aluminum chloride colorimetric method was used for the determination of the total flavonoid content of the sample. For total flavonoid determination, quercetin was used to make the standard calibration curve. Stock quercetin solution was prepared by dissolving 5.0 mg quercetin in 1.0 ml methanol, then the standard solutions of quercetin were prepared by serial dilutions using methanol (5–200 µg/ml). An amount of 0.6 mL diluted standard quercetin solutions or extracts was separately mixed with 0.6 mL of 2% aluminum chloride. After mixing, the solution was incubated for 60 min at room temperature. The absorbance of the reaction mixtures was measured against blank at 420 nm wavelength with a Varian UV-Vis spectrophotometer (Systronics UV-VIS Double beam spectrophotometer). The concentration of total flavonoid content in the test samples was calculated from the calibration plot and expressed as mg quercetin equivalent (QE)/g of dried plant material. All the determinations were carried out in triplicate.

2.2.4 Determination of Antioxidant Activity

2.2.4.1 Preparation of Extract

The extracts were dissolved in dimethyl sulfoxide (DMSO) to make a stock solution of 20 mg/ml. The antioxidant activity of the extracts was measured at a concentration of 500 µg/ml by following two methods.

2.2.4.2 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

The capacity of plant extracts (500 µg/ml) to directly react with and quench free radicals was evaluated as described earlier[25]. A stock solution of DPPH (200 µm) was prepared in ethanol. The assay was performed in 96-well plates. The reaction mixture, containing 100 µl of DPPH and 100 µl of the diluted test sample, was incubated at 37°C for 30 min. The absorbance was measured at 515 nm. Gallic acid was used as a positive control. Percent DPPH radical scavenging activity was calculated as follows for

Percentage radical Scavenging Assay =

$$\left\{1 - \frac{(sample - blank)}{(Control - blank)}\right\} * 100$$

Gallic acid showed 95% radical scavenging activity at 20 µM.

3. RESULTS AND DISCUSSION

3.1 Total Flavanoid and Phenolic Content

The calculation of total Flavanoid content was performed by taking quercetin as Standard. The values for unknown variables were calculated as mg/ml.

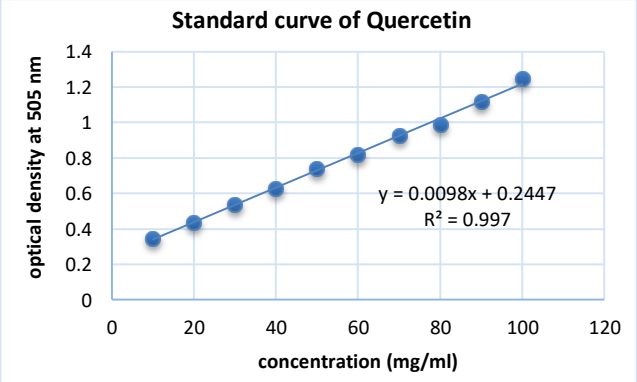

Figure 1: Calibration Plot of Quercetin

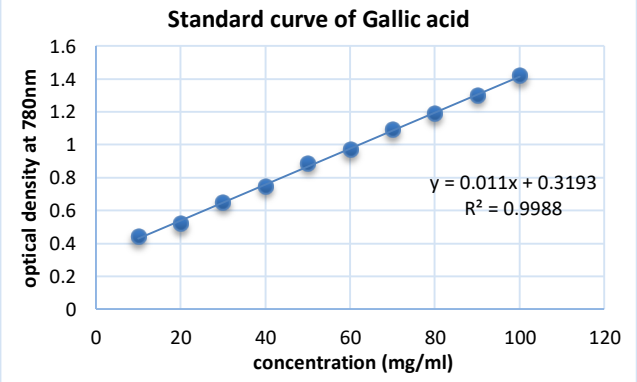

Figure 2: Calibration plot of Gallic acid

The first and foremost experiment of the research investigation to calculate Total Phenolic Content. The phenolic and flavonoid compounds along with the subsequent ascorbic acid contents of the extracts may contribute directly to antioxidative action. The total phenolic content of 70% methanolic extracts of *T. chebula*, and *E. officinalis* were 43.90 mg/ml and 39.87 mg/ml. gallic acid equivalent per 100 mg fruit extract, respectively, whereas the flavonoid contents were 43.78 mg/ml and 39.098 / ml quercetin per 100 mg fruit extract, following the above

order. This result showed that *T. chebula* had quite hiked amount of both Flavanoid and Phenolic content by unit values of 3 mg/ ml, the comparative increase of *Terminalia chebula* contents was both in case. Since our work focused to compare the important Phytoconstituents and check whether relatively they have suitable activities with Antioxidants or not. Antioxidant activity for the extracts of *T. chebula* and *E. officinalis* were calculated using DPPH as reducing radical.

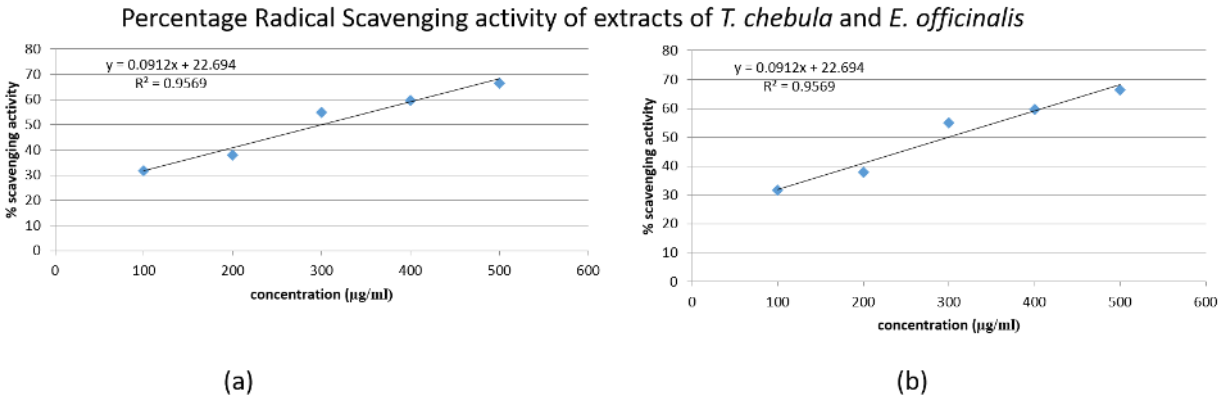

Figure 3 (a) Percentage radical scavenging assay of *T. chebula* (b) percentage radical scavenging assay of *E. officinalis*

Value for IC_{50} for extract was calculated from the graph placing the value of y=50 and calculating for the value of x.

Table 1: Absorbance and %inhibition for different concentration of sample extract

S. No	Concentration (µg/ml)	Absorbance (517nm)	Conc as Ascorbic acid as equivalent	%Scavenging	I_C 50
1	100	0.132	76 µg/ml	31.6062	
2	200	0.12	66.66 µg/ml	37.8238	
3	300	0.087	39.166 µg/ml	54.9222	
4	400	0.078	31.66 µg/ml	59.5854	290 µg/ml
5	500	0.065	20.833 µg/ml	66.3212	

Both of these compounds have good antioxidant potential and their effects on human nutrition and health are considerable. The mechanism of action of flavonoids is through scavenging or chelating process[21]. Phenolic contents are also very important plant constituents because of their scavenging ability due to their hydroxyl groups[22]. Moreover, ascorbic acid acting as a chain breaking antioxidant impairs with the formation of free radicals in the process of formation of intracellular substances throughout the body, including collagen, bone matrix and tooth dentine[23]. From the results, the trend for the ascorbic acid content was found to be *E. officinalis* >*T. chebula*.

CONCLUSION:

The investigation found out that radical scavenging assay revealed that fruit extracts possesses individual strong antioxidant activity with different magnitudes of potency in terms Phytoconstituents (Phenols and Flavanoids). *Terminalia chebula* and *Emblica officinalis* are common Indian herbs generally gives advantages various diseases as well as body grooming products. The wide use of these fruits in the Indian indigenous system of medicine as anti-inflammatory and anti-hepatotoxic may be in part due to their antioxidant potency. Further, the isolation of the compounds responsible for the antioxidant activity has to be taken up which may result in modern drugs from these plants. Also the studies on antioxidant activity of the well-known Ayurvedic formulation, Triphala, a mixture of these fruits, should be carried out. The work is in progress.

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