ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Determination of Total Phenolic, Flavonoid Content and Antioxidant Activity of *Terminalia Chebula* (Fruit).

Kaveti Balaji*, Lim Hui Ni, Bavaneswari Rajindran, Mukesh S Sikarwar, Neeraj Kumar Fuloria, and Shivkanya Fuloria.

Faculty of Pharmacy, AIMST University, Jalan Bedong - Semeling 08100, Bedong, Kedah Darul Aman, Malaysia.

ABSTRACT

The antioxidant activity of methanolic extract of *Terminalia chebula* was evaluated concerning total phenol and flavonoid contents. Three commonly in vitro methods are used to assess antioxidant activity namely Ferric reducing antioxidant power (FRAP assay), DPPH Free radical scavenging ability assay. The results showed that the total phenolic content for 50, 100 and 200 µg/ml extracts were 0.2232, 0.3156 and 0.4170 mg GAE/ gram respectively. The total flavonoid content for 50, 100 and 200 µg/ml extracts were 49, 63, and 83 mg RE/gram respectively. In the DPPH study, the plant extract shown appreciable anti-oxidant potency as the percentage of anti-oxidant activity is more significant compared to the standard, BHT. In the FRAP study, the values of absorbance recorded for both standard and test samples at a concentration of 400 µg/ml are 4.5000 and 3.4889 respectively. From these results, the presence studies indicate that methanolic extract *Terminalia chebula* might have good potential as a source for natural health products due to its antioxidant activities.

Keywords: Antioxidant activity, Methanolic extract, *Terminalia chebula* fruit.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Antioxidants are molecules protect cells from damage caused by unstable molecules are identified as free radicals by reducing or inhibiting the oxidation of other molecules by them [1]. Free radicals are an essential part of any biological process and of aerobic life and metabolism [2]. Antioxidants act by reducing or inhibiting chain reactions of oxidative processes by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves [3].

Natural antioxidants present in foods and other biological materials have fascinated a great interest due to their safety and nutritional and therapeutic effects [4]. Use of synthetic antioxidants been eliminated from many food products, as they required extensive and expensive testing and has to fulfil safety standards. The increasing importance in the search for natural alternates for synthetic antioxidants has led to the antioxidant evaluation of a number of plant sources [5]. *Terminalia chebula* (*T.chebula*) family of Combretaceae is a moderate and native plant that occurs naturally from the sub-Himalayan region of Nepal and Northern India to Sri Lanka, Myanmar, Malaysia, Thailand, Indo-China, and Southern China [6]. It is well known as Harde in India, Black Myroblans in English that is commonly used as a medicinal herb in many ayurvedic preparations. It is a moderate sized growth deciduous tree that is about 25-30 m in height with leaflets, branchlets and young fruits with soft, shinning and rust colored hairs.

The flowers of this plant are dull white or yellow in colour, with an offensive smell. The dried ripe fruit is sub globose to ellipsoid that are blackish in colour traditional folk medicine for bleeding, chronic laryngitis, laryngeal tuberculosis, gastrointestinal bleeding, chronic metritis and dysentery [7-11].

The extract of *T. chebula* has been reported to exhibit a variety of biological activities, including anticancer [12], antidiabetic [13], antibacterial [14], and anticaries effects [15]. A literature reported presence of various types of constituents like tannins, gallic acid, ellagic acid and triterpenoids [16].

Therefore, the objective the present paper was to evaluate antioxidant and total phenolic content of methanol extract of dry fruit of *Terminalia chebula*

MATERIAL AND METHODS

Materials

Plant material of *Terminalia chebula* was collected from local market, Sungai petani, Kedah, Malaysia. 2,2-diphenyl-1-picrylhidrazyl (DPPH), Gallic acid and Rutin were purchased from Sigma Chemical Co. (St.Louis, MO, USA). Folin-Ciocalteu, and other reagents and solvents were bought from E. Merck (Darmstadt, Germany).

Preparation of methanolic extract of *Terminalia chebula*

The powdered plant material macerated with methanol for 7 days with frequent shanking after filtered and evaporated to dryness using vacuum rotary evaporator at 40 ºC.

Analysis of total phenolics content

TPC in extract was determined according to the Folin-Ciocalteu procedure [16]. The extract (0.3 mL, in triplicate) was mixed with of Folin-Ciocalteau's reagent (100 µl, 10%) and sodium carbonate (2.0 mL, 7.5%). The mixture was kept in the dark for 2 hr before measuring the absorbance at 750 nm. Quantification of total phenolic content was done using standard curve of gallic acid as a standard phenolic compound (0.1-0.5 µg/ml) and results were expressed as mg gallic acid equivalent (GAE)/100g plant materials.

Determination of Total Flavonoid Content

Flavonoid contents were determined according to the Aluminium chloride method [17]. The extract (1 ml, different concentration, in triplicate), sodium nitrate (0.7 ml, 5%) and ethanol (10 ml, 30%) were mixed for

five minutes and then aluminium chloride (0.7 ml, 10%) was added and mixed together. Six minutes later, sodium hydroxide (5 ml, 1 mol/L) was added. The solution was then diluted to ethanol (25 ml, 30%). After standing for 10 minutes, the absorbance of the solution was measured at 430 nm using a spectrophotometer. Quantification of total flavonoid content was done using standard curve of rutin (10-100 µg/ml), and expressed as mg rutin per gram dry weight of plant material.

Determination of DPPH free radical scavenging activity

The scavenging activity of DPPH free radical of the methanolic extract of *T.chebula*, was determined according to the procedure [18]. In this assay, alcoholic solution of DPPH (1 ml, 0.3 mM) added to different concentration of plant extracts (2.5 ml). The mixtures were kept in the dark place at room temperature for 30 minutes. Then, the absorbance was measured at 514 nm using a spectrophotometer. The standard graph was plotted using different concentrations of BHT used methanol as blank. The radical scavenging activity was calculated using the following formula:

$$Radical\ scavenging\ activity\ (\%) = \frac{Ac - As}{Ac} x100$$

Where Ac is absorbance of control (DPPH free radical without the addition of test solution), As is sample absorbance (absorbance of DPPH free radical after the addition of test solutions)

Reducing Power Assay

The ferric reducing power of extracts was evaluated using an assay described in method [17]. Different concentration of extracts (1 ml, triplicates), phosphate buffer (2.5 ml, pH 6.6) and potassium ferricyanide (2.5 ml, 1%) were mixed separately and allowed to incubate at 50 ^{o}C for 30 minutes and trichloroacetic acid (2.5 ml,10%) (TCA) was added to the mixtures and centrifuged for 10 minutes at 3000 rpm. About 2.5 ml of the supernatant was diluted with 2.5 ml of water and was shaken with freshly prepared ferric chloride (0.5 ml, 0.1%). The absorbance was measured at 700 nm using a spectrophotometer.

RESULTS AND DISCUSSION

Analysis of phenolic contents

Folin-Ciocalteau (F-C) assay is fast and simple method used for determination of phenolics compounds in plant extract using gallic acid as a standard, and expressed as gallic acid equivalent [16]. This method is based on oxidation of phenolics by F-C reagent (a molybdotungstate) in to produce a colored product (molybdenum blue) having maximum wavelength of 745 – 750 nm. As the concentration of the extract increases, the mean absorbance value also increases. Table 1 expressed the phenolic contents of extract of *T. chebula* expressed as gram Gallic acid equivalent (GAE)/100 gram plant material.

Table 1: Results from total phenolic content (mg GAE/gram)

Concentration of extracts (µg/ml)	Total Phenolic Content (mg GAE/gram)
50	0.2232
100	0.3156
200	0.4170

Determination of Total Flavonoid Content

The total flavonoid content of extract of *Terminalia chebula* was measured [17, 19] by mixing of $NaNO_2$, ethanol and $AlCl_3$. The absorbance of sample was measured at 450 nm using a UV-spectrophotometer. A standard curve was plotted using Rutin as a standard. The amount of total flavonoid compounds that present was reported as RE per gram sample as shown in table 2.

ISSN: 0975-8585

Table 2: Results from total flavonoid content (mg RE/Gram)

Concentration of extracts (µg/ml)	Total Flavonoid Content (mg RE/gram)
50	49
100	63
200	83

Total flavonoid contents of the extract of *Terminalia chebula* showed in 50 µg/ml, (49 mg RE/100 g sample), followed by 100 µg/ml (63 mg RE/100 g sample), 200 µg/ml (83 mg RE/100 g sample)

DPPH free radical scavenging activity

DPPH assay method is standard and an easy colorimetric technique that is used for the assessment of free radical scavenging activity of natural antioxidants [20]. This method mainly used for evaluation of antioxidant activity of pure compound and plant extract. This technique gives data on the reactivity of a stable free radical DPPH with test compound as odd electron of DPPH gives strong absorption band at 517 nm (violet color) and when it is extinguished by the extract, there is a diminish in absorbance [21]. In this study, BHT used as standard, and methanol as solvent due to its capability to produce the sensitive results [22]. It was visually noticeable the discoloration of the violet colour to pale yellow. Extend of DPPH radical scavenged determined by the decrease in the intensity of violet colour in the form of IC_{50}. Thus, the lower the absorbance value, the higher the anti-oxidant activity. The concentration of *Terminalia chebula* required for 50% of inhibition was 4.4197µg/ml.

The plant extract shown appreciable antioxidant potency, as the percentage of antioxidant activity is more significant as compared to the standard, BHT as shown in Fig. 1. Further, it was evident that the standard BHT, at a concentration of 60 µg/ml, has shown an activity of 67.94%, while the plant extracts have exhibited an activity of 93.30% at the same concentration. This significant activity might be due to presence of high amount of polyphenol compounds and flavonoids. The DPPH data were expressed as IC_{50} (mg/ml) as shown in Table no 3.

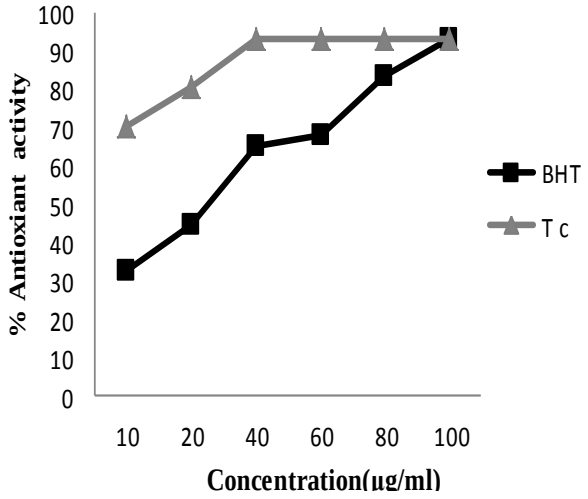

Figure 1: Antioxidant activities of different concentrations methanolic extract *T. Chebula* Fruit (Haritaki) and standard.

Table 3: Antioxidant activity IC50 value of methanolic extract and standard

Sample IC_{50}Value (µg/ml)	Standard IC_{50} Value (µg/ml)
4.4197	29.0297

ISSN: 0975-8585

Reducing Power Assay

FRAP assay is simple and easy to perform method treats the anti-oxidants in the sample as reductant in a redox-linked colorimetric reaction [23]. FRAP measures the reducing power of anti-oxidant to react on ferric and produce blue colour of ferrous form, which can be detected at absorbance 700 nm. The reducing ability of *Terminalia chebula* extract found to be considerable, which increased gradually with the rise in concentration as shown in (Fig. 2) and is comparable with standard (ascorbic acid). Increased absorbance of the reaction indicated increased reducing power.

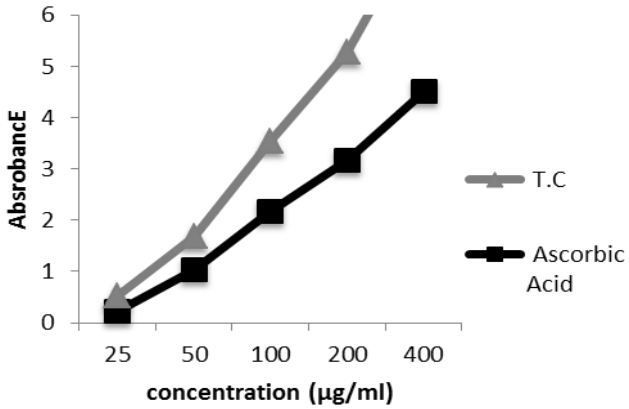

Figure 2: Absorbance of antioxidant activities as measured by the FRAP Method

CONCLUSION

From the results of the study, it was observed that methanolic extract of Terminalia chebula exhibits in vitro antioxidant activity. In vitro antioxidant tests, evidenced methanolic extract of Terminalia chebula as a reducing agent and effectiveness as scavengers of free radicals. Hence, it is worthwhile to isolate and elucidate the bioactive principles that are responsible for the antioxidant activity.

REFERENCES

[1] Khaled R. Hygeia J D Med 2014; 6: 106-110.
[2] Beyhan O, Elmastaş M, Gedikli F, J Med Plants Res 2010;4: 1065-1072 .
[3] Hamid AA, Aiyelaagbe OO, Afr. J. Pure Appl. Chem 2010; 4:142-151.
[4] Reddy PV, Maralinga S, Urooj A, Intl J Medi Che 2014; 7.
[5] Mandal S, Yadav S, Nema RK, J Chem Pharma Res 2009; 1: 102-104.
[6] Rathinamoorthy R, Thilagavathi G, Int J Pharm Tech Res 2014;6: 97-116.
[7] Walia H, Arora S, J Med Plants Res 2012; 7:1351-1361.
[8] Chattopadhyay RR, Bhattacharyya SK, Pharmacognos 2007; 23: 45-150.
[9] Muhammad S, Khan BA, Naveed A, Tariq M, Akhtar RH. Irshad, HB, Khan A, J Med Plants Res 2012; 6: 4772-4775.
[10] Bag A, Subir KB, Chattopadhyay RR, Asian Pac J Trop Biomed 2013; 3:244-252S.
[11] Suryaprakash DV, et al. Int J Res Pharm Biomed Sci 2012; 3: 679-683.
[12] Ammar S, Michael H, Pirkko H, J Ethnopharma 2002; 81:327-336.
[13] Kannan R V, et al. Ame J Drug Discovery Devel 2012: 2;135-142.
[14] Kannan P, Ramadevi SR, Hopper W, Afr. J. Microbiol Res 2009; 3: 180-184.
[15] Carounanidy U, Satyanarayanan R, Velmurugan A, Ind J Dent Res 2007;18: 152-6 .
[16] Rabeta MS, Nabil Z, Int Food Res J 2013; 20:1: 495-500.
[17] Priyanka P, Junaid N, Gagandeep C, Kalia AN, Res J Pharm Biol Chem Sci 2011; 2: 348- 357.
[18] Ali G, Hawa ZE, Jaafar AR, Molecules 2010; 15: 4324-4333.
[19] Maryam Z, Farrukh A, Iqbal A, Int J Pharma Pharma Sci 2009; 1: 88-95.
[20] Moniruzzaman, Afr J Tradit Complement Altern Med 2012; 9: 36-42.
[21] Anandjiwala S, Bagul MS, Parabia M, Rajani M, Ind J Pharm Sci 2008; 70: 31-5.
[22] Arina NB, Rohman A, Int Food Res J 2013; 20:3: 1119-1124.
[23] Nagendra P, Bao Y, Kin WK, Evidence-Based Complem Alt Medi 2013: 9.