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Determination of Free Radical Scavenging Activity of *Emblicaofficinalis* Gaertn., *Terminaliachebula* Retz. And *Terminaliabelerica*Roxb.

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Abstract

The present paper reports about the free radical scavenging activity of *Emblicaofficinalis*, *Terminaliachebula*and *Terminaliabelerica.* The fruits of the above mentioned plants were used in the present study. The study attempts to focus on the antioxidant potential of the selected plant materials. We have undertaken this study by using the most reliable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method followed by the Hydroxyl radical scavenging method. The highest DPPH scavenging activity as per the IC50 values was found to be in *Emblicaofficinalis* (51.00 ± 1.42µg/ml) while *Terminaliachebula* and *Terminaliabelerica* showed (115.29 ± 1.86 µg/ml) and (165.29 ± 2.94 µg/ml), respectively. The highest Hydroxyl radical scavenging activity was found in *Emblicaofficinalis* (10.04 ± 2.92 µg/ml) while *Terminaliachebula* and *Terminaliabelerica* showed (39.65 ± 3.36 µg/ml) and (47.36 ± 2.08 µg/ml), respectively. Among all the plants studied, *Emblicaofficinalis* was found to be the best one with lowest IC50 values in DPPH method as well as Hydroxyl radical method, thus indicating highest free radical scavenging activity. Hence, *Emblicaofficinalis*possess more antioxidant potential as compared to the other two plant materials.

Keywords

Emblicaofficinalis, Terminaliachebula, Terminaliabelerica, DPPH radical and Hydroxyl radical

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INTRODUCTION

Antioxidants help organisms deal with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Several methods are used to measure the antioxidant activity of a biological material. The most commonly used ones are those involving chromogen compounds of radical nature that stimulate the reductive oxygen species. These methods are popular due to their ease, speed and sensitivity. The presence of antioxidants leads to the disappearance of these radical chromogens; the most widely used ones being the DPPH methods[3].

Synthetic antioxidants such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT) and tertiary hydroquinone (TBHQ) are commonly employed as preservatives by pharmaceutical, cosmetic, and food companies. However, they are also suspected of being responsible for liver damage and carcinogenesis[19] and toxicity[10]. Therefore, there is an increasing need to replace synthetic antioxidants with natural, safer compounds. A great number of medicinal plants contain chemical compounds showing antioxidant properties. Natural antioxidants can protect the human body from radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods[8]. Therefore, assessment of the antioxidant properties is an interesting and useful topic, especially in finding new sources of natural antioxidants, functional foods, and neutraceuticals[11].

*Emblicaofficinalis*enjoys a hallowed position in Ayurveda- an Indian indigenous system of medicine. According to the belief in ancient Indian mythology, it is the first tree to be created in the universe. It belongs to the family Euphorbiaceae. It is also named as AmlaorIndian gooseberry. The species is native to India and also grows in tropical and subtropical regions including Pakistan, Uzbekistan, Srilanka, South East Asia, China and Malaysia. It has application as antioxidant, immunomodulatory, antipyretic, analgesic, cyto-protective, antitussive and gastro-protective. Additionally, it is useful in memory enhancing, ophthalmic disorders and lowering cholesterol level. It is often used in the form of Triphla which is an herbal formulation containing fruits of

Emblicaofficinalis, *Terminaliachebula*and*Terminaliabelerica*in equal proportions[15].

*Terminaliabelerica*Roxb.(Family : Combretaceae) referred to as, BelericMyrobalan in English, Bibhitaki in Sanskrit, Locally known as Bahera in India, has been used for centuries in the Ayurveda, a holistic system of medicine originating from India. The dried fruit used for medicinal purposes. It is an integral part of Ayurvedic laxative formulation, Triphala used in treatment of common cold, pharyngitis and constipation. Modern investigations have proved the laxative activity of the oil[9]. It is used in the treatment of fever, cough, asthma, urinary diseases, piles[14], chronic diarrhea, dysentery, flatulence, vomiting, colic and enlarged spleen and liver.

Terminaliachebula Retz.is known as *Myrobalanuschebula*or Hirda belongs to the family Combretaceae. It inhibits the development of duodenal ulcer and appeared to extract a cytoprotective effect on the gastric mucosa[13]. It is good to increase the appetite, as digestive aid liver stimulant, as stomachic, as gastrointestinal prokinetic agent and mild laxative. It is used as an antioxidant, neuroprotective drug and treatment for heart disease, inflammation and brain dysfunction[17]. It has been reported as antioxidant[16], antidiabetic[12], antibacterial[6], antiviral[7], antifungal, anticancerous, antiulcer, antimutagenic, wound healing activities etc. It increases the frequency of stools and has got the property of evacuating the bowel completely. It is used to prevent aging and impart longevity, immunity[1] and body resistance against disease. It has beneficial effect on all the tissues.

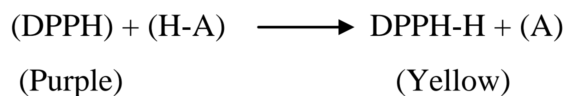
I. In-vitro Antioxidant activity /Free radical scavenging activity (DPPH Assay):

The anti-oxidant potential of any compound is determined on the basis of its scavenging activity of the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical as described[2]. DPPH is a stable free radical usually used in detection of the radical scavenging activity in the chemical analysis with absorption maximum at 517 nm in methanol (Table 1).

The antioxidant activity of the plant extracts were expressed as IC^{50}. The IC50 value is defined as the concentration (μg/ml) of the extracts that inhibits the formation of DPPH radicals by 50%.

Principle:

The scavenging reaction between (DPPH.) and an antioxidant (H-A) can be written as:



(DPPH) + (H-A) ⟶ DPPH-H + (A)

(Purple) (Yellow)

DPPH (2,2-diphenyl-1-picryl hydrazyl), a stable free radical, when acted upon by an antioxidant, is converted into diphenyl-picryl hydrazine with a color change from deep violet to light yellow color. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. This can be quantified spectrophotometrically at 517 nm to indicate the extent of DPPH scavenging activity by the plant extracts. DPPH is very popular for the study of natural antioxidants[18].

II. Determination Of *In Vitro* Antioxidant Activity by Hydroxyl Radical Scavenging Assay:

The hydroxyl radicals are extremely reactive oxygen species that can react with every possible molecule in living organisms, especially with proteins, DNA, and lipids. Hydroxyl radicals are capable of rapid initiation of the lipid peroxidation process by extracting hydrogen atoms from unsaturated fatty acids.

MATERIALS AND METHODS

Fruits of *Emblicaofficinalis*,*Terminaliachebula*,*Terminaliabelerica* were used in the present studies. All the plant materials selected for the study were selected on the basis of available literature, easy and abundant availability of the plant material in our locality, medical formulations, traditional knowledge, the ethnobotanical data and discussions with various doctors for their anti-oxidant potential.

I. In-vitro Antioxidant activity /Free radical scavenging activity (DPPH Assay):

Preparation of the Standard Solution

Required quantity of the Ascorbic Acid was dissolved in methanol to give the concentration of 1mg/ml. From this solution concentrations of 5, 10, 15, 25, 50 and 60 µg/ml were prepared.

Preparation of the Test Sample

Stock Solutions of the samples were prepared by dissolving 10 mg of the dried hydroalcoholic extract in 10 ml of methanol to give the concentration of 1 mg/ml and from this solution, concentrations of 5, 10, 15, 25, 50 and 60 µg/ml were prepared.

Preparation of DPPH Solution

3.9 mg of DPPH was dissolved in 3 ml of methanol; it was protected from light by covering the test tubes with aluminium foils.

Procedure:

Protocol for estimation of DPPH scavenging activity

The stock solution of the DPPH was prepared as mentioned above such that 75µl of it in 3 ml methanol gave an initial absorbance of 0.9. The decrease in the absorbance of the sample extracts and the standard at different concentrations was noted after 30 minutes. A blank reading was taken using methanol instead of the sample extract. IC50 was calculated from the % inhibition. This IC50 value denotes the concentration of sample required to scavenge 50 % of the DPPH free radicals. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{DPPH Scavenged (\%)} = \frac{\text{A Control - A Test}}{\text{A Control}} \times 100$$

Where,

A Control = Absorbance of DPPH alone

A Test = Absorbance of DPPH and different concentrations of thesample extracts.

IC50 was calculated from equation of line obtained by plotting a graph of concentration versus % inhibition.

II. Determination Of *In Vitro* Antioxidant Activity by Hydroxyl Radical Scavenging Assay:

Scavenging of the hydroxyl free radical was measured by the method[4-5]. To 1.5 ml of varying concentration of the extract, 60 µl of (1 mM) Ferrous chloride, 90 µl of (0.2 M) Phosphate buffer (pH 7.8) and 150 µl of (0.17 M) Hydrogen peroxide was added and incubated at room temperature for 5 minutes. After incubation at room temperature for 5 min, the absorbance of reaction mixture was noted at 560 nm. The hydroxyl radicals scavenging activity was calculated according to the following equation and compared with ascorbic acid as standard:

$$\%\text{Inhibition} = (A_{Blank} - A_{Samples})/A_{Blank} \times 100$$

Where, A_{Blank} was the absorbance of blank (without extract) and

$A_{Samples}$ was the absorbance of tested samples.

Absorbance was read at 560 nm. % inhibition was calculated. (Table 2)

OBSERVATION

Table 1 Free radical scavenging activity (DPPH Assay) of Anti-haemorrhoidal plant materials

Sr. No.	Sample	Part Used	Concentration (µg/ml) and % Inhibition					IC50 (µg/ml)
			20	40	60	80	100	
1	*Emblicaofficinalis*	Fruits	65 ± 0.72	68.88 ± 0.85	73.55 ± 0.46	78 ± 0.83	82.66 ± 0.66	51.00 ± 1.42
2	*Terminaliachebula*	Fruits	55 ± 0.14	62 ± 0.77	70 ± 0.32	75 ± 0.39	85.33 ± 0.26	115.29 ± 1.86
3	*Terminaliabelerica*	Fruits	65 ± 0.11	71.22 ± 0.28	75 ± 0.14	79.11 ± 0.40	85 ± 0.27	165.29 ± 2.94
4	Ascorbic Acid	-	26.39 ± 0.11	39.55 ± 0.33	49.75 ± 0.14	61.59 ± 0.28	72.88 ± 0.85	58.17 ± 3.75

Table 2 Hydroxyl Radical Scavenging Assay of Anti-Haemorrhoidal Plant Materials

Sr. No.	Sample	Part Used	Concentration (µg/ml) and % Inhibition					IC50 (µg/ml)
			25	50	75	100	150	
1	*Emblicaofficinalis*	Fruits	62 ± 0.72	69.29 ± 0.85	76.11 ± 0.46	81.28 ± 0.83	89.39 ± 0.66	10.04 ± 2.92
2	*Terminaliachebula*	Fruits	59.15 ± 0.14	72.38 ± 0.77	81.33 ± 0.32	87.85 ± 0.39	93.59 ± 0.26	39.65 ± 3.36
3	*Terminaliabelerica*	Fruits	68 ± 0.11	74.95 ± 0.28	85.58 ± 0.14	89.03 ± 0.40	92.43 ± 0.27	47.36 ± 2.08
4	Ascorbic Acid (Standard)	-	18.33 ± 0.11	46.00 ± 0.33	68.33 ± 0.14	79.19 ± 0.28	86.65 ± 0.85	11.69 ± 3.99

RESULTS AND DISCUSSION

DPPH analysis is one of the best-known, accurate, and frequently employed methods for evaluating antioxidant activity. It is a stable free radical because of its spare electron delocalization over the whole molecule. The donation of H^+ to the DPPH radicals made a corresponding change from violet colour to pale yellow in the solution. The DPPH scavenging also made a proportionate decrease in its absorbance at 517nm.

According to the Observation table 1, the positive control, ascorbic acid showed maximum scavenging effect at very low

concentration and its IC50 value was found to be 58.17 ± 3.75µg/ml. The highest DPPH scavenging activity as per the IC50 values was found to be in *Emblicaofficinalis* (51.00 ± 1.42µg/ml) while *Terminaliachebula* and *Terminaliabelerica* showed (115.29 ± 1.86 µg/ml) and (165.29 ± 2.94 µg/ml) respectively. The proton radical scavenging action is known to be one of the important mechanisms for measuring antioxidant activity. This assay determines the scavenging of stable radical species DPPHby antioxidants compounds present in the extracts.

According to the Observation Table 2, *Emblicaofficinalis* (10.04 ± 2.92µg/ml) shows the highest hydroxyl radical scavenging activity followed by *Terminaliachebula*(39.65 ± 2.36µg/ml) and *Terminaliabelerica* (47.36 ± 1.08µg/ml). The positive control, ascorbic acid showed maximum scavenging effect at very low concentration and its IC50 value was found to be 11.69 ± 3.99µg/ml.

Hence, in both the free radical scavenging methods, i.e. DPPH as well as Hydroxyl radical, *Emblicaofficinalis*excells the list among the three plant materials. Thus, it has more antioxidant potential followed by *Terminaliachebula*and*Terminaliabelerica*.

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