

**Research Article****Comparative evaluation of antioxidant power of hydroalcoholic extract of *Terminalia bellerica* and *Emblca officinalis* plant**Akanksha Tripathi^{1*}, Ritu Thakur Bais²¹Department of Microbiology, Barkatullah University, Bhopal, India²Department of Botany, Govt. M.L.B. Girls P.G. (Auto.) College, Bhopal, India**ARTICLE INFO:****Article history:**

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T. bellerica, *E. officinalis***ABSTRACT**

The present study was aimed to evaluate antioxidant activity in hydroalcoholic extract of fruits of *Terminalia chebula* and *Emblca officinalis*. Antioxidant activity of the extracts was determined using diphenylpicrylhydrazyl (DPPH), NO and Total antioxidant assays. Extracts of both medicinal plant exhibited antioxidant potential but *E. officinalis* proved more active. The presence of antioxidant activity in the extracts showed that these plants have the potential to be an alternate source of natural antioxidants. In vivo study is needed for successful commercialization and to benefit the food and pharmaceutical industries.

Introduction

Free radicals and other reactive oxygen species like hydroxyl radical, superoxide anion, singlet oxygen and hydrogen peroxide (H_2O_2) can cause oxidative damages to biological macromolecules which can lead to initiation and/or progression of various diseases. Such as cellular and metabolic injury, cancer, atherosclerosis, inflammation, aging, immunosuppression, diabetes, ischemic heart disease and neurodegenerative disorder such as Alzheimer's and Parkinson's disease [1]. ROS include superoxide radical, hydrogen peroxide, and hydroxyl free radical, all of which have one or more unpaired electrons that potentially cause damage to the respiring cells. All these reactive species are highly toxic and mutagenic [2]. Antioxidants are necessary for body, serves to inhibit the damage caused by reactive oxygen species (ROS) on membrane lipid, DNA and protein. ROS comprise hydrogen peroxide (H_2O_2), superoxide anion and free radicals: hydroxyl and peroxy, singlet oxygen and peroxynitrite unstable and highly reactive molecules. ROS damage cells by chain reactions like lipid peroxidation that contribute to chronic disease development, such as cancer, heart disease and cerebrovascular [3]. A number of spices and herbs contain antioxidants chemical compounds. Such compounds include vitamins, carotenoids, terpenoids, alkaloids, flavonoids, lignin, simple phenols, phenolic acids and etc. Likewise, nutmeg

as a spice plants and herbs has antioxidant properties [4]. Many Indian plants have previously been investigated for their beneficial use in different types of diabetes; however majority of the rich diversity of Indian medicinal plants is yet to be scientifically evaluated for their biological/antioxidant properties [5]. Natural antioxidants such as flavonoids and polyphenols are believed to possess antioxidant properties due to their reducing and chelating capabilities. Flavonoids and polyphenols are secondary plant metabolites that are widely distributed in fruits, leaves, bark, and other parts in plants with free radical scavenging abilities. The genus *Terminalia* (fam. Combretaceae), comprising 250 species, is distributed across tropical countries worldwide, and the Indian traditional system of medicine has documented several books and literatures on the medicinal values of many of its species, of which *Terminalia bellerica*, *T. chebula* and *T. arjuna* are prime examples [6]. It has been reported that these species are rich in flavonoids and polyphenols. In "Ayurveda," a herbal formulation combining the dried fruits of *T. chebula*, *T. bellerica* (*R.*) and *E. officinalis* (*L.*) by the name of "Triphala" has been used as a food and dietary supplement to derive several health benefits such as laxation, detoxification, liver protection, anti-aging, and as a rejuvenator of the body [7]. The combination has also been found to have

antidiabetic and cholesterol-lowering activities. The fruit extracts of *T. chebula* and *T. bellerica* (*R.*) have been shown to contain antioxidants, and *T. arjuna* bark extracts have been reported to possess cardioprotective, antioxidant, and antimutagenic abilities [8]. Despite reports of the antioxidant and antidiabetic activities of the fruit and bark of *Terminalia* species, which are a rich source of flavonoids, tannins, and many phenolic derivatives, concrete evidence supporting the relationship with regard to the leaves of the genus is lacking [9].

Materials & methods

Total antioxidant activity

Total antioxidant activity was estimated by phosphomolybdenum assay [10]. 1ml each of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate were added in 20 ml of distilled water and made up volume to 50 ml by adding distilled water. All extracts of both plants in different concentration ranging from 200 µg to 1000 µg were added to each test tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. These tubes were kept incubated at 95°C for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm. Mean values from three independent samples were calculated for each extract. Ascorbic acid was used as positive reference standard.

Nitric oxide scavenging activity

$$\% \text{ DPPH radical scavenging} = \frac{[\text{OD of Control} - (\text{OD of Sample})]}{\text{OD of Control}} \times 100$$

The results were expressed as mean of three independent experiments in triplicates. The concentrations of the samples versus % DPPH radical scavenging activity were plotted for the IC₅₀ values.

Statistical analysis

The data acquired from in vitro analysis were communicated as mean standard error (±S.E.M.).

Results and Discussion

Nitric Oxide Scavenging Assay

All the polyherbal extracts exhibited a good NO scavenging activity in vitro and the scavenging activity was better than the positive control used. The hydroalcoholic extract of *E. officinalis* (*L.*) showed a concentration dependent NO scavenging that reached a peak of 74.15% at 1000 µg/ml and remained unaltered thereafter. The scavenging activity of *E. officinalis* (*L.*) was greater than that of the concurrent concentration of *T. bellerica* (*R.*) (Table 1 & Figure 1).

The procedure was based on the method, where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10mM) in phosphate buffered saline (PBS) was mixed with different concentrations of all extracts dissolved in methanol and incubated at room temperature for 150 minutes. The same reaction mixture without the extracts but the equivalent amount of methanol served as the control. After the incubation period, 0.5 ml of Greiss reagent [1% sulfanilamide, 2% H₃PO₄ and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride] was added. The absorbance of the chromophore formed was read at 546 nm using a spectrophotometer [11].

DPPH Assay

The free radical scavenging activity was assayed spectrophotometrically as per the method described by Blois and modified as per Nia [12]. Briefly, 9 X 10⁻⁵ M solution of DPPH in methanol was added 1:1 to the test samples. The stock concentration of 10mg/ml was diluted to different testing concentrations for each sample. The reaction was allowed to proceed at room temperature under dark conditions and the OD was read at 519nm after 30 minutes in the UV/Vis spectrophotometer (Cary UV 50). Ascorbic acid was used as the positive control. Appropriate blanks of the test samples were used. The control reaction was carried out with the solvent replacing the test sample. The calculation was as follows:

It is to be noted that *T. bellerica* (*R.*) extract have a significant inhibition comparative to other plant extract but less than ascorbic acid which has shown 97.23% inhibition of NO. The maximum NO scavenging of *T. bellerica* (*R.*) was 49% with IC₅₀ value 1037.5 µg/ml (Table 2) respectively.

The plant/plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in vivo [13]. Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and is involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases. NO is generated in biological tissues by specific nitric oxide synthesis (NOSs), which metabolizes arginine to citrulline with the formation of NO via a five electron oxidative reaction [14]. Nitric oxide is an essential bioregulatory molecule required for several physiological processes like neural signal transmission, immune response, control of vasodilation and control of blood pressure etc [15]. However, the elevation of the nitric oxide results in several pathological conditions including cancer. Moreover in the pathological conditions, nitric oxide

reacts with superoxide anion and form potentially cytotoxic molecules, peroxy nitrite. Nitric oxide inhibitors have been shown to have beneficial effects on some aspects of inflammation and tissue damage seen in inflammatory diseases. The level of nitric oxide was significantly reduced in this study by the crude extract. These observations further highlight the importance of plant extract in preventing physiological deleterious caused by NO radicals and O₂ radicals.

Total antioxidant assay

The present study was focused on identifying the most antioxidant extract of *Terminalia bellirica* (R.) and *E. officinalis* (L.) using a wide range of solvent systems. The total antioxidant activity was determined on the basis of the reduction of Mo (VI) to Mo (V) followed by the subsequent formation of green phosphate/Mo (V) complex [16]. All the extracts demonstrated good oxygen radical absorption capacity, IC₅₀ values are listed in Table 4. *E. officinalis* (L.) exhibited lower IC₅₀ values (534.7mg/mL) than the other species. *T. bellerica* (R.) had an IC₅₀ value of 1191 mg/mL, whereas positive control (ascorbic acid) had the lowest IC₅₀ value (528.9mg/mL) (Table 4). Therefore, the results attribute *T. bellerica* (R.) extracts with the highest oxygen radical absorption capacity (Table 3 Figure 2). The reducing power of extract of *T. bellerica* (R.) and *E. officinalis* (L.) was found remarkable and the reducing power of the extract was observed to rise as the concentration of the extract gradually increased. Earlier authors Sarwar have observed a direct correlation between antioxidant activity and reducing power of certain plant extracts [17]. The reducing properties are generally associated with the presence of reductones [18], which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [19]. Higher plants have the capacity to produce large number of organic phytochemical known as secondary metabolites. Phenolics are the good source of natural antioxidants and. *Terminalia bellerica* (R.) and *Embllica* fruit extracts showed higher phenolics and high antioxidant activity as compared to leaves. However, leaves showed higher amount of flavonoid as compared to that of fruits. Several studies have shown that the antioxidant potential is attributed to the polyphenolic compounds in medicinal plants [20]. Katalinic et

al., [21] Petridis et al., [22] showed strong relationship between total phenolic content and antioxidant activity, whereas Hazra et al., [23] showed strong correlation between flavonoid contents with its antioxidant capacity.

DPPH Antioxidant Assay

A freshly prepared DPPH solution exhibited a deep purple color with a maximum absorption at 517 nm. This purple color disappears when an antioxidant is present in the medium [24]. Figure 5 illustrates a significant decrease in the concentration of DPPH radicals due to the scavenging ability of *E. officinalis* (L.) extract. This activity was dose dependent. Maximum scavenging activity (75.93 %) was observed at 1000 µg/ml concentration and the IC₅₀ value of *E. officinalis* (L.) extract and ascorbic acid were found to be 523.7µg/ml and 446.4µg/ml respectively (Table 6). Sumalatha et al.,[25] reported the similar results about *Embllica* extract. They found the percentage decrease of DPPH standard solution 71.75% for *Phyllanthus emblica*. Singh et al.,[26] evaluated the Pharmaceutical Properties of *E. officinalis* (L.) and *Phyllanthus emblica* Extracts. The fruit extract of both *E. officinalis* (L.) and *Phyllanthus emblica* was proved to be more effective than the leaf extract.

The percentage radical scavenging activity of selected plants was measured using DPPH assay test and it ranged from 11.32 to 50.15%. It was significantly lower in extract of *T. bellerica* (R.). Hydroalcoholic solvent extracts of *Terminalia bellerica* Roxb. was assayed for their radical scavenging activity using the DPPH colorimetric test. Figure 3 & Table 5 shows the antioxidant activities of the two extracts compared to that of ascorbic acid. Concentration of sample at which the inhibition percentage reaches 50% is its IC₅₀ value. The lower the IC₅₀ value, the higher is the antioxidant activity of the tested sample. *T. bellerica* (R.) exhibited higher IC₅₀ value 1024.4 µg/ml. These results are in agreement with the previously reported data [27]. The obtained results reveal that there is a moderate correlation between the total phenols content and the antioxidant activities of these extracts [28]. In a related context, free radicals play a complex role in the inflammatory cascade [29], thus antioxidants would logically be implicated in an anti-inflammatory function [30].

Table 1: Percentage scavenging of nitric oxide radical through different extract

Concentration (µg/ml)	Ascorbic acid	<i>E. officinalis</i> (L.)	<i>T. bellerica</i> (R.)
200	38.73	32.25	37
400	53.71	45.36	38
600	68.34	60.71	45
800	82.75	72.54	46
1000	97.23	74.15	49

Table 2: IC50 value for nitric oxide scavenging assay

S. no.	Extract	IC50 value ((µg/ml))
1	Ascorbic acid	351.5
2	<i>E. officinalis</i> (L.)	478.1
3	<i>T. bellerica</i> (R.)	1037.5

Table 3: Total antioxidant assay of *E. officinalis* (L.) and *T. bellerica* (R.) extract

Concentration (µg/ml)	Ascorbic acid	<i>E. officinalis</i> (L.)	<i>T. bellerica</i> (R.)
200	28.73	12.25	37.25
400	43.71	25.36	38.17
600	58.34	30.71	55.5
800	62.75	32.54	66.11
1000	77.23	44.15	69.45

Table 4: IC50 value for total antioxidant assay

S. no.	Extract	IC50 value ((µg/ml))
1	Ascorbic acid	528.6
2	<i>E. officinalis</i> (L.)	534.7
3	<i>T. bellerica</i> (R.)	1191

Table 5: DPPH antioxidant assay of *E. officinalis* (L.) and *T. bellerica* (R.) extract with standard

Concentration (µg/ml)	Ascorbic acid	<i>E. officinalis</i> (L.)	<i>T. bellerica</i> (R.)
200	35.62	29.78	11.32
400	47.28	39.09	24.1
600	59.65	53.46	29.65
800	70.59	76.3	37.92
1000	79.35	75.93	50.15

Table 6: IC50 value for DPPH antioxidant assay

S. no.	Extract	IC50 value ((µg/ml))
1	Ascorbic acid	446.4
2	<i>E. officinalis</i> (L.)	523.7
3	<i>T. bellerica</i> (R.)	1024.4

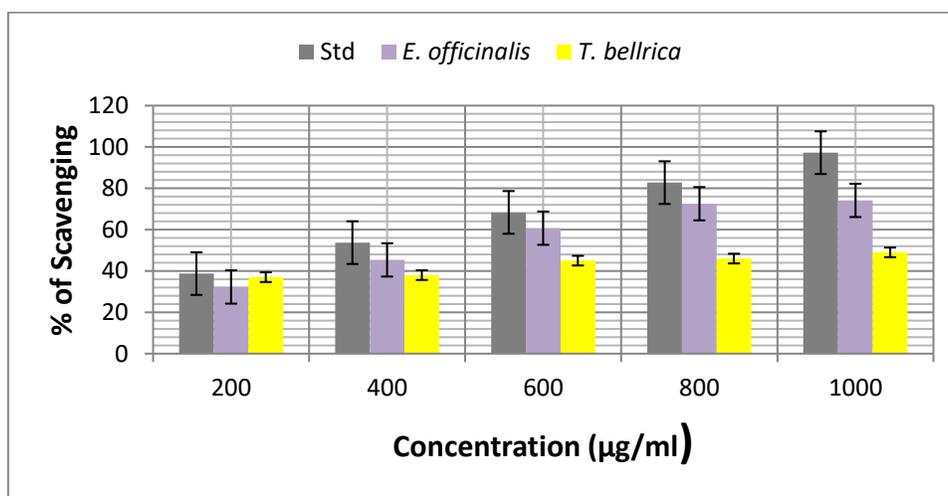


Figure 1: Nitric oxide scavenging potential of Ascorbic acid, *Emblica* and *Terminalia* extract

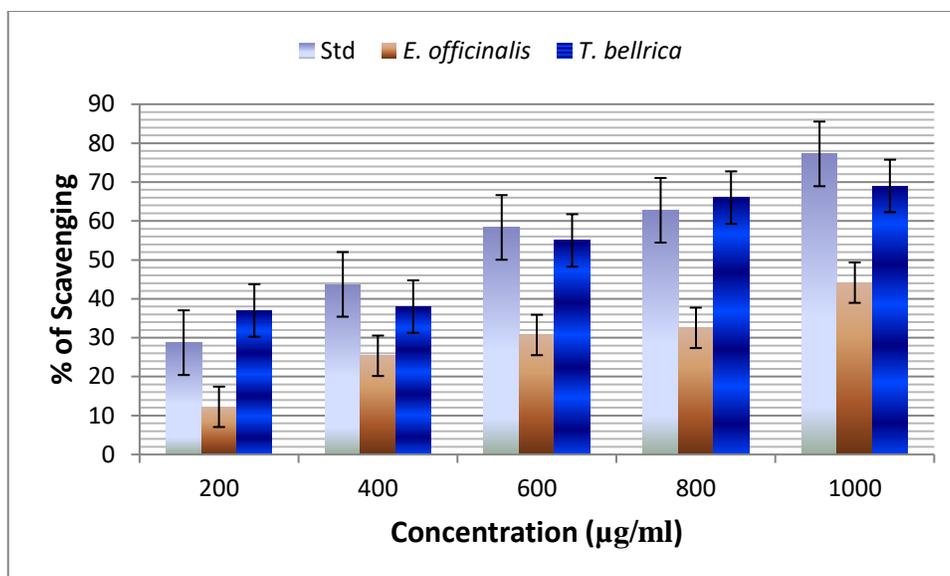


Figure 2: Total antioxidant assay of *E. officinalis* (L.) and *T. bellerica* (R.) extract

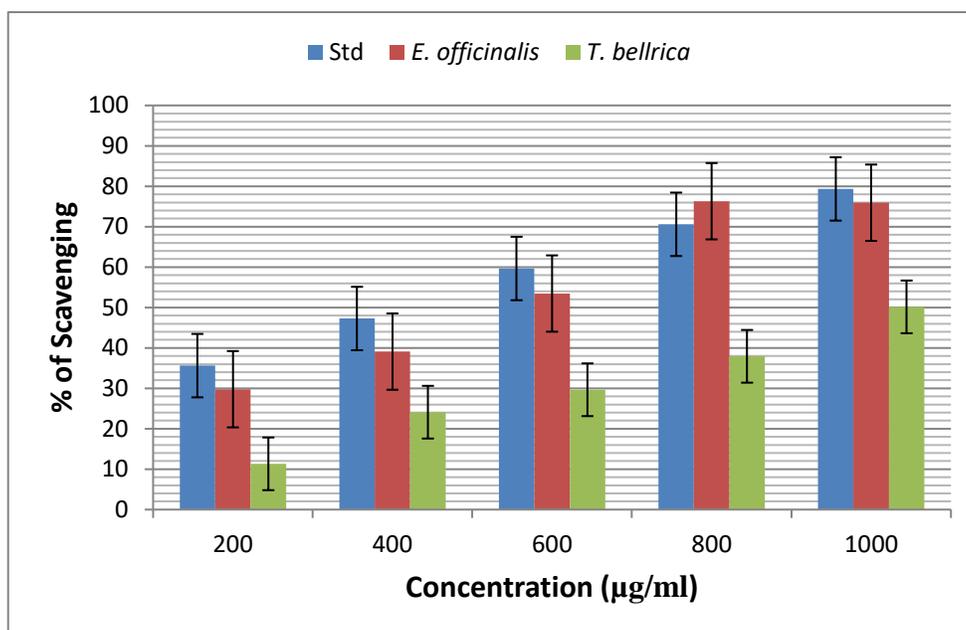


Figure 3: DPPH antioxidant assay of *E. officinalis* (L.) and *T. bellerica* (R.) extract with standard

Conclusion

This study showed that the extracts of *E. officinalis* (L.) contain high amount of phenol and flavonoid content than *T. bellerica* (R.) extracts. Due to the presence of polyphenols in extracts both plants are also found to have a significant antioxidant property, which can be industrially exploited to produce many useful pharmaceutical products to tackle the serious damaged caused by free radicals. The present study indicates that the extracts of the selected *Terminalia bellerica* (R.) and *E. officinalis* (L.) species possess antioxidant activities, which is probably due to their

phenolic groups, and brings new hope to research on the management of oxidative stress conditions. Hence it can be concluded that the hydroalcoholic extract showed strong antioxidant activity towards hydroxyl, DPPH and nitric oxide. Hence, the *Terminalia bellerica* (R.) was found to be a good antioxidant compound and the extract can be exploited in future for medicinal use. However future studies have to be carried out to evaluate the antioxidant potential of the extract in an *in vivo* system using animal models.

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