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# Isolation and quantification of active phytochemicals in hydroalcoholic extract of *Emblica officinalis* and *Terminalia bellerica*

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**Abstract**
*Terminalia bellerica* (Bahera) and *Emblica officnalis* (Amla) are well-known medicinal plants with enormous usage in Ayurvedic herbal formulation to treat various ailments like fever, diabetes and cancer. In the current study, we correlated the phenolic and flavonoids contents with antioxidant potential of the fruits of both species. Quantitative phytochemical analysis was performed to assess the total phenolic and flavonoids content in hydroalcoholic extract using Folin-Ciocalteau and aluminium chloride assay respectively. *E. officinalis* contained higher fraction of total phenol content as compared to the *T. bellerica*, i.e., 7.73 mg/g tannic acid equivalents and 2.43 mg/g tannic acid equivalents. However total flavonoids (TFC) appeared to be in similar frcations in both plant species. The study therefore suggested potentioal antioxidant potential for the plant species, keeping in the mind the fact that the fruits could be used as a source of natural antioxidant in food and pharmaceutical industry.

**Keywords:** antioxidant, ayurveda plants, total phenol content, *T. bellerica*, *E. officinalis*

## Introduction
Natural antioxidants are believed to possess antioxidant properties due to their reducing and chelating capabilities (Sowndhararajan and Kang, 2013) [6]. These include flavonoids and polyphenols, which are secondary plant metabolites and are widely distributed in fruits, leaves, bark with free radical scavenging abilities (Kumar and Pandey, 2013) [20]. The genus *Terminalia* (fam. Combretaceae), comprising 250 species, is distributed across tropical countries worldwide, The Indian traditional system of medicine (also known as Ayurveda) has established extensive literatures on the medicinal values of its species for instance, *Terminalia bellerica*, *T. chebula* and *T. arjuna* (Walia *et al*., 2011) [19]. It has been reported that these species are rich in flavonoids and polyphenols (Asha *et al*., 2012) [21]. 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 (Jeong *et al*., 2004) [11]. 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 (Devi, 2005) [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 (Mathen *et al*., 2014) [13].
In present work fruits, extracted in hydroalcohol of two plants**,** *E. officinalis* & *T. bellerica* were investigated for the presence of phenol content & flavonoid content in a comparative way.

## Materials and Methods
### Sample Collection
*Terminalia bellerica (R.)* fresh fruits and *E. officinalis (L.)* fruits *were collected from Ahuza spice store, Bhopal. Collected samples washed in tap water and disinfectant to remove impurities and shade dry to prepare powder.* These plant materials extract out with hydroalcohol (50:50) water and ethyl alcohol by hot extraction method. After that, the extracts were evaporated in water bath at 50°C to obtain crude for phytochemical analysis (Singh *et al.*, 2012) [16]

**Fig 1:** A *Terminalia bellerica* (R.) fruit powder (B) *E. officinalis* powder (L.)

### Determination total flavonoid content
The total flavonoid content (TFC) of the extracts was determined using the aluminium chloride assay through spectrophotometer (Samatha *et al*., 2012) [14]. An aliquot (0.5 ml) of extracts was taken in different test tubes, then 2 ml of

distilled water was added, followed by the addition of 0.15 ml of sodium nitrite (5% $NaNO_2$, w/v) and allowed to stand for 6 min. Later 0.15 ml of aluminium trichloride (10% $AlCl_3$) was added and incubated for 6 min, followed by the addition of 2 ml of sodium hydroxide (NaOH, 4% w/v) and volume was made upto the 5ml with distilled water. After 15 min of incubation the mixture turns to pink whose absorbance was measured at 510 nm using a spectrophotometer. Distilled water was used as blank. The TFC was expressed in mg of Quercetin equivalents (QE) per gram of extract. All the determinations were carried out three times.

**Determination of total phenolic content**

The total phenolic content (TPC) of the crude extracts of *E. officinalis (L.) (L.)* and *T. bellerica (R.) (R.)* plants were determined using the method of Singleton *et al*., (1999) with slight modifications. To 0.5 ml of test sample, 1.5 ml (1:10 v/v diluted with distilled water) Folin Ciocalteau reagent was added and allowed to stand for 5 min. After 5 min, 2.0ml of 7.5% of sodium carbonate was added. These mixtures were incubated for 90 min in the dark with intermittent shaking. After incubation, development of the blue colour was observed. Finally absorbance of blue colour in different samples was measured at 725 nm using spectrophotometer. The phenolic content was calculated as Tannic acid equivalents TA/g on the basis of standard curve of Tannic acid. The results were expressed as tannic acid equivalents TA/g of the plant material. All the determinations were carried out in triplicates.

**Results & Discussion**

The total phenolic content for hydroalcohol extracts of *E. officinalis (L.)* and *T. bellerica (R.) (R.)* was estimated by Folin Ciocalteu's method using tannic acid as standard Figure 1 (Guleria *et al*., 2016) [9]. Hydroalcoholic extract of *E. officinalis (L.) (L.)* possess higher amount of phenolics (7.73 mg/g Tannic acid equivalents) as compared to *T. bellerica (R.) (R.)* extract. Anbuselvi and Jha, 2015 reported the similar findings of *E. officinalis (L.) (L.)* extracts with the references of Total phenol. The high contents of phenolic compounds and significant linear correlation between the values of the concentration of phenolic compounds and antioxidant activity indicated that these compounds contribute to the strong antioxidant activity (Gupta *et al*., 2015) [10]. The total phenolic content was found to be 2.55 mg/g of extract in their study. Table 1showed that hydroalcoholic extract of *T. bellerica (R.) (R.)* had 2.43 mg TAE/g of phenolic content. In contrast to our study, Kathirvel *et al* (2012) found that methanolic extract of leaves of *T. bellerica (R.) (R.)* possess 7.62 mg/gm GAE of phenolic content. Total phenolic content of the extract of *T. bellerica (R.) (R.)* was calculated from standard curve of tannic acid (y=0.3x-2E-16, $R^2 = 1$. There was increase in total phenolics and flavonoids with increase in concentration of extracts (0.2-1 mg). In the previous study showed that methanolic extract of fruits of *T. bellerica (R.) (R.)* had higher amount of phenolic content as compared to that of leaves, however higher phenolic content was not always accomplished with high amount of flavonoids (Chandel *et al*., 2016) [5]. Phenolic compounds are known to be powerful chain breaking antioxidants and are important constituents of plants. Phenolic compounds may contribute directly to antioxidative action. It is suggested that phenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when ingested up to 1.0 gm daily from a diet rich in fruits and vegetables (Amir *et al*., 2011) [2].

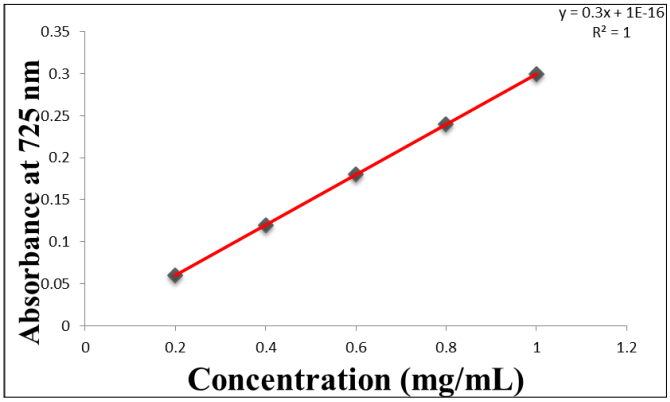


**Fig 2:** Standard graph of tannic acid for total phenol content

**Table 1:** Total phenol content in *E. officinalis (L.) (L.)* and *T. bellerica (R.) (R.)* extract

| S. No | Extract | Total Phenol (mg TA/g Extract) |
|---|---|---|
| 1 | *E. officinalis* (L.) | 7.73 |
| 2 | *T. bellerica* (R.) | 2.43 |

**Total flavonoid content**

Total flavonoid content expressed as quercetine equivalent (mg /g QE) in different extracts of *T. bellerica (R.)* and *E. officinalis (L.)* has been summarized in Table 2. For *T. bellerica (R.)* and *E. officinalis (L.)* extract, total flavonoid content was maximum in hydroalcohol extract of *E. officinalis (L.)* (0.457 mg g-1 QE). The quercetin solution of concentration (0.2-1 mg) conformed to Beer's Law at 510 nm with a regression co-efficient (R2 ) = 0.9901. The plot has a slope (m) = 3.37 and intercept = 0.1664. The equation of standard curve is y = 3.317x + 0.1664 (Figure 2). Agarwal *et al*., 2012 reported the extraction of polyphenol, flavonoid from *E. officinalis (L.), Citrus limon, Cucumis sativus* and evaluation of their antioxidant activity. All the three activities determined were found maximum in *E. officinalis (L.)*. Badoni *et al*., 2016 reported the maximum flavonoid content in methanolic extract of *E. officinalis (L.).*

The summary of the quantity of flavonoids detected in the tested extract is shown in Table 2. The concentration of flavonoid in *T. bellerica (R.)* extract was 0.436 mg QE/g extract. Chavan *et al*., 2010 reported the antioxidant availabiltiy of beheda (*Terminalia bellerica (Roxb.)*) in relation to its medicinal uses. Moreover, reports on medicinal plant extracts state that concentration of phytochemicals anchorage a positive correlation with antioxidant activity of extracts (Venkatesan *et al*., 2017) [18]. Present results clearly indicates, the presences of high quantity phytochemicals namely, phenols and flavonoids, are play important role in antioxidant potential of these extracts.

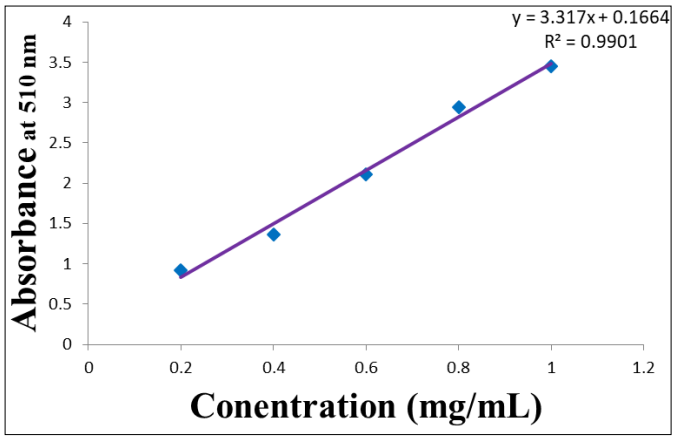


**Fig 2:** Standard graph of quercetin for total flavonoid content

**Table 2:** Total phenol content in *E. officinalis (L.)* and *T. bellerica (R.)* extract

| S. no. | Extract | Total Flavonoid (mg QE/g Extract) |
|---|---|---|
| 1 | *E. officinalis (L.)* | 0.457 |
| 2 | *T. bellerica (R.)* | 0.436 |

## 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

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