

# Comparative study of Ascorbic Acid content in *Aegle marmelos* & *Terminalia belerica* Fruit Pulp

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**ABSTRACT-** Indian Medicinal plants are considered a vast source of several pharmacologically active principles and compounds, which are commonly used in home remedies against multiple ailments. *Aegle marmelos* and *Terminalia belerica* are another Indian medicinal plants, which have enormous traditional values against various diseases and many bioactive compounds have been isolated from these plants. The aim of the current study is to investigate the ascorbic acid content in *Aegle marmelos* and *Terminalia belerica* fruit extract as nutritional value. *Terminalia belerica* is a very rich source of vitamin C shown in results, which indicates a higher nutritional importance of both plants in pharmaceutical field.

**KEYWORDS:** Ascorbic Acid, DNPH, HPLC, Antioxidant.

## INTRODUCTION

Plant and plant products are being used as a source of medicine since long time. Among the most important constituent of edible plant produce, low molecular weight antioxidants are the most important species. It is known that consumption of fruits and vegetables is essential for normal health of human being. Vegetarian diet can reduce the risk of cancer, atherosclerosis, *etc.*

*Aegle marmelos* (L.) Correa commonly known as Bael or Bilva belonging to the family Rutaceae has been widely used in indigenous systems of Indian medicine due to its various medicinal properties (Bose TK, et. al., 1985). *Aegle marmelos* (L.) tree is held sacred by Hindus and offered in prayers to deities Lord Shiva and Parvati and thus the tree is also known by the name Shivaduma (the tree of Shiva). *Terminalia belerica* also referred to as, Beleric Myrobalan 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.

Most of the antioxidant compounds in a typical balanced diet are derived from plant sources with a wide variety of biological and chemical properties (Scalbert et al., 2005). L-Ascorbic acid, also known as L-xylo-ascorbic acid, 3-oxo-L-gulofuranolactone (enol form), L-3-ketothreohexuronic acid lactone, anti-sorbic vitamin and vitamin C, has the chemical formula C<sub>6</sub>H<sub>8</sub>O<sub>6</sub> and a molecular weight of 176.12. This water-soluble vitamin is important in forming collagen, a protein that gives structure to bones, cartilage, muscle, and blood vessels. Ascorbic acid is widely distributed in nature, mostly rich in fresh fruits and leafy vegetables such as guava, mango, papaya, cabbage, mustard leaves and spinach (Tee *et al.*, 1997). Ascorbic acid is well known for its ability to scavenge free radicals and thereby reduce oxidative stress. Ascorbic acid is needed for various functions in the body, though its function at the cellular level remains unclear.

Ascorbic acid is needed for collagen synthesis, a production of certain hormones and of neurotransmitters, metabolism of some amino acids and vitamins, detoxification of toxic substances in the body, and proper function of the immune system. People with hypertension are at a high risk of developing cardiovascular diseases. The supplement of vitamin C intake helps in lowering the body's blood pressure. Vitamin C has effectively resulted in the proper dilation of blood vessels in the cases of atherosclerosis, congestive heart failure, high cholesterol, angina pectoris, and high blood pressure. Results have been found that supplements of vitamin C improve blood vessel dilation and protect cardiovascular health.

## **MATERIAL AND METHODS**

### **PLANT MATERIAL**

Aegle marmelos & Terminalia bellerica plant were collected from G.B. Pant University of Ag. & Technology, Pantnagar, U.K.

### **PREPARATION OF EXTRACTS**

100 gm of shade dried powdered plant material (fruit) was extracted successively using the Chloroform (60°C) and finally with methanol in a Soxhlet extractor. Each time before extracting with next solvent powdered was dried in an air oven below 50°C. Finally, marc was macerated with chloroform water for 24 hour to obtain the aqueous extract. The extract was concentrated by distilling off the solvent and then evaporating to dryness on a water-bath. The extract was weighed and its percentage was calculated in terms of air-dried weight of the plant material. The colour and consistency of the extract was noted. The colour and consistency of the extract was noted.

### **ESTIMATION OF ASCORBIC ACID (HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD)**

Standards - 98%, Sigma-Aldrich

Oxalic acid - 4% concentration in water

Thiourea - 10% concentration in water.

DNPH - 2% concentration was prepared by dissolving 2g of Di-nitro-phenyl-hydroxine (DNPH) in 100ml of 0.5N H<sub>2</sub>SO<sub>4</sub> and filtered.

H<sub>2</sub>SO<sub>4</sub> - 80% concentration in water.

Bromine water - Few drops of liquid bromine was dissolved in water.

### **CHROMATOGRAPHIC CONDITION**

Column – C18 3 µm, 3.0 × 150 mm

Column Temperature - 25 °C

Mobile Phases –

- A. 25 mM Phosphate buffer (dissolve 3.4 g KH<sub>2</sub>PO<sub>4</sub> in 1000 mL water, and adjust pH to 3.6 with H<sub>3</sub>PO<sub>4</sub>)
- B. CH<sub>3</sub>CN-mobile phase A (7:3, v/v)

Flow Rate –1 ml/min.

UV Detection – UV at variable wavelengths

Injection Volume – 10  $\mu$ L

### STANDARD PREPARATION

Ascorbic acid was dissolved Deionized water in a standard flask. Working standard was prepared by dissolving standard solution with Deionized water. The concentration was 100 $\mu$ g/g.

### SAMPLE PREPARATION

1g of dried fruit powder (sample) was ground well in a pestle and mortar with Deionized water. After 15 min of ultrasonic extraction, add water to the mark Known volume of (25ml). The above was changed to de-hydro form using the procedure adopted for working standard. Prior to injection, sample was filtered through a 0.2  $\mu$ m filter.

### METHOD PERFORMANCE (REPRODUCIBILITY, LINEARITY, AND DETECTION LIMITS)

The method reproducibility was estimated by making consecutive injections of standards, respectively. Excellent RSDs for retention time and peak area were obtained.

### RESULT AND DISCUSSION

The freshly prepared fruit extract of was qualitatively tested for the presence of ascorbic acid constituents by HPLC method and result shown in figure Maximum ascorbic acid (305.10 $\mu$ g/g) was recorded in Terminalia belerica fruit extract (Figure-1).

Except Aegle marmelos seeds everything is useful for consuming. Taking 5-6 leaves regularly keeps the body and mind healthy. Because of its medicinal qualities it cures a lot of ailments. That is why Aegle marmelos is also known as *Mahaphala* or Great fruit. Terminalia belerica is now known as the richest source of vitamin C of any fruit in the world, with levels over 900 times higher than the same weight of blueberries.

As the induction of oxidative stress vitamin C is known to be associated with some cancers, cardiovascular disease, neuro-degeneration disorders, diabetes and obesity, the high levels of ascorbic acid associated with Terminalia belerica fruit may also have beneficial health related bioactivities Brake, (1997). Phytochemical studies of the nutritional value of Terminalia belerica fruit have also shown it to also be high in other important polyphenolic antioxidants including Ellagic and Gallic acids.

Studies have also shown that increased consumption of vitamin C is connected with a decreased possibility of cancers of the lungs, mouth, vocal chords, throat, colon, rectum, stomach, and esophagus. In two rodent studies, addition of ascorbic acid to the diet reduced the size and number of dermal neoplasms and skin tumors induced by chronic UV exposure.

It is generally assumed that frequent consumption of plant-derived phytochemicals from vegetables, fruit, tea, and herbs may contribute to shift the balance toward an adequate antioxidant status. Thus interest in natural antioxidant, especially of plant origin, has greatly increased in recent years (Jayaprakash and Rao, 2000).

Ascorbic acid plays an important role as a component of enzymes involved in the synthesis of

collagen and carnitine; however, its most vital role is as a water-soluble vitamin in the human body (Sies & Stahl, 1995; Levine *et al.*, 1995).

The role of vitamin C in the prevention and control of disease states is encouraging, since it is a cheaper way of enhancing vitamin C status through natural fruits, instead of use of supplements that may be quite expensive.

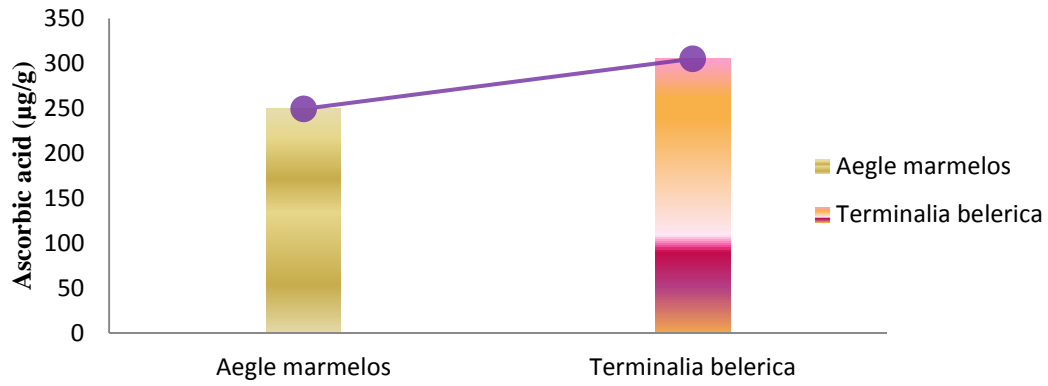
## CONCLUSION

A number of chemical constituents and various therapeutic effects of fruit of *Aegle marmelos* and *Terminalia bellerica* have been reported by different workers. The most important pharmacological activity of the fruit has been found to be its antioxidant activity. The diet formulated by including these powders can significantly decrease the incidence of environmental stress and increase the immune competence promoting growth and survival.

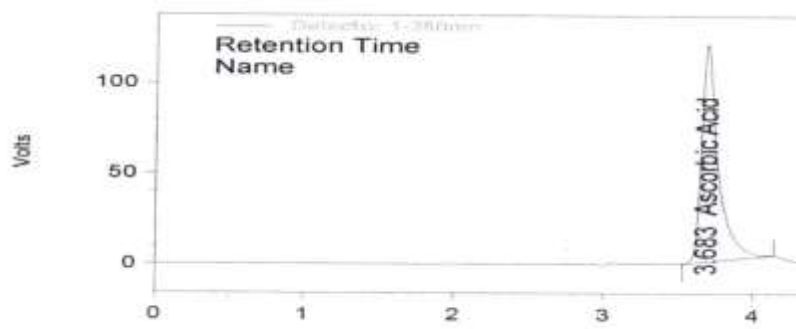
The understanding of vitamin C's antioxidant activity, and of its potential protective effects on tissues and different health outcomes, may also require further research, as well as whether a vitamin C intake beyond the PRI affects relevant biomarkers of oxidative damage to lipids, DNA and proteins in intervention studies, including an assessment of dose-response relationship where relevant.

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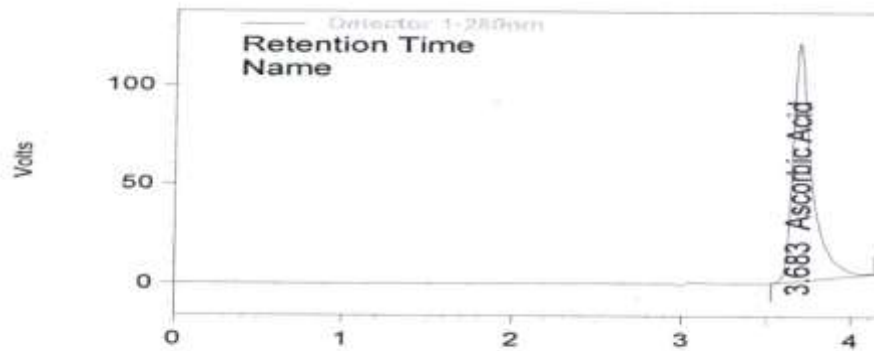
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**Fig 1. Ascorbic Acid content in fruit extract of Aegle marmelos and Terminalia belerica**



**Fig 2. HPLC CHROMATOGRAM OF AEGLE MARMELOS SAMPLE (FRUIT EXTRACT)**



**Fig. 3 HPLC CHROMATOGRAM OF TERMINALIA BELERICASAMPLE (FRUIT EXTRACT)**