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# Evaluation of total antioxidant potential of tamarind (*Tamarindus indica*) of different ripening stages

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**Abstract**
The present study disclosed about the difference between two different varieties of tamarind (*Tamarindus indica*) based upon their ripening stages, viz. brown (ripe) and green (unripe). This was achieved by performing a body of antioxidant and antimicrobial assays. Antioxidant assays performed were ABTS and DPPH radical scavenging assays, determination of total phenolic contents, FRAP assay and hydroxyl radical scavenging assay. It was observed that ripe tamarind was better than unripe variant as it neutralized 20 to 50% more oxidants in assays like DPPH, FRAP and total phenolic contents. However, the activity of green tamarind was 50% less in its' hydroxyl radical scavenging ability. The results indicated that ripe tamarind was better than the unripe variety regarding their physiological efficacies. Antimicrobial activities of the two variants were also tested against bacteria like *Bacillus cereus* (MTCC 1272), *Escherichia coli* (MTCC 1610), *Staphylococcus aureus* (MTCC 9542) and *Klebsiella aerogenes* (MTCC 9544). Results of this assay indicated that both the extracts were effective against all the four tested strains. Both the extracts showed profound effect against *Bacillus cereus*. Effect against *Staphylococcus aureus* was next on the list. The study indicated not only the comparative activities of ripe and unripe tamarind against free radicals responsible for aging, but also effective against food borne pathogens and should be incorporated in daily dishes.

**Keywords:** tamarind, *Tamarindus indica* antioxidant, antimicrobial, polyphenols

## Introduction

Recent trends showed that antioxidative and antimicrobial properties of many plant extracts are utilized greatly in both theoretical and practical standpoints of food science, since they can be utilized as natural supplements that can replace synthetic antioxidants. A growing trend in researches about medicinal plants is thus seen nowadays due to their abilities to cure many diseases, low costs and low side effects when compared to synthetic drugs. In recent times, there has been a fast increase in the standardization of medicinal plants with significant potential as therapeutics due to their specific remedial properties and prospective actions. Tamarind, commonly known as *Imli* in Hindi and referred as *Chincha* or *Amlika* in Ayurveda is one such plant (Panara *et al*., 2014) [23]. It is astonishing to know that every part of Tamarind (i.e. wood, root, leaves, bark and fruits) has nutritional as well as medicinal value, with a number of industrial and commercial applications like juice and jam making (De Caluwe *et al*., 2010) [11]. That is why, Tamarind has its' utility as a multipurpose tropical fruit, which are eaten fresh or processed, used as a seasoning or spice, or the fruits and seeds are processed for non-food uses (Anu *et al.*, 2014) [5].

Tamarind is an arboreal fruit of *Tamarindus indica* L. which belongs to family Leguminosae or Caesalpiniaceae. The tree is mostly found in the Asian countries like India, Thailand, Bangladesh, Sri Lanka and Indonesia. In America, Mexico and Costa Rica are the biggest producers (De Caluwe *et al*., 2010) [11]. In India the tree is particularly abundant in states of Madhya Pradesh, Bihar, Andhra Pradesh, Chhattisgarh, Karnataka, Tamilnadu and West Bengal (Muzaffar & Kumar 2017) [20]. Many parts of tamarind plant have long been used in traditional medicines for the treatment of a wide variety of disorders and diseases like jaundice and gastrointestinal disorders. A remedial drink prepared from the fruit pulp is used in febrile conditions, convalescence, bowel complaints, bilious disorders, dysentery and rheumatism. Pulp extract as juice is also administered to cure sunstroke, Datura poisoning and alcoholic intoxication (Adeola *et al.*, 2010) [1]. Apart from the fruits, other parts of the plant also show activities like - antibacterial, antifungal, anti-cholesterolemic, antihepatotoxic, antiinflammatory and antimutagenic effects (Khanzada *et al.*, 2008; Ramos *et al.*, 2003; Rimbau *et al.*, 1999) [16, 26, 27].

As natural products are replete with biochemicals of medicinal utilities, a thorough study of such pharmacophores are essential to adjudicate their wise use in human therapeutics. It is also understandable that expression of such biomolecules would be different in different maturity stages of the herb. This was deciphered in part in some previous researches where different expression of the bioactives was linked with their pharmacognostic activities (Balamurugan, 2014; Bhattacharjee *et al.*, 2015; Keles *et al.*, 2016; Lloki *et al.*, 2013; N'Dri *et al.*, 2010; Pramanik *et al.*, 2014) [6, 9, 15, 17, 21, 24]. In a previous study, different ripening stages of tamarind were also evaluated for their antioxidant and browning capacities, although antioxidant studies were performed with the phenolic-enriched portion of the fruits (Obulesu & Bhattacharya, 2011) [22]. The present study was designed to delineate total antioxidant capabilities of tamarind of two different maturity stages (viz. green and brown) using some common *in vitro* antioxidant assays. To our knowledge,

antioxidant activities of total tamarind fruit pulp of different ripening stages were evaluated for the first time in the present study. This would enable us to compare use of tamarind pulps of different maturity stages in cuisines judiciously.

## Materials and Methods

2,2′-Diphenyl-1-picryl hydrazyl (DPPH) were obtained from Himedia, India. Analytical grades of 2-Deoxy-D-ribose, thiobarbituric acid (TBA), ascorbic acid, gallic acid, Folin-Ciocalteau's solution, sodium hydroxide and sodium carbonate were obtained from Merck, India. 2,4,6-Tris(2-pyridyl)-*s*-triazine (TPTZ) was procured from SRL, India. All other chemicals used were of AR grade. Deionized distilled water was used in the entire study.

### Collection of samples and extraction

Fresh tamarind fruits were collected from local market of Barasat, Kolkata. The samples were checked for dirt or any visible damages prior to the study. Such samples were discarded. Pulps were separated and dried at 50±2ºC to a constant weight. About 5 gms of the pulp were suspended in 50 ml of 60% ethanol in water and extracted at 80±5ºC for 15 minutes. The solution was filtered and the filtrate was used for further studies.

### ABTS radical decolorization assay

The assay was performed using a previously described procedure (Chakraborty & Bhattacharyya, 2014) [10]. The oxidant was generated by persulfate oxidation of 2,2′-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid. The oxidant solution was mixed with the sample/standard solutions in such a way that total volume of the solution reached 1 ml. The absorbance at 734 nm in a Systronics spectrophotometer (model – 2202) was read at room temperature, 4 minutes after mixing. The results were expressed as Gallic acid equivalents (µg/gm pulp).

### DPPH radical decolorization assay

The DPPH assay was performed using a previously described procedure (Chakraborty & Bhattacharyya, 2014) [10]. 1 ml DPPH solution (3 mg in 25 ml ethanol) was mixed with 0.5 ml of sample or standard and the decrease in absorbance of the mixture after 20 minutes of incubation in the dark was monitored at 517 nm in a Systronics spectrophotometer (model – 2202). Gallic acid was used as positive control and the results were expressed as Gallic acid equivalents (µg/gm pulp).

### Estimation of total phenolic content

Total phenolic compound contents were determined by the Folin-Ciocalteau method (Sarkar *et al*., 2014) [28]. Sample or standards (0.5 ml) were mixed with Folin-Ciocalteau reagent (5 ml, of 1:10 diluted sample with distilled water) for 5 min and aqueous sodium carbonate (4 ml, 1 M) was then added. The absorbance of the reaction mixture was then measured at 765 nm with a UV-Vis spectrophotometer (model – Systronics 2202). Gallic acid was used as standard. The results were expressed in terms of mg gallic acid equivalent/gm pulp.

### Ferric reducing antioxidant potential: FRAP

Ferric reducing potentials of the samples were estimated with a previously established procedure (Aktar *et al*., 2016) [3]. Briefly, a maximum of 100 µl of sample or standard was mixed with FRAP reagent to a final volume of 2 ml and incubated at 37ºC for 30 mins. After the incubation, absorbance was measured at 593 nm in a UV-Vis spectrophotometer (model – Systronics 2202). Gallic acid is used as standard. Results are expressed as Gallic acid equivalents (GAE, µg/gm pulp).

### Hydroxyl radical scavenging assay

Hydroxyl radical scavenging potentials of the samples were estimated with a previously described procedure (Banik *et al*., 2017) [7]. Briefly, 10 mM each of $FeSO_4.7H_2O$, EDTA, 2-deoxy-D-ribose and $H_2O_2$ solutions were prepared in water. 0.2 ml each of above four and 0.2 ml sample and/or standard solution was mixed in a test tube and incubated at 37ºC for 90 mins. $H_2O_2$ solution was added last. After the incubation, 1 ml of 2.8% (w/v) aqueous TCA solution and 1 ml of 1% (w/v) aqueous TBA solution were added to the reaction mixture and kept at boiling water bath for 20 mins. Development of the pink chromophore was measured at 532 nm in a UV-Vis spectrophotometer (model-Systronics 2202). Gallic acid is used as standard. Results were expressed as Gallic acid equivalents (GAE) in terms of µg gallic acid equivalent/gm pulp.

### Estimation of ascorbic acid content

Estimation of ascorbic acid content of the samples was estimated with a previously described method (Bhattacharyya *et al*., 2016) [9]. Briefly, a maximum of 100 µl sample (or standard) was mixed with 400 µl 5% metaphosphoric acid solution. Then another 500 µl of 10% metaphosphoric acid solution was added followed by 300 µl of citrate buffer (pH 4.15) and 300 µl of 2,6-DCP-IP solution. Absorbance was read at 520 nm in a UV-Vis spectrophotometer (model – Systronics 2202) within 1 min.

### Antimicrobial activity

The antibacterial activity was measured by agar well diffusion method (Mitra *et al*., 2018) [19]. Each bacterial isolates was previously grown on sterile Muller Hinton Agar plate at 35°C for 24 hours. After that, each of the isolates was inoculated with 100µl of standardized inoculums of each bacterium (in quadruplicates) and spread with sterile cotton swabs. Wells are 6 mm sizes were made with sterile borer into agar plates containing the bacterial inoculums. Different working dilutions of the pulp extracts were prepared in sterile water. From these different dilutions, 50µl each sample was poured into the wells of the respective culture plates. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extract into the agar. After incubation for 24 hrs at 35ºC, the plates were observed. If antibacterial activity was present on the plates, it was indicated by an inhibition zone surrounding the well containing the different dilutions of extracts. The zone of inhibition was measured and expressed in millimeters. Antibacterial activity was recorded if the zone of inhibition was greater than 6 mm. The antibacterial activity results were

expressed in term of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially active; while13-18mm as active and >18mm as very active.

## Statistical analysis

Experimental results are expressed as mean ± SD of four individual samples. The statistical analysis was done by using the software 'SPSS Statistics 17.0' (IBM Corporation, USA).

## Results & Discussion

The two free radicals that are commonly used in *in vitro* antioxidant assay protocols are 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), ABTS, and 2,2′-Diphenyl-1-picryl hydrazyl (DPPH). DPPH and ABTS scavenging methods are the most commonly used ones to estimate the antioxidant activity of compounds since those are simple, rapid, sensitive and reproducible (Lu *et al*., 2010) [18]. However, these radicals are not of biological origin and hence cannot be directly correlated to physiological systems. The DPPH radical is capable of accepting an electron as well as a hydrogen as numerous data support that the latter mechanism is predominant (Ahmed *et al*., 2015) [2]. Since most of the plant derived biomolecules like flavonoids are good hydrogen atom donors, DPPH assay for natural products are more relevant to adjudicate their antioxidant potentials. This has been observed in earlier studies (Prior *et al*., 2005) [25]. On the other hand, ABTS method has the flexibility of use in different pH conditions and thus is useful for determination of antioxidant potential of plant extracts, where compounds with various proton donating capabilities are admixed. This method has an extra advantage as this radical is soluble in both aqueous and organic solvents and therefore, could be used for samples with different polarities (Shalaby & Shanab, 2013) [31]. Using these two methods, a preliminary idea of the nature of the tamarind extract has been obtained in the present study.

The present study indicated that ABTS radical scavenging abilities of the two varieties of tamarind were nearly at par (Fig. 1). Since ABTS assay system is based on electron transfer mechanism, it could be deduced that both the extracts contained compounds like phenolic acids and other carboxylic acids, which have good electron donating capabilities.

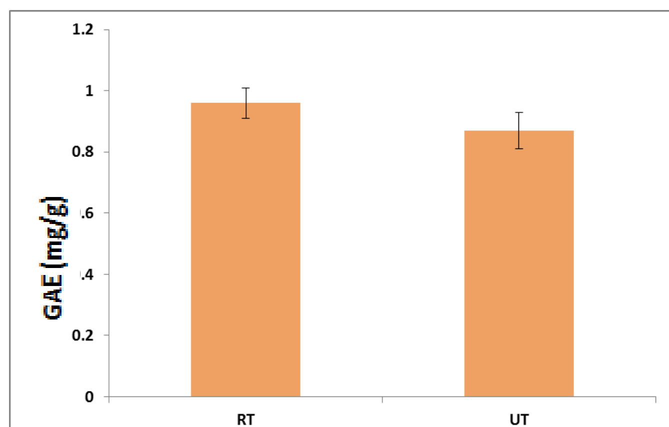


**Fig 1:** Comparative ABTS radical scavenging abilities of ripe and unripe pulps. Results (Mean±SD) are expressed as Gallic acid equivalents (GAE, mg/gm). RT – Ripe tamarind pulp extract, UT – unripe tamarind pulp extract.

However, in DPPH assay system, ripe tamarinds showed considerably better radical scavenging compared to unripe variants (Fig. 2). Since this system works better for hydrogen atom donating antioxidants, it can be concluded that ripe variants contain more of the hydrogen donating biomolecules, including flavonoids. The biomolecules acted better in less polar medium of DPPH assay indicating probable presence of less polar biomolecules in the ripe pulp.

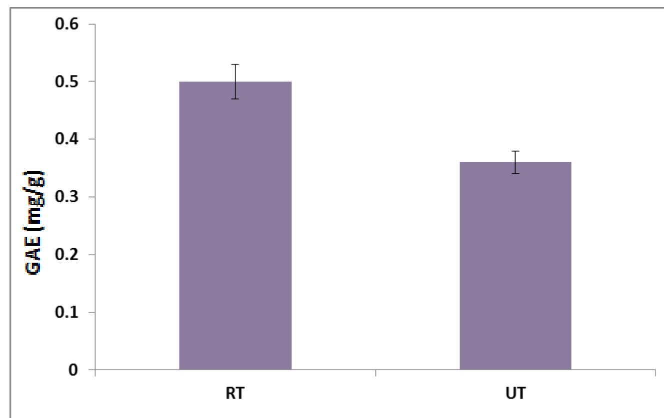


**Fig 2:** Comparative DPPH radical scavenging abilities of ripe and unripe tamarind pulps. Results (Mean±SD) are expressed as Gallic acid equivalents (GAE, mg/gm). RT – Ripe tamarind pulp extract, UT – unripe tamarind pulp extract.

Total polyphenolics content was greater in ripe variant (Fig. 3). Earlier studies reported that this content changed during ripening of fruits of *Rosa laevigata Michx* (Xie *et al.*, 2016) [32]. Under the alkaline reaction conditions, a phenol loses an $H^+$ ion to produce a phenolate anion, which reduces Folic-Ciocalteu reagent in the assay system. The result thus also indicated that ripe variant contains more polyphenolics than the unripe ones. As phenolics (including many flavonoids) contain less polar phenolic hydroxyl group/s, the result was thus commensurate with the results obtained from DPPH assay. Reports also indicated that a gradual decreased acidity with an increase in total soluble solids was also observed during ripening of *Eugenia jambolana* which imparted the characteristic flavour of the fruit (Balamurugan, 2014) [6].

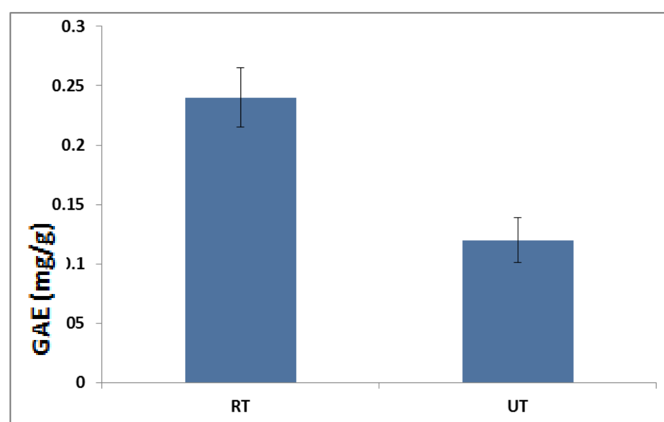


**Fig 3:** Comparative total phenolic contents of ripe and unripe tamarind pulps. Results (Mean±SD) are expressed as Gallic acid equivalents (GAE, mg/gm). RT – Ripe tamarind pulp extract, UT – unripe tamarind pulp extract.

Ferric reducing antioxidant capacities of the two extracts were given in Fig. 4. The results commensurate with the phenolic contents of the extracts, clearly indicating predominance of the electron donating antioxidants in the extracts.

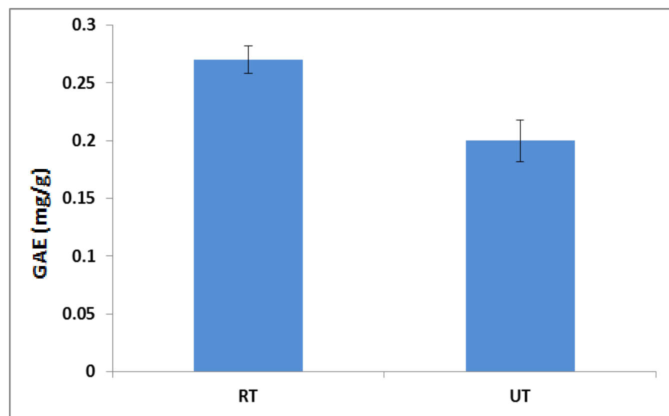


**Fig 4:** Comparative ferric reducing potential of ripe and unripe tamarind pulps. Results (Mean±SD) are expressed as Gallic acid equivalents (GAE, mg/gm). RT – Ripe tamarind pulp extract, UT – unripe tamarind pulp extract.

Vitamin C contents of the two variants were also at par (Fig. 5). Vitamin C is not one of the most important bioactives present in tamarind pulp. It might form by the conversion of reducing sugars in the fruits. However, during maturity, conversion rate might not be that high which produced fair amount of vitamin C after ripening. In fact, most of the reducing sugars were used to produce Maillard reaction products during ripening, as was reported in the earlier studies (Obulesu & Bhattacharya, 2011) [22]. This was one of the reasons of darkening of the fruits during maturation.

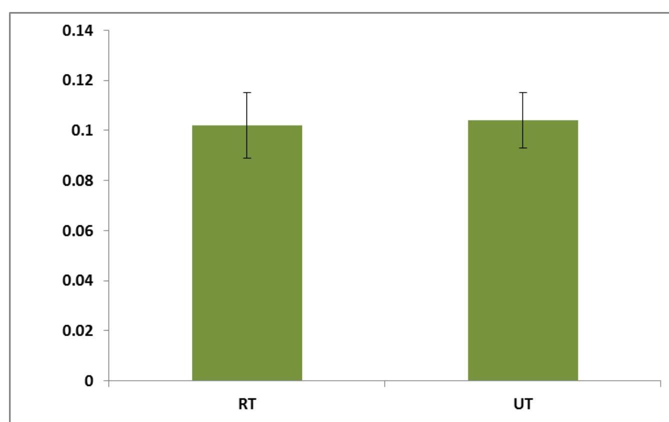


**Fig 5:** Comparative Vitamin C contents of ripe and unripe tamarind pulps. Results (Mean±SD) are expressed in mg/gm. RT – Ripe tamarind pulp extract, UT – unripe tamarind pulp extract.

Natural product extracts with potential hydroxyl radical scavenging ability is the most sought after problem in the field of pharmacognosy. Among all the oxygen-centered free radicals, hydroxyl radical is the most reactive chemical species known. Biomolecules such as proteins, DNA and other nucleic acids are prone to hydroxyl radical induced oxidative damage (Hussein, 2011) [14]. Hydroxyl radical was generated *in vitro* by Fenton reaction, which degrades deoxy-ribose to produce malonedialdehyde (MDA) (Gayathri *et al.*, 2014) [12]. Even $Fe^{2+}$-ascorbic acid mixture is known to stimulate lipid peroxidation in animal liver *in vitro* by producing deleterious hydroxyl radicals (Anu, 2014) [4]. Production of this radical subsequently might produce $H_2O_2$, which is also toxic due to its ability to penetrate into biological membranes (Sasikumar & Kalaisezhiyen, 2014) [29]. In the present study, it can be observed that unripe pulp was better hydroxyl radical scavenger than the other (Fig. 6). Ripening of the fruits induces formation of Maillard reaction products (Obulesu & Bhattacharya, 2011) [22]. This might be one of the reasons of lowering of hydroxyl radical scavenging potential of the ripe pulp.

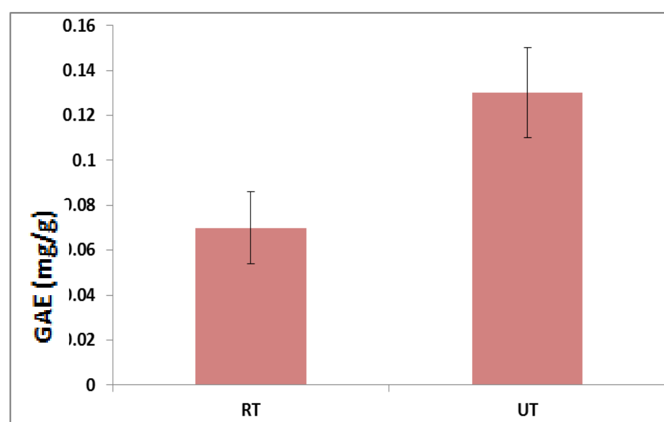


**Fig 6:** Comparative hydroxyl radical scavenging potential of ripe and unripe tamarind pulps. Results (Mean±SD) are expressed as Gallic acid equivalents (GAE, mg/gm). RT – Ripe tamarind pulp extract, UT – unripe tamarind pulp extract.

Antimicrobial activities of the aquo-alcoholic extracts of tamarind pulps were furnished in the antibiogram of Table 1. The results indicated that the extracts effectively inhibited the growth and multiplication of the tested bacterial strains - *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, and *Klebsiella aerogenes.* Between two variants of the tamarind, unripe one was most effective against *Bacillus cereus* and *Staphylococcus aureus* as was observed by their zone of inhibition at the concentration level of 200□g/ml. The extracts were almost equi-effective against the other two tested strains. *Bacillus cereus* causes various types of food poisoning that include diarrheal syndromes, and a variety of local and systemic infections (Schoeni & Wong, 2005) [30]. On the other hand, staphylococcal food poisoning (SFP) is one of the most common food-borne diseases in the world caused by ingestion of staphylococcal enterotoxins (SEs) that are produced by coagulase-positive staphylococci (CPS), mainly *Staphylococcus aureus* (Henekkine *et al.*, 2012) [13]. Since food is an important factor for development of antimicrobial resistance, cuisines prepared with unripe tamarind pulps or its extract could lower prevalence of infections caused by the above two strains effectively.

**Table 1:** Bacteriostatic activities of ripe and unripe tamarind pulp extracts against common food borne bacteria. RT – Ripe tamarind pulp extract, UT – unripe tamarind pulp extract. Values are given as Mean±SD.

| Concentration (µg/ml) | Sample | Mean diameter of zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | *E.coli* | *B.cereus* | *S.aureus* | *K.aerogenes* |
| 200 | RT | 14±0.3 | 17±0.3 | 16±0.5 | 13±0.5 |
| | UT | 14±0.6 | 24±0.5 | 18±0.3 | 12±0.4 |
| 100 | RT | 13±0.3 | 16±0.4 | 14±0.4 | 10±0.4 |
| | UT | 10±0.5 | 21±0.5 | 16±0.4 | 10±0.4 |
| 50 | RT | 10±0.2 | 11±0.4 | 12±0.7 | 8±0.4 |
| | UT | 9±0.2 | 19±0.6 | 15±0.6 | - |
| 25 | RT | - | - | 9±0.3 | - |
| | UT | - | 13±0.6 | 9±0.6 | - |

## Conclusion

The present study lent credence to the fact that unripe tamarind might be a better choice over its' ripe variant due to better hydroxyl radical scavenging property of its' aquo-alcoholic extract. *In vitro* radical scavenging activities indicated that the ripe variant contained higher proportions of less polar antioxidative bioactives with better electron donating capabilities. Unripe tamarind extract was also highly effective against two most deadly food borne pathogens - *Bacillus cereus* and *Staphylococcus aureus*, as was observed by their zone of inhibition at the concentration level of 200g/ml. Since food is an important factor for development of antimicrobial resistance, cuisines prepared with unripe tamarind pulps or its extract could lower prevalence of infections caused by the above two strains effectively.

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