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## Evaluation of antioxidant property of West Coast Tall variety tender coconut water and synthetic Trans – Zeatin – An *in-vitro* study

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**Abstract**

**Background:** Coconut (*Cocos nucifera* L.) has been described as the most important and extensively grown palm tree worldwide and had been widely used for human consumption in tropical countries because of its nutritional properties and low cost. Various studies had proved *Cocos nucifera* water was found to be effective in maintain electrolytic balance in body. trans-Zeatin on other hand is a cytokine abundantly found in TCW had shown significant anti-ageing, anti-carcinogenic, and anti-thrombotic effects.

**Materials and methods:** Phytochemical analysis, Total phenolic content, Free Radical Scavenging by DPPH Assay and ABTS assay, FRAP Assay and Total antioxidant capacity of west coast tall variety TCW (West Coast Tall Variety) 3 to 4 months old .was done using standard methods.

**Results and Conclusion:** Phytochemical analysis of West coast tall variety TCW (TCW) showed the presence of Carbohydrates, Proteins, Flavonoids, Terpenoids, Glycosides, Phenolic Compounds and Steroids. *In vitro* study also proved that the TCW is a rich source of antioxidants, both TCW and synthetic trans-Zeatin exhibits antioxidant activity by exhibiting its free radical scavenging potential. TCW is a rich source of phenolic compounds. The study concludes that the effective free radical scavenging ability of TCW might be attributed due the presence of phenolic compounds.

**Keywords:** Phytochemicals, Phenolic compounds, Free radicals, trans-Zeatin, Tender Coconut water

**1. Introduction**

Coconut (*Cocos nucifera* L.) has been described as the most important and extensively grown palm tree all over the world. It belongs to the family *Arecaceae*. Traditionally TCW was used as an emergency glucose supply in absence of sterile glucose. It was used as intravenous fluid to counteract the effect of drug overdose, poisoning and adverse drug reaction, extensively used as the refreshment drink after physical exercise [1]. Till date it has been known that TCW(TCW) is a good source of various biomolecules like Sugar, Vitamins, Electrolytes, Plant hormones like Zeatin, Kinetin etc., [2] Various studies have proved that the cytokines present in tender coconut, in addition to various plant-related functions also showed significant anti-ageing, anti-carcinogenic, and anti-thrombotic effects [3,4]. It has been reported that the coconut water is useful in conditions like digestive disturbances, kidney stones, urinary tract infections, and sterility [4].

Zeatin, one of the important class of the phytohormone present abundantly in the TCW has been extensively used in plant tissue culturing. It has also been proved to be a potential agent for the management of skin photo aging [5]. Studies suggests that zeatin might be useful for protecting cognitive dysfunction, as well as for reducing the activation of Acetyl Choline Esterase in dementia [6].

Free radicals are known to play a definite role in a wide variety of pathological manifestations of pain, inflammation, cancer, diabetes, alzheimer, hepatic damage etc. Antioxidants fight against these free radicals either by Scavenging the reactive oxygen species or protecting the defense mechanisms [6] and protect us from various diseases [7].

Various studies have been done to evaluate the composition of coconut water, but less research has been done to know the composition of Tender Coconut in costal belt of Karnataka. Therefore the current study aimed to evaluate the phytochemicals components present in TCW and the *in-vitro* Further no significant work is done to evaluate the properties of trans-Zeatin which is the important phytohormone present in TCW. Antioxidant properties of both TCW

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and trans-Zeatin which provides a solid base for further studies.

## 2. Materials and Methods

The experiment was carried out in Nitte University Center for Science Education and Research, K. S. Hegde Medical Academy, Deralakatte, Mangalore.

### 3. Selection criteria for TCW

Fresh tender coconut (West Coast Tall Variety) of 3 to 4 months were obtained from the local market, Mangalore. Care was taken to avoid coconut with solid endosperm (coconut meat or copra).

#### A. Phytochemical analysis

Test for various components like Carbohydrates, Amino acids, Lipids, Alkaloids, Tannins, Saponins, Flavonoids, Terpinoids, Glycosides, Steroids/Quinones and Phenols were carried out in fresh TCW to identify various constituents using standard methods of Sofowora, Trease and Evans and Harbone with slight modifications [8].

#### B. Test for total Phenolic content of TCW

The entire experiment was carried out using Maurya S *et al.*, [9] method with slight modification. The amount of total phenolics in extracts was determined with the Folin-Ciocalteu reagent taking Ellagic Acid as a standard and the total phenolics were expressed as mg/g Ellagic Acid equivalents (EAE)

#### C. Evaluation of Antioxidant properties

##### Sample preparation

Three different dilutions of TCW (1:10, 6:10, Undiluted) and three concentrations of trans-Zeatin (0.1, 0.05, 0.25 mg/ml) purchased from Richchem Internationals Enterprise Co., Ltd. was used for the present study. For each sample 2 duplicates were kept for the present study.

##### i. DPPH Assay of TCW

The assay was done according to the Brand-Williams *et al.* (1995) [10] with some modifications. The Decrease in the absorbance was measured at 515 nm after 30 minutes using the spectrophotometer. A control having only Methanolic DPPH was maintained. The percentage of inhibition was calculated from the following equation,

$$\% \text{ Inhibition} = \left[ \frac{(\text{absorbance of control} - \text{absorbance of test sample})}{\text{absorbance of control}} \right] \times 100$$

##### ii. Estimation of Free Radical Scavenging potential by ABTS Assay

For ABTS assay, the procedure followed the method of Arnao *et al.* (2001) [11] with some modifications. The absorbance was taken at 734 nm using the spectrophotometer. The percentage of inhibition was calculated from the following equation,

$$\% \text{ Inhibition} = \left[ \frac{(\text{absorbance of control} - \text{absorbance of test sample})}{\text{absorbance of control}} \right] \times 100$$

##### iii. Determination of FRAP – ferric reducing power [12]

FRAP is a simple direct test of capacity. FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored FeII-tripyridyltriazine compound from colorless oxidized FeIII form by the action of electron donating. The absorbance was obtained at 593 nm. The EC 50 values were then calculated.

##### iv. Estimation of the Total Antioxidant Capacity by Phosphomolybdenum method [13].

This quantitative assay is based on the conversion of Molybdenum (Mo VI) by reducing agents like to molybdenum (Mo V), which further reacts with phosphate under acidic pH resulting in the formation of a green coloured complex, the intensity of which can be read spectrophotometrically at 695 nm and results were expressed in mM/L equivalent to Ascorbic acid.

## 4. Results

### A. Phytochemical analysis

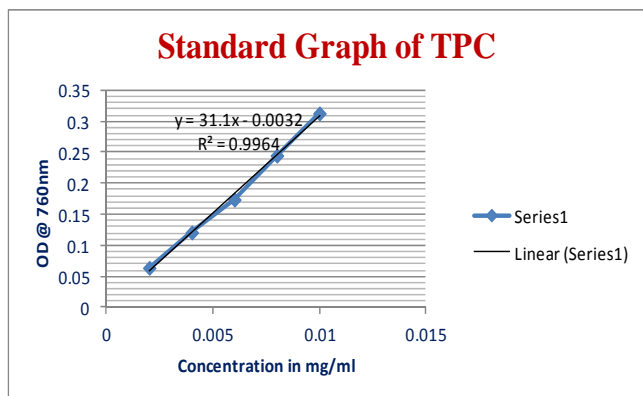
Table 1: Results of Phytochemical Analysis

Test	Result
<b>Test for carbohydrate</b>	
I. Fehlings Test	+
II. Benedict's Test	+
III. Iodine Test	-
<b>Test for Proteins</b>	
I. Millons Test	-
II. Biuret Test	-
<b>Test for Lipids</b>	
Saponification Test	-
<b>Test for Alkaloid</b>	
I. Mayers Test	-
II. Wagner's Test	-
III. Dragendroff Test	-
<b>Test for Tannins</b>	-
<b>Test for Saponins</b>	-
<b>Test for Flavonoids</b>	+
<b>Test for Terpenoids</b>	+
<b>Tests for glycosides</b>	
I. Borntrager's Test	+
II. Keller- Killiani Test	+
<b>Tests for steroids/quinons</b>	+
<b>Test for Phenols</b>	+

Note: “-” symbol represents absence of component where as “+” symbol represents presence of compound.

According to the results obtained, TCW is found to be a rich source of Carbohydrates, Proteins, Flavonoids, Terpinoids, Glycosides, Steroids and Phenolic Compounds.

**B. Test for total Phenolic content of TCW**



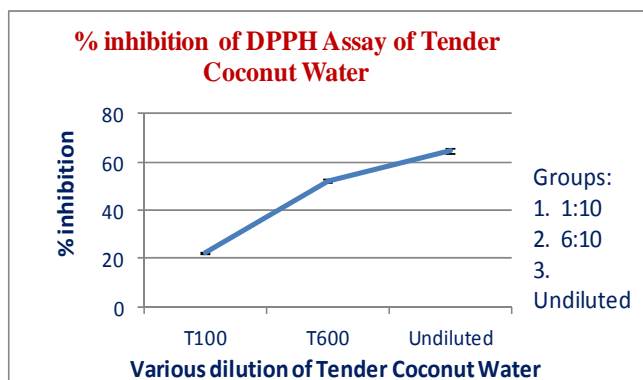
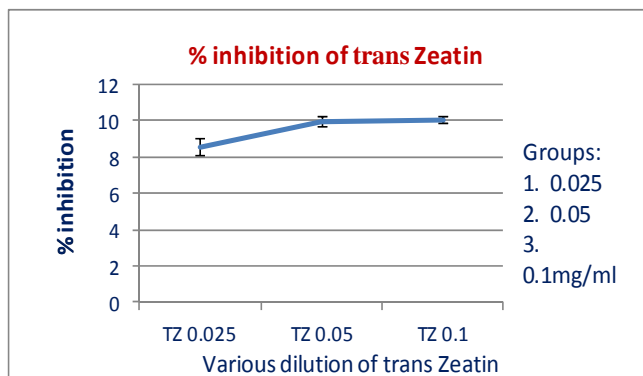
**Fig 1:** Standard graph of Ellagic acid

1:1 diluted TCW Showed  $0.827 \pm 0.0014$  mg equivalent to Ellagic acid indicating TCW is rich with phenol contents.

**C. Evaluation of *in-vitro* antioxidant properties**

**i. DPPH Assay of TCW**

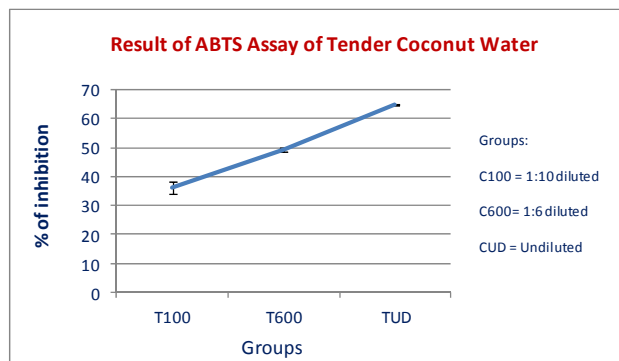
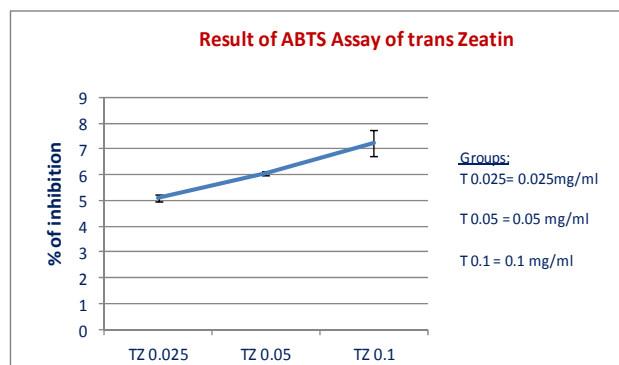
Undiluted TCW showed very good i.e., 64.53% inhibition for the free radical formation whereas 0.1 mg/ml trans-Zeatin showed 10% inhibition for the free radical formation.



**Fig 2:** Graphical representation of % inhibition of DPPH Assay

**ii. Estimation of Free Radical Scavenging potential by ABTS Assay**

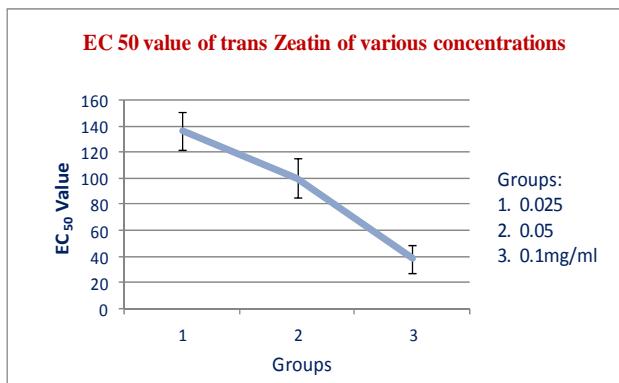
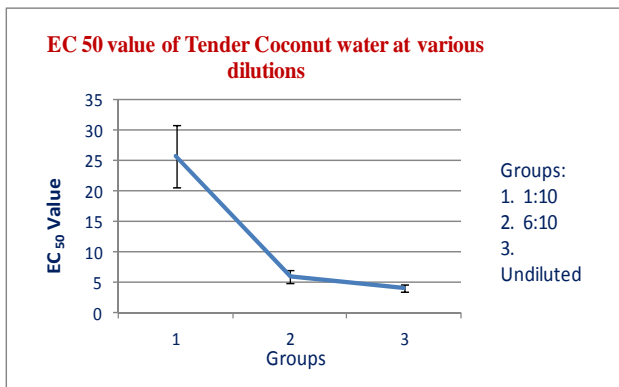
Undiluted TCW showed very good i.e., 65% inhibition for the free radical formation whereas 0.1 mg/ml trans-Zeatin showed 7.3% inhibition for the free radical formation.



**Fig 3:** Graphical representation of % inhibition of ABTS Assay

**iii. Determination of FRAP – ferric reducing power**

Undiluted TCW showed EC 50 value of 3.9 whereas 0.1 mg/ml trans-Zeatin showed EC 50 value of 38.23.



**Fig 4:** Graphical representation of % inhibition of FRAP Assay

#### iv. Estimation of the Total Antioxidant Capacity by Phosphomolybdenum method

Undiluted TCW showed  $1.11 \pm 0.035$  mM/L equivalent to Ascorbic acid whereas 0.1 mg/ml trans-Zeatin showed  $0.093 \pm 0.003$  mM/L equivalent to Ascorbic acid.

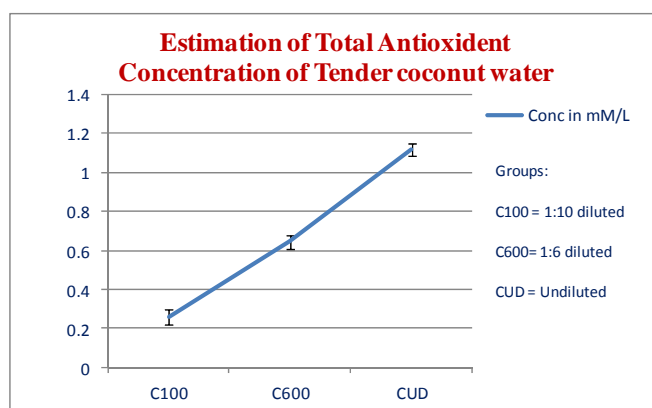
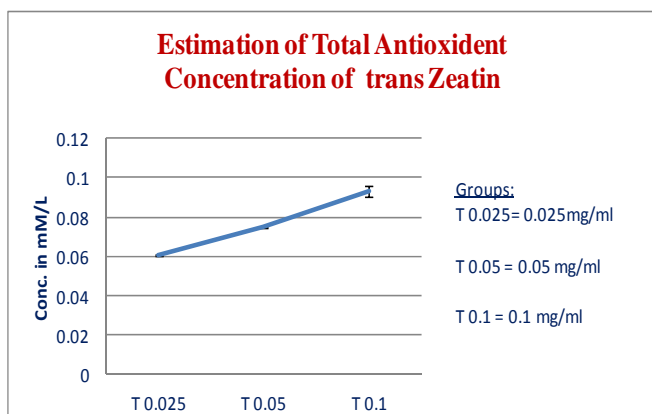


Fig 5: Graphical representation of results of Total Antioxidant Capacity

#### 5. Discussion

The current study was done to evaluate various phytochemicals present in the TCW and also to evaluate the in-vitro antioxidant properties of both TCW and trans-Zeatin using standard assays like DPPH, ABTS scavenging assays, FRAP and Total antioxidant capacity assays.

The important bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. They are organic substances and could be obtained in both primary and secondary metabolic processes. They also provide a source of medicine since the earliest time [14, 15].

Tender *Cocos nucifera* L. (Palmacea) water, variety Chandrasankara, was tested for its ability to scavenge free radicals, inhibit lipid peroxidation and protect hemoglobin from nitrite-induced oxidation. The activity was most significant for fresh samples of CW and diminished significantly upon heat, acid or alkali treatment or dialysis. Maturity of coconut drastically decreased the scavenging ability of CW against DPPH, ABTS and superoxide radicals [16].

According to a study conducted by Anders Brathe *et al* [17] the 6-aminopurines BAP, kinetin and trans-Zeatin were not at all able to scavenge DPPH at 667mM concentration. None of

the synthetic cytokines analogues was highly active DPPH scavengers, but several compounds were significantly more active than BAP, kinetin and trans-Zeatin.

Present study was carried out using fresh Tender coconut (West Coast Tall Variety) aged 3 to 4 months. Phytochemical analysis of TCW showed the presence of Carbohydrates, Proteins, Flavonoids, Terpenoids, Glycosides, Phenolic Compounds and Steroids. The total Phenolic contents of TCW was estimated. Quantitative assay for Total phenolic content showed that TCW (1:1 diluted) had  $0.827 \pm 0.0014$  mg equivalent to Ellagic Acid. According to the *in-vitro* antioxidant studies, it is found that undiluted TCW shows 64.3% inhibition of DPPH, 65% inhibition of ABTS free radical formation, FRAP value of 3.9 and total antioxidant capacity was found to be  $1.11 \pm 0.035$  mM/L equivalent to Ascorbic acid. 0.1 mg/ml trans-Zeatin showed 10% inhibition of DPPH, 7.3% inhibition of ABTS free radical formation and FRAP value of 38.23 and total antioxidant capacity was found to be  $0.093 \pm 0.003$  mM/L equivalent to Ascorbic acid.

#### 6. Acknowledgement

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