Antioxidant Activity in Aqueous Extracts of *Terminalia chebula* Stored for Six Months at Room Temperature and At 4°c Using 1, 1- Diphenyl-2-Picrylhydrazyl (Dpph) Assay

Thilageswary Kumutharanjan[a], Ramaiyah Sivakanesan[b], Vasanthy Arasaratnam[c]
[a]*Unit of Siddha Medicine, University of Jaffna,* [b]*Department of Biochemistry, Faculty of Medicine, University of Peradeniya,* [c]*Department of Biochemistry, Faculty of Medicine, University of Jaffna*

Abstract - The objective of this study was to quantitative the Antioxidant activity of the skin of the seeds of *Terminalia chebula (T. chebula).* The cold and hot extracts were obtained from the powder of skin of the seeds of *T. chebula* stored at room temperature and at 4° C in monthly interval for six months. The initial Total Antioxidant Capacity (TAC) of cold and hot water extracts were 8.98, 4.0µg/ml dry weights respectively. When the powder was stored at room temperature for a month and the TAC was analyzed, the cold and hot water extracts contained 18.28, 14.20 µg/ml dry weight respectively.When the skin of the *T. chebula* seed powder was stored at room temperature for 6 months, TAC of cold and hot aqueous extracts were 230.6, 204.10 µg/ml dry weights respectively, While the TAC of cold and hot aqueous extracts of the skin of the seeds powder stored at 4°C for six months respectively were 206.1, 177.0µg/ml dry weight. Extraction of antioxidant activity was better with hot water than with cold aqueous. TAC of the skin of the seeds powder decreased when stored both at room temperature and at 4°C. At 3rd month the decline in TAC of the powder stored at Room temperature was higher than that stored at 4°C. In Siddha Medicine the life span of 'Chooranam' which is prepared from herbs is used for 3 months. However based on these results researchers recommend that freshly prepared powder should be used for the preparation of the 'Chooranam'.

Keywords - DPPH (1, 1-Diphyenyl 1-2- Picryl Hydrazyl radical Scavenging Assay), siddha medicine, *terminalia chebula,* total antioxidant capacity,

I. Introduction

Small amount of Reactive Oxygen Species (ROS), such as 0_2, H_20_2, OH, are constantly generated in aerobic organisms as a consequence of aerobic respiration and substrate oxidation [1];[2]. ROS are involved in cell growth, differentiation, progression and death [3]. Free radicals are highly reactive and unstable compounds produce in the body during the normal metabolic functions or introduced from the external environment such as pollution and cigarette smoke. Human bodies are protected from oxidative damage of free radicals through some complex defense systems which are called antioxidants [4]. Oxidative stress in cells and tissues results from the increased generation of reactive oxygen species and / or from decreases in antioxidant defense potential antioxidants works to maintain the oxidant at optimum level and to reduce free radical before disturbs living cells in our body. Plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones [5]. Indigenous system of medicine which is the oldest health system in the world appreciates and uses *T. chebula* to treat a host of diseases and promote positive health. It is extensively used as a rejuvenator in Siddha, Ayurveddha and Unani. *T. chebula* is commonly called as black myrobalam, ink tree. It belongs to the family of compretacea. In English it is called as Chebulic myrobalan. In Tamil it is called as Kadukkaai, In Sinhala it is called as Aralu. The Objective of this study was to determine the antioxidant capacity. Of aqueous extracts of *T. chebula at* different storage condition. It posses anti diabetic activity [6]and It posses the antioxidant and oxygen species scavenging properties in the extracts of the fruits of *T. chebula* [7] it is recommended for the treatment of Diabetes mellitus, gastric ulcer, constipation, arthritis, cancer, skin diseases and anti-ageing [8]. It has been reported that *T. chebula* has antioxidant status in the liver and kidney of young and aged rats [9]. Agasthiyar's verse about *T. chebula* is "Mother flourishes the child by feeding food but *T. chebula* flourishes the child by relieving them from the diseases". Therefore *T. chebula* has been given more significance than the mother. It has been reported to possess anti diabetic [10] and antibacterial [11] agents. It is used to prevent aging and impart longevity and Immunity [8]; [12]. Reported that has an antioxidant activity.

II. Materials And Methods

Collection of Plant material

Thirty Matured fruits of the *T. chebula* were collected from *Meesalai* area of Jaffna peninsula.

Preparation of plant extract

Skin of the Seeds of the *T. chebula* were cleaned, washed and dried under shade at room temperature. Then these were powered and sieved with muslin cloth and stored in airtight container. 3 mg quantities packets were kept in the refrigerator and at room temperature. The cold and hot aqueous extracts using stored powder were prepared at monthly interval. Dissolved in 20ml distilled water and one part was kept in room temperature, other part was kept in water bath at 100°C for 5 minutes. Then these were centrifuged at 10,000 rpm for 10 minutes. Supernatant was taken from the centrifuged extract.

Estimation of DPPH Radical Scavenging activity

The Free Radical Scavenging of plant extracts evaluated by DPPH assay according to the procedure described by Blois (1958). Briefly, 0.1mM solution of DPPH in methanol prepared and 500µL of this solution added to 1 mL of extract containing 0.1, 0.2, 0.3, 0.4, 0.5 mg/ml. The mixture allowed standing at room temperature for 30 minutes. Then the absorbance measured at 517nm with UV- Vis spectrophotometer. In order to measure the absorbance of the control, 500 µL of DPPH mixed with 1 mL of distilled water and then the absorbance was taken as in the case of samples.

III. Results And Discussion

Inhibition concentration 50% (IC 50) values are inversely proportional to the antioxidant activity. Initially the Total Anti oxidant Capacity of the Skin of the Seeds of the *T. chebula powder* of cold and hot extracts were 8.98, 4.0 µg/ml dry weight respectively (Table 1). The TAC was better extracted with hot aqueous than with cold aqueous and hence when compared with the cold extracts, the hot extracts contained higher TAC (Table 1).

When the powder was stored at room temperature for a month and the TAC was analyzed, the cold and hot aqueous extracts contained 18.28, 14.20 µg/ml dry weight respectively. The skin of the seeds powder stored at room temperature for six months showed TAC of 230.6, 204.10 µg/ml dry weights respectively in cold and hot aqueous extracts. The skin of the seeds powder stored at 4°C for six months showed TAC of 206.1, 177 µg/ml dry weights respectively in cold and hot aqueous extracts. With time, the TAC of the skin of the seeds powder stored at room temperature decreased (Table2)

When the powder was stored at 4°C for a month and the TAC was analyzed, the cold and hot aqueous extracts contained 14.8, 8.10 µg/ml dry weight respectively (Table 1). When compared with the cold extracts, the hot extracts contained higher TAC than cold extract (Table 1).The skin of the seeds powder stored at 4°C for six months showed TAC of 206.1, 177.0µg/ml dry weights respectively in cold and hot aqueous extracts. With time, the TAC of the skin of the seeds powder stored at 4°C decreased (Table 2).

The skin of the seeds powder stored at room temperature for three months showed TAC of 52.6, 43.7 µg/ml dry weights respectively in cold and hot aqueous extracts. The skin of the seeds powder stored at 4°C for six months showed TAC of 41.7, 29.7 µg/ml dry weights respectively in cold and hot water extracts. With time, the TAC of the skin of the seeds powder stored at room temperature decreased (Table 2)

The cold and hot aqueous extracts of the dried powder of the skin of the seeds of *T. chebula* possess antioxidant activity. When compared with the cold extracts of *T. chebula* powder with hot extracts, hot extracts contained higher antioxidant activity than cold extracts. Antioxidant activity was higher at 4°C than stored at Room temperature. Antioxidant activity decreased according to the month of storage, when stored at either room temperature or 4°C. But decreasing activity is higher at room temperature than 4°C (Table 2).

IV. Conclusion

This study showed that the *T. chebula* powder could be used for '*Chooranam*' preparation immediately after the preparation of the *T. chebula* powder. Under emergency situations, the powder stored for three months at room temperature could be used, but not the powder preparation stored for more than 3 months.

Table 1: Total Antioxidant activity by 1, 1-Diphyenyl 1-2- Picryl hydrazyl radical Scavenging Assay (DPPH) of cold and hot water extracts of the Terminalia chebula seed skin powder stored at Room Temperature and at $4^{o}C$

Time (Month)	1-Diphyenyl 1-2- Picryl hydrazyl radical Scavenging Assay (DPPH) µg/ml			
	Stored at Room Temperature		Stored at $4^{o}C$	
	Cold extract	Hot extract	Cold extract	Hot extract
0	8.98	4.0		
1	18.28	14.2	14.5	8.1
2	30.9	22.5	24.3	15.5
3	52.6	41.7	41.7	29.7
4	101.5	85.8	83.9	67.2
5	161.7	141.6	141.8	118.8
6	230.6	204.1	206.1	177.2

Table 2

Time (Months)	Decrease, 1-Diphyenyl 1-2- Picryl hydrazyl radical Scavenging Assay(DPPH) of *Terminalia chebula* seed skin powder of the Antioxidant Power (µmol/g)							
	Stored at Room Temperature				Stored at $4^{o}C$			
	Cold water extract		Hot water extract		Cold water extract		Hot water extract	
	Loss	Cumulative loss	Loss	Cumulative loss	Loss	Cumulative loss	Loss	Cumula-tive loss
1	9.3	9.3	5.2	5.2	5.5	5.5	4.1	4.1
2	12.6	21.9	8.3	13.5	9.8	15.3	7.4	11.5
3	21.7	43.6	19.2	32.7	17.4	32.7	14.2	25.7
4	48.9	92.5	44.1	76.8	42.2	74.9	37.5	63.2
5	60.2	152.7	55.8	132.6	57.9	132.8	51.6	114.8
6	68.9	221.6	62.5	195.1	64.3	197.1	58.4	173.2

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