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TOTAL PHENOLIC CONTENT OF'MATHUMEHA CHOORANAM' STORED FOR SIX MONTHS AT 4^{O}C AND ROOM TEMPERATURE

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ABSTRACT

Siddha system is the ancient traditional system of Medicine helping to bring good health and cure diseases. 'Mathumeha chooranam'is used in the treatment of Mathumeham (Diabeticmellitus). It is prepared from the leaves of *Gymnema sylvestre*, skin of the seeds of *Terminalia chebula*, fruit of *Phyllanthus emblica* and leaves of *Murrya keonigii*respectively in 0.5:1:1:1 ratio. Numerous studies have indicated that the herbal medicines have antioxidant properties and provide protection against oxidative stress induced diseases and disorders. Hence this study was initiated to evaluate the antioxidant activity of the aqueous extracts of 'Mathumeha chooranam'in terms of TotalPhenolic Content (TPC).The initial TPC of cold and hot water extracts of'Mathumeha chooranam' was 194.3&200.1µg TAE/g dry weight respectively. When 'Mathumeha chooranam' was stored at room temperature for 6 months, TPC of cold and hot water extracts was104.8&127.4µg TAE/g dry weight respectively while the TPC of cold and hot water extracts of that stored at 4^{o}C for six months was 118.5&133 µg TAE/g dry weight respectively. Extraction of TPC was better with hot water than with cold water. TPC of'Mathumeha chooranam' decreased when stored both at room temperature and at 4^{o}C. Both cold as well as hot water extracts exhibited antioxidant activity even after storing for 6 months. At 3 months the decline in the TPC of the powder stored at Room temperature is higher than that stored at 4^{o}C. In the Siddha Medicine the lifespan of 'Chooranam' which is prepared from herbsis used for 3 months. Hence 'Mathumeha chooranam' can be stored for 3 months and used for medicinal purpose.

KEY WORDS:Antioxidant: Diabetic mellitus,'Mathumeha chooranam', SiddhaMedicine, TotalPhenolic Content

INTRODUCTION

Plants used in traditional medicine usually constitute biologically active compounds. Numerous useful drugs have been discovered from higher plants by following their medicinaluses (Fabricant *et al*., 2001). Medicinal plants play a key role in the human health care. About 80% of the world populations rely on the use of traditional

medicine which is predominantly base on plant materials (W.H.O., 1993).

Diabetes mellitus has been shown to be a state of increased free radical formation. Oxidative stress may increase in diabetes owing to a higher production of reactive oxygen species as well as due to the deficiency of antioxidant defense systems (Bayane and Thorpe, 1999). Thus the antioxidant actions are key to prevent and its complications (DefFronzo, 1999). The symptoms of diabetes mellitus are correlated with 'Mathumeham'.In Siddha system of Medicine varies 'chooranams' are used to treat 'Mathumeham' and 'Mathumeha chooranam' is widely used in Siddha hospitals and Dispensaries in the Northern and Eastern parts of Sri Lanka. 'Mathumeha chooranam' is prepared from the leaves of *Gymnema sylvestre*, skin of the seeds of *Terminalia chebula*, fruit of *Phyllanthus emelica*, and leaves of *Murrya keonigii* in 0.5:1:1:1 ratio. Hence a study was initiated to evaluate the antioxidant activity in the aqueous extracts of 'Mathumeha chooranam'in terms ofTotal PhenolicContent (TPC).

Gymnema sylvestre is a herb, native to the tropical forests of southern & central India and Sri Lanka. It has been used to treat the diabetes mellitus for nearly two millennia (Gurmar, 2011).It belongs to the family of Asclepiadaceous. In Tamil it is called as 'Chakkaraikolli' or Sirukurinja', in English 'small Indian epecacuanha'and in Sinhala 'Bin nuga'. *Gymnema sylvestre* is said topossessinsulinotrophic activity of human islets of Langerhans (Liu, 2009) and regenerates the islets of Langehans in streptozotocin induceddiabetic rats (Shanmugasundram *et al*., 1990).

Phyllanthus emblica belongs to the family of Euphorbiaceae. In Tamil it is called 'Peru nelli', in English it is called as 'Indian gooseberry', and in Sinhala called as 'nelli'.It has an antioxidant property (Chakarabarty *et al*., 1997). Another study with alloxan – induced rats given *P.emblica* extract has shown significant decrease of the blood glucose, as well as triglyceridemic levels and an improvement of liver function (Qureshi *et al*., 2009). It is believed that the major constituent responsible for these activities is vitamin C (ascorbic acid). Ascorbic acid shows antioxidant, anti inflammatory and anti mutagenic properties (Levine, 1986; Shah *and Bhattachariya*, 1982). *P.emblica* has been reported to contain antioxidant activity in scavenging superoxide anions from the system (Rao*et al*., 2005; Naik *et al*., 2005; Bhattacharya *et al*., 2002; Anila and Vijayalakshmi, 2002; Scartezzini and Speroni,2000; Maulik *et al*., 1996&1997). Other*in vivo* studies show that the antioxidant activities of amla cannot be due to ascorbic acid and that the overall effect is due to other polyphenols such as ellagic acid, gallic acid, tannins, etc. (Bhattacharya*etal*., 1999; Ihantola-Vormish*et al*., 1997; Santos *et al*., 1999). Amla was established to be a potent scavenger of free radicals, superoxide dismutase (SOD) (Ghosal*et al*., 1996).

Terminilia chebula belongs to the family of Compretacea.In English it is called as Chebulic myrobalan,in Tamil 'Kadukkaai', in Sinhala 'Aralu'. It was reported to posses anti diabetic (Kumar*et al*., 2006)and antioxidant activity (Chia-Lin and Lin, 2010) and reactive oxygen species scavenging properties (Hazra*et al*., 2010).

Murrya keonigii belongs to the family of Rutaceae. In English it is called as curry leaves, in Tamil 'Karivepillai' and in Sinhala it is called as 'curryppincha'. It reported that *Murrya keonigii* posses antidiabetic activity (Tembhurne and Sakarkar, 2009a), hypocholestrolemic property (Tembhurne and Sakarkar, 2009a & b), increaseing the gastrointestinal motility (Tembhurne and Sakarkar, 2009c & 2010) andantioxidant properties due to the presence of carbozole alkaloids (Iyer and Uma, 2008; Chakarbarty *et al*., 1997; Tachibana *et al*., 2003).

Usually 'Mathumeha chooranam' is stored for 3 months. The aim of this study was to find whether the total antioxidant activity of 'Mathumeha chooranam' is lost with the storage period of6 months and its hot water and cold water extracts were analysed for total phenolic content (TPC).

MATERIALS AND METHODS

Plant material

Leaves of *Gymnema sylvestre*, leaves *Murrya keonigii*, seeds of the *Terminalia chebula* and fruits of *Phyllanthus emblica* were collected from Karaveddy and Meesalai of Jaffna peninsula.

Preparation of plant extract

Leaves of *Gymnema sylvestre*, leaves *Murrya keonigii*, seeds of the *Terminalia chebula* and fruit of *Phyllanthus emblica* were cleaned, washed and dried under shade at room temperature for 10 days. The individual parts were powered and sieved with muslin cloth and stored in airtight containers either at room temperature or at 4^{o}C. 'Matumeha chooranam' was prepared from the powders by mixing in 0.5:1:1:1 ratio respectively. 'Mathumeha chooranam' (5mg) of was mixed with 10ml of distilled water and one part was kept at room temperature and the other part was kept in a water bath at 100^{o}C for 5 minutes. Then these were centrifuged at 10,000 rpm for 10 minutes. Supernatants wereused for the analysis of the Total Phenolic Content (TPC was determined using Folin-Ciocalteu reagent as an oxidizing agent and tannic acid as standard monthly intervals for six month (Mc Donald *et al*., 2001).

RESULTS AND DISCUSSION

Several years of experience has shown that of 'Mathumeha chooranam'has hypoglycemic effect on "Mathumeham' patients. The 'choranam' is prepared by the Siddha native physicians and administered to the of 'Mathumeham' patients 5g twice a day.as a dose. During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen

species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation (Aragno, *et al*., 1999; Bonnefont Rousselot,*et al*., 2000; Robertson, 2004). The levels of the antioxidants critically influence the susceptibility of various tissues to oxidative stress and are associated with the development of complications in diabetes. This is particularly dangerous to the beta islets, which is among those tissues which have the lowest levels of intrinsic antioxidant defenses (Grodsky, *et al*., 1982; Lenzen, *et al*., 1996; Robertson, 2004; West, 2000). There is believe in the Siddha medicine that *Gymnema sylvestre* and other ingredients in the 'Mathumeha chooranam' can regenerate the beta islets (Liu, *et al*., 2008; Shanmugasundaram *et al*., 1990; Abdul *et al*., 2014). Oral administration of *Gymnema sylvestre*increased the number of pancreatic islets and beta cells (Kaczmar, 1998).Ethanol extract of the fruits of *Terminelia chebula* has shown significant morphological changes in the mitochondria and endoplasmic reticulum of beta cells (Kumar *et al*., 2006). Further one of the ingredients of *Phyllanthus emblica* Linn has anti-inflammatory effect (Jaijoy *et al*., 2010).

Initially the Total Phenolic Content of 'Mathumeha chooranam'in cold and hot extracts was194.3 and 200.1µg TAE/g dry weight respectively (Table 1). The TPC was better extracted with hot water than with cold water and hence when compared with the cold extracts, the hot extracts contained higher TPC (Table 1). Initially the extraction of TPC with hot water was (1.03 times) more than that obtained with cold water. TPC extraction from*Murrya keonigii*was better with ethanol: water (1:1) at ambient temperature than with other organic solvents (Sasitharan and Menon, 2011).TPC extraction from *Murrya keonigii* leaf powderwas also found to be better with hot water was (1.11 times) more than that with cold water (Kumutharanjan, *et al*., 2015).

Measurement of Total Antioxidant activity by FRAP method

The FRAP (Ferric reducing antioxidant power assay) procedure described by Benzie and Strain was followed (Benzie and Strain, 1999).

RESULTS AND DISCUSSION

Among ingredients of the Mathumeha chooranam, *T.chebula* showed highest TAC in µmol/mg dry weight in cold as well as in hot extracts (8602.6)(9191.1) µmol/mg at first day. And lowest TAC in µmol/mg dry weight in cold as well as in hot extracts of *G.sylvestrae* (81.32), (94.48) µmol/mg at first day. (Table 3) The TAC of Mathumeha chooranam (2424.8) (2867.3) µmol/mg at first day. After 6 month storage The TAC *T.chebula* in cold as well as in hot extracts (5152.8)(6128.1) µmol/mg at room temperature. And at 4^{o}C the values were (5688.3) (6568.8). µmol/mg (Table3). TAC of *G.sylvestrae* in cold as well as in hot extracts were (55.47) (72.27) µmol/mg at room temperature. And at 4^{o}C the values were (60.96) (76.4) µmol/mg (Table3). The values in descending order of Antioxidant activity was skin of the seeds of the *Terminalia chebula*, Fruit of the *Phyllanthus embelica*,

Mathumeha chooranam Leaves of the *Murrya koenigii,* and *Gymnema sylvestrae.* The cold and hot aqueous extracts of the dried powder of the Mathumeha chooranam and its ingredients possess Antioxidant capacity. Among the four various ingredients of the Mathumeha chooranam *T.chebula* ,*Phyllanthus embelica* were found to possess higher amount of Antioxidant capacity than other ingredients. Antioxidant activity of ingredients Mathumeha chooranam and compound medicine decreased when stored at either room temperature or at 4^oC,

Determination of Antioxidant Activity

Total Anti oxidant Activity was determined based on Ferric .Reduction method by using. A spectrophotometric method (Yildirm, *et al*., 2001) was used for the measurement of reducing power, at monthly intervals for six month

RESULTS AND DISCUSSION

Highest (lowest EC 50 values(reverse order of antioxidant activity) and Lowest antioxidant (Highest EC 50 value) were observed in skin of the seeds of the *Terminalia chebula* (17.7) (14.4) µg/ml and *Gymnema sylvestrae* (581),(527) µg/ml on first day (Table 7) and When the powders were stored at room temperature for a month and the TAC were analysed, the cold and hot water extracts contained (34.4),(29.75) µg/ml dry weight respectively and *Gymnema sylvestrae* (942.5),(496.8) µg/ml dry weight cold as well as hot extract(Table 7). The powders stored at room temperature for six months showed TAC of *Terminalia chebula* was (87.34),(72.88) µg/ml dry weight respectively in cold and hot water extracts and *Gymnema sylvestrae.(*3358.6),(2658.3) µg/ml dry weight respectively in cold and hot water extracts(Table7) The powders stored at 4^oC for six months showed TAC of *Terminalia chebula* was (30.71),(23.7)µg/ml dry weights respectively in cold and hot water extracts. and *Gymnema sylvestrae* (3017.9),(2717.9) µg/ml dry weight cold as well as hot extract(Table 7). *Terminalia chebula* was found to possess higher amount of antioxidant capacity than other ingredients. The values in descending order of Antioxidant activity was skin of the seeds of the *Terminalia chebula,* Fruit of the *Phyllanthus embelica,* Mathumeha chooranam, *Leaves of the Murrya keonigii, and Gymnema sylvestrae*. Antioxidant activity was higher at 4^o C than stored at Room temperature. Mathuheha chooranam possess antioxidant activity. When compared with the cold extracts of mathumeha chooranam and its ingredients with hot extracts, hot extracts contained higher antioxidant activity than cold extracts. Antioxidant activity was higher at 4^o C than stored at Room temperature. Among the ingredients of Mathumeha chooranam *Terminalia chebula, Phyllanthus embelica* showed the highest antioxidant activity compared to the other plant parts.(*Murrya keonigii, and Gymnema sylvestrae)*

Total phenolics content

TPC was determined spectrophotometrically (Mc Donald *et al*.2001),

RESULTS AND DISCUSSION

TPC content of 'Mathumeha chooranam' when stored at room temperature

When the 'Mathumeha chooranam'was stored at room temperature for a month and the TPC was analysed; the cold and hot water extracts contained 188&197.5 µg TAE/g dry weight respectively. When the *Murrya keonigii* leaf powderwas stored at room temperature for a month and the TPC was analysed; the cold and hot water extracts contained 21.01& 23.53µg TAE/g dry weight respectively (Kumutharanjan, *et al*., 2015). As the 'Mathumeha chooranam'contains the*Murrya keonigii* leaf powder as one of the ingredients, the loss of TPC in 'Mathumeha chooranam'was 6.3 µg TAE/g could be due to the loss of TPC content of 1.59 µg TAE/g of the*Murrya keonigii* leaf powder.

'Mathumeha chooranam' stored at room temperature for three months showed TPC of 166.5 and 179.2 µg TAE/g dry weight respectively when extracted with cold and hot water(Table 1). After three months of storage at room temperature, the loss of TPC observed in cold-water extract and hot water extract was 27.8 and 20.9 µg TAE/g dry weight respectively and the TPCs retained were 85.74 and 89.56% of the initial TPC content respectively (Table 2). With time, the TPC of 'Mathumeha chooranam' stored at room temperature decreased. *Murrya keonigii* leaf powder stored at room temperature for three months showed TPC of 16.9 and 19.95 µg TAE/g dry weight respectively when extracted with cold and hot water, which were 78.45 and 9.55% of the initial TPC content respectively (Kumutharanjan, *et al*., 2015).

'Mathumeha chooranam' stored at room temperature for six months showed TPC of 104.8 and 127.4 µg TAE/g dry weight respectively when extracted with cold and hot water(Table 1). After six months of storage at room temperature, the loss of TPC observed in cold-water extract and hot water extract was89.5 and 72.7 µg TAE/g dry weights respectively and was 45.9 and 36.33% of the initial TPC content respectively (Table 2). With time, the TPC of the 'Mathumeha chooranam' stored at room temperature decreased. *Murrya keonigii* leaf powder stored at room temperature for six months showed TPC of 9.02 and 12.9 µg TAE/g dry weight respectively when extracted with cold and hot water and hence the TPCs retained were 39.98 and 51.75% of the initial TPC content respectively (Kumutharanjan, *et al*., 2015).

TPC content of 'Mathumeha chooranam' when stored at 4^{o}C

When the powder was stored at 4^{o}C for a month and the TPC was analysed, the cold and hot water extracts contained190.2&196.3µg TAE/g dry weight respectively (Table 1). When compared with the cold extracts, the hot extracts contained higher TPC than cold extract (Table 1). The loss of TPC observed in cold-water extract and hot water extract was 4.1 and 3.8 µg TAE/g dry weight respectively and the TPC retained was 97.9 and 98.1% of the initial TPC content respectively (Table 1).

When the *Murrya keonigii* leaf powder was stored at 4^{o}C for a month and the TPC was analysed; the cold and hot water extracts contained 21.01 & 23.53 µg TAE/g dry weight respectively (Kumutharanjan, *et al*., 2015). As the 'Mathumeha chooranam' contains the *Murrya keonigii* leaf powder as one of the ingredients, the loss of TPC in 'Mathumeha chooranam' was 6.3 µg TAE/g could be due to the loss of TPC content of 1.59 µg TAE/g of the *Murrya keonigii* leaf powder.

'Mathumeha chooranam' stored at 4^{o}C for three months showed TPC of 170.8 and 180.1µg TAE/g dry weight respectively when extracted with cold and hot water(Table 6). After three months of storage at 4^{o}C, the loss of TPC observed in cold-water extract and hot water extract was 23.5 and 20 µg TAE/g dry weight respectively and the TPCs retained were 76.5 and 80% of the initial TPC content respectively (Table 2). With time, the TPC of 'Mathumeha chooranam' stored at 4^{o}C decreased. *Murrya keonigii* leaf powder stored at 4^{o}C for three months showed TPC of 18.50 and 22.53 µg TAE/g dry weight respectively when extracted with cold and hot water, which were 87.9 and 89.83% of the initial TPC content respectively (Kumutharanjan, *et al*., 2015).

'Mathumeha chooranam' stored at 4^{o}C for six months showed TPC of 118.5 and 133 µg TAE/g dry weight respectively when extracted with cold and hot water(Table 1). After six months of storage at 4^{o}C, the loss of TPC observed in cold-water extract and hot water extract was 75.8 and 67.1 µg TAE/g dry weight respectively and was 60.99 and 66.5% of the initial TPC content respectively (Table 2). With time, the TPC of the 'Mathumeha chooranam' stored 4^{o}C decreased. *Murrya keonigii* leaf powder stored at 4^{o}C for six months showed TPC of 11.05 and 15.2 µg TAE/g dry weight respectively when extracted with cold and hot water and hence the TPCs retained were 51.2and 79.2% of the initial TPC content respectively (Kumutharanjan, *et al*., 2015).

Comparison of the TPC content of 'Mathumeha chooranam' when stored at room temperature and at 4^{o}C

Cumulative loss of TPC from 'Mathumeha chooranam'when stored at room temperature and 4^{o}C from one month to six months was more at room temperature than at 4^{o}C (Table 2). Whether the TPC was extracted with cold water or cold water, the loss was very much similar (Table 2).

Table 1: Total Phenolic Content in cold and hot water extracts of 'Mathumeha chooranam' stored at Room Temperature and at 4°C.

Time (Month)	Total Phenolic Content (µg TAE/g dry weight)			
	Room Temperature		4°C	
	Cold water	Hot water	Cold water	Hot water
0	194.3	200.1	194.3	200.1
1	188	197.5	190.2	196.3
2	178	189.4	182.1	189.2
3	166.5	179.2	170.8	180.1
4	148.8	165.5	156.7	167.7
5	128.6	148.3	134.7	152.4
6	104.7	127.4	118.5	133.0

Table 2: Decrease in the Total Phenolic Content of Mathumeha chooranam stored at Room Temperature and at 4°C for analysis the TPC was extracted with cold and hot water.

Time (Months)	Decrease in TPC (µg TAE/g dry weight)							
	Stored at Room Temperature				Stored at 4°C			
	Cold water extract		Hot water extract		Cold water extract		Hot water extract	
	Loss	Cumulative loss	Loss	Cumulative loss	Loss	Cumulative loss	Loss	Cumulative loss
1	6.3	6.3	2.6	2.6	4.1	4.1	3.8	3.8
2	10.0	16.3	8.1	10.7	8.1	12.2	7.1	10.9

3	11.5	27.8	10.2	20.9	11.3	23.5	9.1	20
4	17.7	45.5	13.7	34.6	14.1	37.6	12.4	32.4
5	20.2	65.7	17.2	51.8	17	54.6	15.3	47.7
6	28.8	89.5	20.9	72.7	21.2	75.8	19.4	67.1

Table 3

Ferric Reducing Antioxidant Power(FRAP) of cold and hot water extracts of the *Terminalia chebula* seed skin powder stored at Room Temperature and at 4^{o}C

Time (Month)	**Ferric Reducing Power(µmol/g)**			
	Stored at *Room Temperature*		**Stored at 4^{o}C**	
	Cold extract	**Hot extract**	**Cold extract**	**Hot extract**
0	8602.6	9119.1	8602.6	9119.1
1	8355.1	8912.1	8400.6	8956.2
2	7977.2	8547	8161.5	8762.9
3	7521.4	8170.9	7803.6	8509.9
4	6926.4	7619	7218.6	8025.5
5	6084.5	6916.1	6520.3	7365.8
6	5152.8	6128.1	5688.3	6568.8

Table 4

Time (Months)	Decrease in the Ferric Reducing Antioxidant Power							
	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	Cumulative loss
1	247.5	247.5	207	207	202	202	162.9	162.9
2	377.9	625.4	365.1	572.1	239.1	441.1	193.3	356.2
3	455.8	1081.2	376.1	948.2	357.9	799	253	609.2
4	595	1676.2	551	1499.2	585	1384	484.4	1093.6
5	841.9	2518.1	702.9	2202.1	698.3	2082.3	659.7	1753.3
6	931.7	3449.8	788	2990.1	832	2914.3	797	2550.3

Table 5

Total Antioxidant activity by FRAP method

µmol/mg

at roomtemperature & 4°C

Month.	*Terminalia chebula*				*Phylanthus emblica*				*Murrya keomigii*				*Gymnema sylvestrae*				Mathumeha chooranam			
	Room temperature		**4° C**		**Room temperature**		**4° C**		**Room temperature**		**4° C**		**Room temperature**		**4° C**		**Room temperature**		**4° C**	
	cold ex	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**
0	8602	9119.1			6414.6	6699.2			176.7	206.2			81.3	94.5			2424.8	2867.3		
1st	8355.1	8912.1	8400.6	8956.2	6284.2	6575.6	6303	6597.4	170.6	200.3	171.0	200.9	79.7	92.9	79.82	93.1	23277.5	2779.2	2347.8	2805.8
2nd	7977.2	8547	8161.5	8752.9	6072.2	6409.6	6112.4	6435.7	163.6	193.5	164.5	194.8	76.8	90.5	77.8	91.3	2225	2680.0	2264.9	2727.3
3rd	7521.4	8170.9	7803.6	8509.9	5795.6	6164.1	5855.5	6224.2	155.4	185.6	157.3	187.9	73.7	87.6	74.9	89.2	2104.8	2572.1	2159.8	2634.4
4th	6926.4	7619	7218.6	8025.5	5480.5	5885.9	5577.1	5978.5	144.7	175.4	147.7	173.8	69.1	83.6	71.6	86.0	1957.9	2451.3	2035.4	2519.2
5th	6084.5	6916.1	6520.3	7365.8	5068.2	5504.4	5197.9	5631.7	131.9	163.1	135.7	168.7	63.3	78.7	66.9	81.9	1794.5	2305.8	1897.6	2385.2
6th	5152.8	6128.1	5688.3	6568.8	4533.3	5024.6	4728	5160.4	116.9	148.2	121.2	154.9	55.5	72.3	60.9	76.4	1608	2130.5	1731.1	2224.1

Table 6

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Total phenolic content (µgTAE/g dry weight)																			
Month	***Terminalia chebula***				***Phylanthus emblica***				***Murrya keomigii***				***Gymnema sylvestrae***				**Mathumeha chooranam**			
	Room temperature		**4° C**		**Room temperature**		**4° C**		**Room temperature**		**4° C**		**Room temperature**		**4° C**		**Room temperature**		**4° C**	
	cold ex	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**
0	359.1	365.6			186.5	198.4			22.60	25.08			10.45	11.55			194.3	200.1		
1st	358.4	365.1	358.7	365.3	185	195.4	185.2	197.5	21.01	23.53	21.9	24.73	9.91	11.21	10.12	11.32	188	197.5	190.2	196.3
2nd	353.1	360.9	354.5	362.1	178.3	192.5	180.0	194.5	19.0	21.78	20.31	23.67	9.1	10.65	9.4	10.71	178	189.4	182.1	189.2
3rd	344.4	355.4	346.9	356.6	170.2	185.5	172.1	187.5	16.9	19.95	18.50	22.53	8.2	9.90	8.57	9.99	166.5	179.2	170.8	180.1
4th	332.5	345.2	336.8	347.4	158.6	176.3	164	180.0	14.43	18.03	16.15	20.53	7.0	9.1	7.68	9.24	148.8	165.6	156.7	167.7
5th	316.7	333.9	325.8	337.3	144.2	165.0	150.5	168.3	11.9	15.57	13.75	18.4	5.3	8.1	6.68	8.33	128.6	148.3	134.7	152.4
6th	297.2	318.3	299.4	323.0	126.4	150.2	134.5	153.3	9.02	12.98	11.05	15.2	3.8	6.68	5.38	7.31	104.7	127.4	118.5	133.0

Table 7: Antioxidant activity of by Ferric Reducing Power.

Antioxidant activity

(µg/ml)

6	***Terminalia chebula***				***Phylanthus emblica***				***Murrya keomigii***				***Gymnema sylvestrae***				**Mathumeha chooranam**			
	E C 50 at Room temperature		**E C 50 at 4º C**		**E C 50 at Room temperature**		**E C 50 at 4º C**		**E C 50 at Room temperature**		**E C 50 at 4º C**		**E C 50 at Room temperature**		**E C 50 at 4º C**		**E C 50 at Room temperature**		**E C 50 at 4º C**	
	cold ex	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**	**cold ex**	**Hot ex**
0	17.7	14.4			29	25.45			290.2	155.8			581	527			38.08	27.9		
1st	18.04	14.63	17.9	14.51	34.4	29.75	32.7	28.65	312.5	174.2	310.0	173.0	942.5	946.8	901.0	825.5	44.03	32.9	42.31	31.0
2nd	19.14	15.53	18.5	15.0	41.71	35.76	37.91	32.75	407.7	254.5	396.3	248.4	1337.8	852.5	1246	1128.9	51.93	38.92	47.51	35.5
3rd	21.5	16.58	20.4	15.9	50.63	42.66	44.21	37.75	543.3	357.0	493.7	329.0	1748.3	1247.0	1641.4	1474.6	59.83	46.02	53.81	40.6
4th	25.0	18.88	23.2	17.4	61.43	50.76	52.31	44.85	743.6	521.8	681.0	479.5	2198.5	1657.0	2051.7	1860.4	70.33	55.04	60.82	47.6
5th	29.2	25.5	26.7	20.3	74.33	60.78	62.61	53.35	1024.2	715.3	914.3	669.8	2758.6	2147.7	2507.4	2280.5	82.43	65.04	70.02	55.2
6th	34.3	30.1	30.71	23.7	87.34	72.88	75.11	64.55	1374.5	975.7	1206.5	920.0	3358.6	2658.3	3017.9	2717.9	96.44	77.54	82.03	66.5

CONCLUSION

In Siddha Medicine, the plant materials are used for the 'Chooranam' preparation. Usually the 'Chooranam' is used for 3 months. The loss of TPC from'Mathumeha chooranam' stored at room temperature for 3 months decreased by 14.29 and 10.44% respectively when extracted with cold and hot water. On the other hand, when 'Mathumeha chooranam' was stored at 4^{o}C, the loss was12.09 and 9.9%, when extracted with cold and hot water respectively. On the other hand the loss of TPC at 6 months was observed to be 46.06&36.33% and 39.01& 33.53% respectively in the powder stored at room temperature and 4^{o}C, and extracted with cold and hot water. Thus the loss of TPC is more when stored at room temperature than when stored at 4^{o}C.

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