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ANTIOXIDANT STUDY OF ONE AYURVEDIC PREPARATION AMRITHAMEHARI CHURNAM

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Abstract

Amrithamehari churnam is an Ayurvedic preparation for the treatment of diabetes. The present study is to understand the antioxidant activity of this medicine. Three different types of antioxidant studies were conducted namely, ABTS, DPPH and FRAP. The results clearly indicated that Amrithamehari choornam has antioxidant activities only at higher concentrations. Thus it seems that its antioxidant action could be one of its antidiabetic role. Further work is in progress in this regard.

Key words: Ayurvedic, Amrithamehari Churnam, Antioxidant, ABTS, DPPH, FRAP, Antidiabetic.

Introduction

The medicinal role and mechanisms of action of Ayurvedic medicines is a challenging factor towards validating and authenticating them with modern scientific methods. Due to the lack of study on the scientific efficacy by standard procedures the Ayurvedic and Sidha medicines are lagging behind as compared to their allopathic counterparts. Thus there is an urgent need to prove the efficacy of these medicines and recently some reports are forthcoming. [1 -12]

We have reported the GC MS pattern of Amrithamehari Choornam. (Edel Queen *et al*, 2016) [13] The present study is another step towards validating it scientifically, by studying the antioxidant activities of the same medicine by FRAP, ABTS and DPPH assays.

Amrithamehari churnam is in herbal powder form used for the treatment of diabetes. It is also known as Amritadi churnam. Amrithamehari Churnam consists of the following ingredients.

Each 10 gm of Amrithamehari churnam contains:

Amrita *(Tinospora cordifolia*)- 1.667 gm.

Meharimula (*Gymnema sylvestre*)- 5.000 gm.

Dhatri – Amalaki (*Embelica officinalis*)- 1.667 gm.

Ratri – turmeric (*Curcuma longa*)- 1.667 gm.

The dosage ranges from 3 to 10 grams depending on the blood sugar levels of the patient. This medicine is prescribed along with some adjuvant like hot water, Dhanwantara ghritam, Vastyamayantakam ghritam etc. The medicine should be taken strictly, according to the prescription of an ayuvedic practitioner.

MATERIALS AND METHODS

The present study encompasses three different antioxidant assays, namely, ABTS, DPPH and FRAP. The FRAP assay was performed by Pulido *et al*, (2000), ABTS assay was done following the method of Re *et al*, (1999) and the DDPH assay was done by the method of Blios *et al*, (1958). [14-16]

FRAP Assay (Ferric Reducing/Oxidant Power)

Amrithamehari Choornam was dissolved in Ethanol. Triplicates had been put for all the Processes.

Conc. = Concentration of the sample

OD = OD of the sample

Linearity (y) = mx + c

M = Slope

C = The point x crosses y axis

X = OD – c value / m value

mM Fe/mg = X value / concentration x 1000

Mean = Average of mM Fe/mg

STDEV = Standard Deviation for mM Fe/mg.

ABTS Assay

ABTS and potassium persulfate were dissolved in distilled water to a final concentration of 7 mM and 2.45 mM respectively. These two solutions were mixed and the mixture allowed to stand in the dark at room temperature for 16 h before use in order to produce ABTS radical (ABTS•+). This was incubated with the Amrithamehari choornam at different concentrations and the reaction mixture which was blue became colourless due to the presence of antioxidants present in the medicine. This was change in color was estimated spectrophotometrically.

DPPH Assay (1, 1-diphenyl-2-picrylhydrazyl)

The sample was dissolved in Ethanol in 1mg/ml concentration and used as stock. From the stock, various concentrations (100, 200, 300, 400mg) were taken for further analysis.

Respective solvents were taken as negative control.

Conc. = Concentration of the sample

OD = OD of the sample

Neg. Control = The Solvent

Activity = Neg. Control – OD / Neg. Control

% of Activity = Activity/100

IC50 = 50 – c value / m value

IC50/ml = IC50/3 (3 ml of DPPH for the assay. To find the activity in 1 ml, the value had been divided by 3).

Results and Discussion

Tables 1, 2 and 3 and Figures 1, 2 and 3, represent the antioxidant profiles of the three assays namely FRAP, ABTS and DPPH, respectively for Amrithamehari choornam.

Table 1. Indicates the FRAP antioxidant activity of Amrithamehari choornam with Ascorbic acid as control.

Sl. No	Amrita mehari Choornam Concentration (µg)	Absorbance (%)	Standard Deviation	Vitamin C	Absorbance (%)	Standard Deviation
1.	5	1.23967	0.41322	5	31.2672	1.79063

2.	10	2.20386	0.27548	10	58.0303	0.56809
3.	20	5.64738	0.41322	20	60.5096	0.88273
4	40	8.35629	0.91713	40	68.1405	0.56926
5.	80	12.213	0.57346	80	80.6244	0.17765
6.	100	18.9164	0.48373	100	85.0689	0.49663

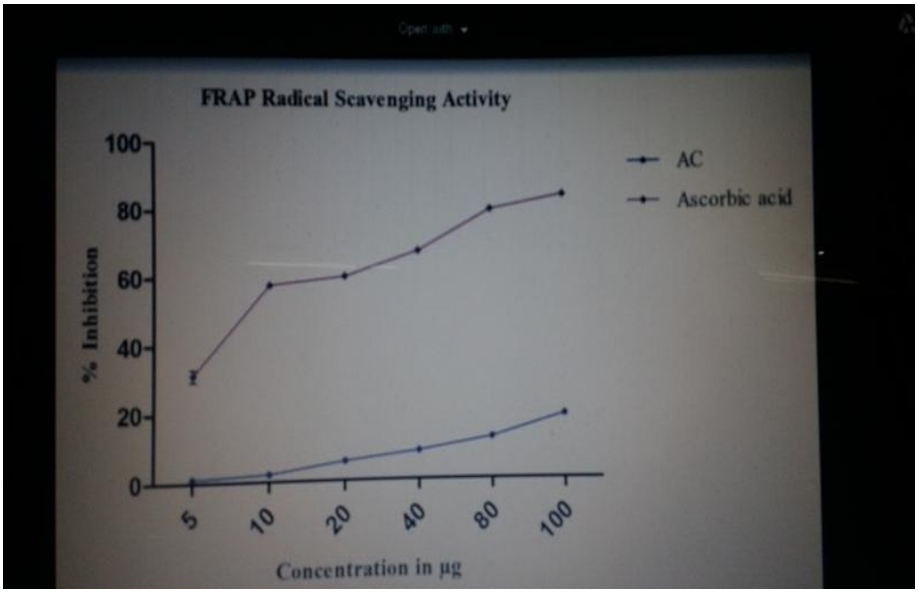

Figure 1. Represents the comparative graphical profile for the Amrithamehari choornam and Ascorbic acid with respect to FRAP assay, which indicates that Amritamehari choornam does have antioxidant activity only at higher doses as compared to Ascorbic acid.

Table 2. Indicates the ABTS antioxidant activity of Amrithamehari Choornam assay with Ascorbic acid as control.

Sl. No	Amrithamehari Choornam Concentration (µg)	Absorbance (%)	Standard Deviation	Vitamin C	Absorbance (%)	Standard Deviation
1.	5	0.8804	0.36675	5	24.8094	0.96288
2.	10	1.8407	0.30211	10	47.0588	0.55013
3.	20	2.4010	031762	20	71.1485	0.36675
4	40	4.8419	0.30211	40	89.0877	0.20195
5.	80	8.1032	0.42159	80	91.0764	0.13493
6.	100	10.0840	0.36014	100	95.1621	0.11003

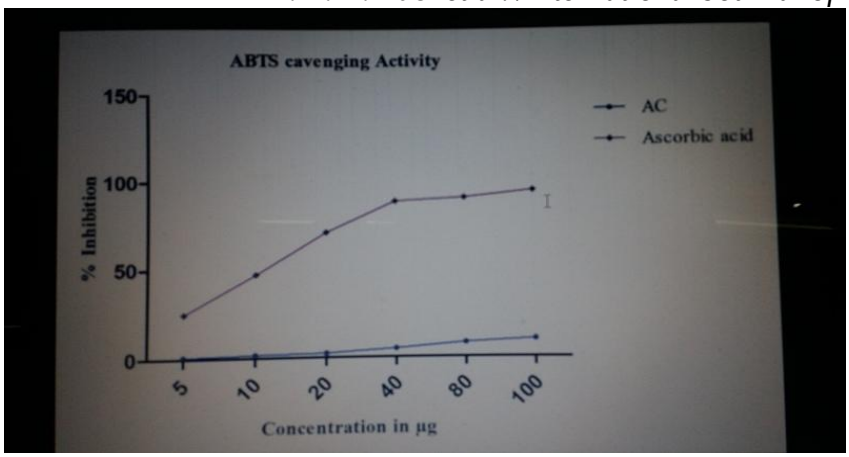

Figure 2. Represents the comparative graphical profile of Amritamehari choornam and Ascorbic acid with respect to ABTS assay, which indicates that Amritamehari choornam does have antioxidant activity only at higher doses as compared to Acorbic acid.

Table 3. Indicates the DDPH antioxidant activity of Amritamehari choornam with Ascorbic acid as control.

Sl. No	**Amrita mehari Choornam Concentration (µg)**	**Absorbance (%)**	**Standard Deviation**	**Vitamin C**	**Absorbance (%)**	**Standard Deviation**
1.	5	1.0204	0.226757	5	29.2517	0.408793
2.	10	1.8896	0.458214	10	51.17158	0.398173
3.	20	4.6107	0.398173	20	75.85034	0.631266
4	40	5.7823	0.299972	40	89.95465	0.092804
5.	80	19.6901	0.398173	80	92.89872	0.028533
6.	100	23.0915	0.511253	100	95.98262	0.097751

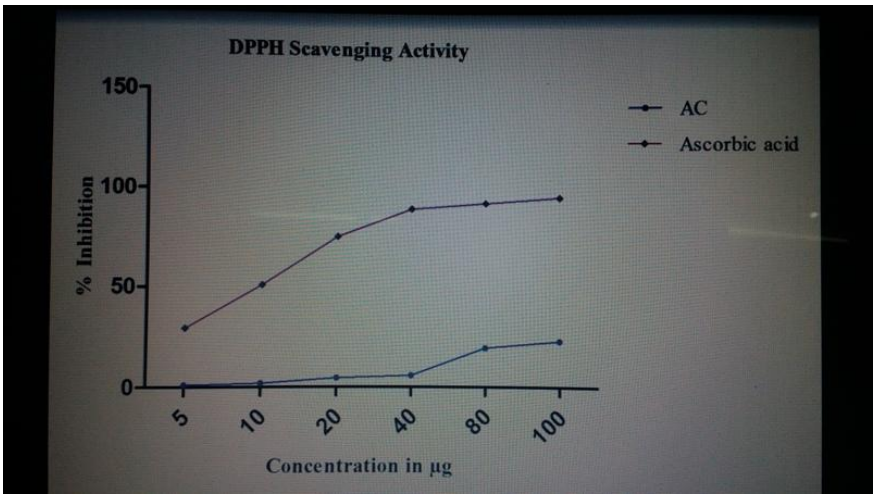

Figure 3. Represents the comparative graphical for the Amrithamehari choornam and Ascorbic acid with respect to DPPH assay, which indicates that Amrithamehari choornam does have antioxidant activity only at higher doses as compared to Ascorbic acid. From the above antioxidant profiles it is clear that Amrithamehari choornam has antioxidant activities only at higher concentrations which do not seem to be much significant. All the constituents of Amrithamehari choornam (*Tinoapora cordifolia*, *Gymnema sylvestre*, *Embelica officinalis, Curcuma longa)* have been reported to have antioxidant activities (Upadhayay *et al*, 2010; El Shafey *et al*, 2013; Malik *et al*, 2007; Malik *et al*, 2010; Bhide and Nitave 2014; Sikha *et al*, 2015; Liu *et al*, 2014). [17-23] Among the 50 types of molecules that were shown in the GC MS analysis of this medicine, only a few namely, 4-Isoquinolinecarboxylic acid, 2, 3, 5, 6, 7, 8-hexahydro-3-oxo- and Benzene propanoic acid, 3, 5-bis (1,1-dimethylethyl)-4-hydroxy- were reported to have antioxidant activities. The low antioxidant activity of this medicine could be due to synergy among the various components or the antidiabetic property of Amrithamehari choornam could be due to some other mechanisms which are to be probed further.

Conclusion

From the above results and discussion it is evident that Amrithamehari choornam does not show any significant antioxidant activity in all the three assay methods namely, FRAP, ABTS and DPPH. The antidiabetic role of this medicine could be one of the supporting molecular mechanisms which need further work.

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