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Antioxidant Study of One Ayurvedic Preparation Katakakhadiradi Kashayam

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INTRODUCTION

Ayurvedic and sidha medicines are the traditional and age old medical practices of India. Due to the advent of modern medicine these forms of medical practices have become the back benchers. There is an implied need to resurrect these forms of treatment due to the simple fact that they are easily available, cost effective and considered to have lesser side effects. The modern medicine, although effective, is beset with negative aspects like side effects, costly and not available to masses easily. Since last two decades some studies have been done to prove the medicinal efficacy of Ayurvedic and Sidha formulations in the light of modern scientific techniques. This trend must continue to establish the age old systems back to their glory. [1-13]

The present work deals with the antioxidant study of one Ayurvedic medicine, namely, Katakakhadiradi Kashayam, which is used to cure mainly Diabetes and urinary ailments. It is helpful to relieve complications of diabetes such as neuropathy. It is supposed to control both Vata and Kapha related diseases. This medicine is taken before food once or twice a day at a dose of 5 to 10 ml or as advised by medical practitioner. The medicine is also available in capsule form, which can be taken twice a day before food, two at a time. This medicine is taken along Niruryadi gulika, Swetha gunjadi gulika, Mehasahari gulika etc as adjuvant. The reference of this medicine is found in the Ayurvedic treatise Sahasrayoga, Kashaya Prakarana, Pramehahara Kashaya. We have reported the GC MS patterns of this medicine. [14] This work is the second step in understanding the mechanism of action of this medicine.
Katakakhadiradi Kashayam is an herbal decoction prepared from 10 grams each of the following ingredient plants. Kataka – *Strychnos potatorum*, Khadira – *Acacia catechu*, Dhatri- Amla- *Emebelica officinalis*, Darvi- Daruharidra- *Berberis aristata*, Samanga - *Biophytum sensitivum*, Vidula – *Barringtonia acutangula*, Abda- *Cyperus rotundus*, Vairi – *Salacia reticulata*, Rajani- Turmeric- *Curcuma longa*, Abhaya – *Terminalia chebula*, Chootabija – Mango Seed- *Mangifera indica*.

MATERIALS AND METHODS

The present study encompasses three different antioxidant assays, namely, ABTS, DPPH and FRAP of Katakakhadiradi Kashayam. The medicine was procured by standard Ayurvedic vendor at Chennai. 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). [15-17]

FRAP Assay (Ferric Reducing/Oxidant Power)

Katakakhadiradi Kasyam was mixed 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 Katakakhadiradi Kashaym 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 colour 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

Figure 1, 2, 3 and Table 1, 2 and 3 represent the results of FRAP, ABTS and DPPH assay results of Katakakhadiradi Kashayam.

FRAP ASSAY

Table 1. Indicates the FRAP antioxidant activity of Katakakhadiradi kashayam with Ascorbic acid as control.

Sl. No	Katakakhadiradi Kashayam Concentration (µg)	Absorbance (%)	Standard Deviation	Vitamin C	Absorbance (%)	Standard Deviation
1.	5	23.4619	1.14692	5	31.2672	1.79063
2.	10	39.6235	1.72222	10	58.0303	0.56809
3.	20	60.5096	0.88273	20	60.5096	0.88273
4	40	71.8136	0.91795	40	68.1405	0.56926
5.	80	80.6244	0.17765	80	80.6244	0.17765
6.	100	83.7373	1.27983	100	85.0663	0.49663

ABTS ASSAY

Table 2. Indicates the ABTS antioxidant activity of Katakakhadiradi Kashayam assay with Ascorbic acid as control.

Sl. No	Katakakhadiradi kashayam Concentration (µg)	Absorbance (%)	Standard Deviation	Vitamin C	Absorbance (%)	Standard Deviation
1.	5	9.766391	0.42159	5	24.8094	0.96288
2.	10	13.4454	0.31762	10	47.0588	0.55013
3.	20	35.1341	0.30211	20	71.1485	0.36675
4	40	56.5426	0.43284	40	89.0876	0.20195
5.	80	79.3117	0.36675	80	91.0764	0.13493
6.	100	82.0328	0.42159	100	95.1621	0.11003

DDPH ASSAY

Table 3. Indicates the DDPH antioxidant activity of Katakakhadiradi Kashayam with Ascorbic acid as control.

Sl. No	Katakakhadiradi Kashayam Concentration (µg)	Absorbance (%)	Standard Deviation	Vitamin C	Absorbance (%)	Standard Deviation
1.	5	23.84732	0.3981473	5	29.2517	0.408793
2.	10	49.92441	0.692755	10	51.17158	0.398173
3.	20	73.80952	0.599944	20	75.85034	0.631266
4	40	87.64172	0.340136	40	89.95465	0.092804
5.	80	91.72336	0.56693	80	92.89872	0.028533
6.	100	94.27438	0.207827	100	95.98262	0.097751

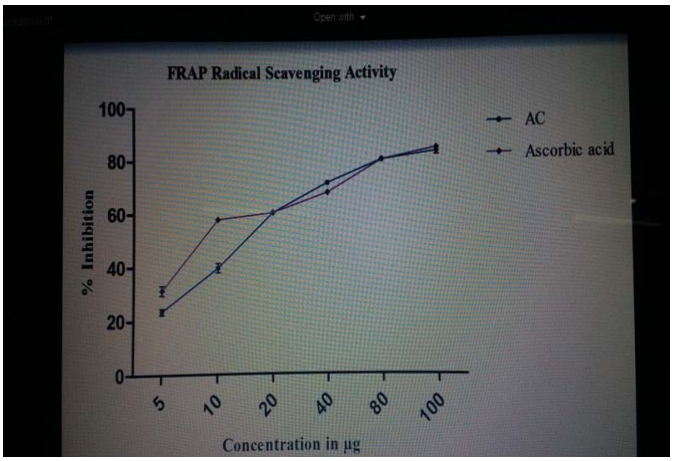

Figure 1. Indicates the graph of FRAP assay of Katakakhadiradi Kasyahm as compared with Ascorbic acid.

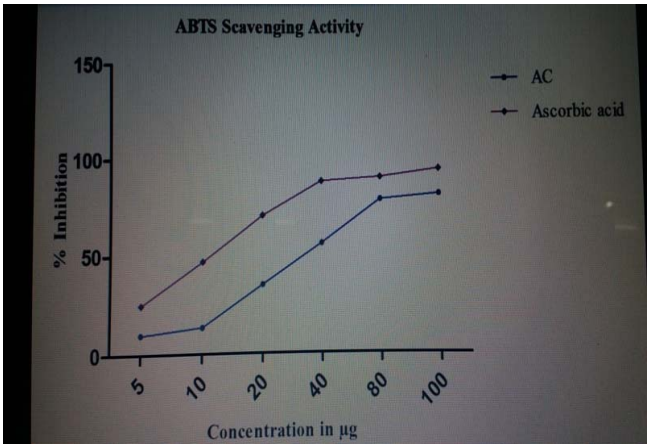

Figure 2. Indicates the ABTS assay graph of Katakakhadiradi Kashyam as compared with Ascorbic acid.

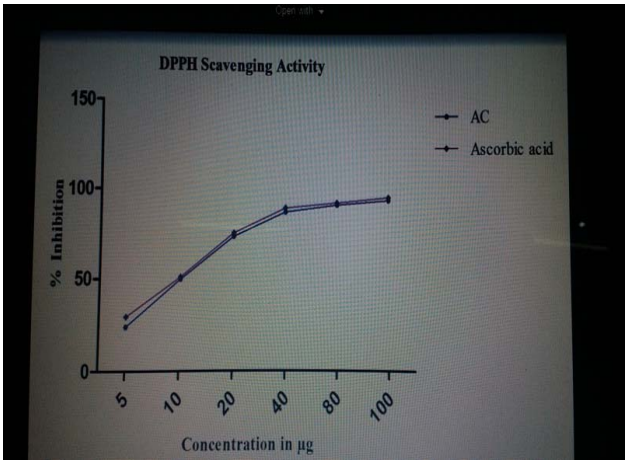

Figure 3. Indicates the DPPH assay graph of Katakakhadiradi Kashyam as compared to Ascorbic acid.

The results of the three assays indicate that

a. The FRAP graph indicates that there is very good antioxidant activity of Katakakhadiradi Kashayam as compared to ascorbic acid
b. ABTS assay results indicate the antioxidant role to moderate as compared to Ascorbic acid.
c. The DPPH assay results are almost at par when compared to the ascorbic acid.

Most of the ingredients of Katakakhadiradi Kashayam have been reported to have antioxidant activates. [18-29] These positive results of the antioxidant study of Katakakhadiradi Kashayam is one step in the right direction in understanding its scientific role in treating diabetes. Further parameters need verification to prove the efficacy of this medicine as antidiabetic.

CONCULSION

From the above results and discussion the antidiabetic role of Katakakhadiradi kashaym could be due to its antioxidant properties. More parameters for its scientific validity need be done.

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