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INVITRO ANTIOXIDANT PROPERTY OF A SIDDHA DRUG KASTHURI MATHIRAI

Vanitha A.*[1], Praveena R.[2], Seethalakshmi G.[3], Meenakshi Sundaram M.[4] and Muthukumar N. J.[5]

[1]Siddha Physician, Chennai.

[2]Lecturer, Pura maruthuvam Department, National Instituite of Siddha, Chennai.

[3]Siddha Physician, Chennai.

[4]HOD, Kuzhandhai Maruthuvam Department, National Institute of Siddha, Chennai.

[5]Director i/c, National Institute of Siddha, Chennai.

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***Corresponding Author**
Vanitha A.
Siddha Physician, Chennai.

ABSTRACT

The siddha system of medicine is deeply rooted on concepts of body, mind and the universe.[1] The main intention of Siddhars to attain eternity and to prevent infirmity. The sample drug Kasthuri Mathirai is used to treat acute and chronic respiratory diseases.[2] The authors of this paper have attempted to explore the antioxidant property of the sample drug Kasthuri Mathirai by the estimation of DPPH and Hydroxyl scavenging activity.

KEYWORDS: Kasthuri Mathirai, Antioxidant, DPPH, Hydroxyl.

INTRODUCTION

The Siddha medicinal preparations are known to contain a variety of natural antioxidants that insulate and safeguard the physical and metabolic integrity of the cell.[3] The antioxidants are moderating the ageing process by limiting biochemical consequences of oxidation. Antioxidants helps to counterbalance the free radicals in our bodies and to upgrade the immune system of our overall health.[4]

“EXTINCTION IS THE RULE, SURVIVAL IS THE EXCEPTION”

-Carl sagan

The present study is carried out to figure out the antioxidant property of Kasthuri Mathirai.

MATERIALS AND METHODS

Preparation of Kasthuri Mathirai

Ingredients

1. Kasthuri – 1part
2. Kungumapoo – 2part
3. Korosanai - 3part

Preparations

The above said drugs are purified and ground with lemon juice and rolled it as 130mg pill. The pills are stored in an airtight container.

Dosage: 1 to 2 pills twice a day with appropriate adjuvant.

Indication: Thodam, Pitham, Kabam and Vaayu.

Estimation of antioxidant activity of kasthuri mathirai

Hydroxyl radical scavenging activity

Principle

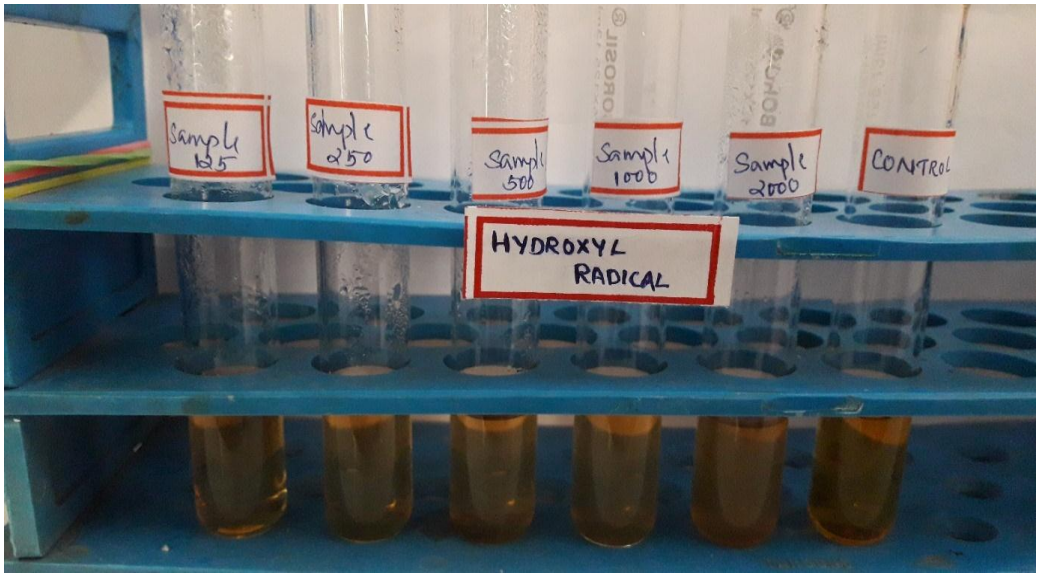
This assay is based on the qualification of the degradation product of 2 deoxy ribose by condensation with TBA (Elizabeth and Rao, 1990). Hydroxyl radical was generated by the Fe^{3+} - ascorbate- EDTA –H_2O_2 system (The Fenton reaction). Gallic acid (10mg/mL DMSO) was used as reference.[5]

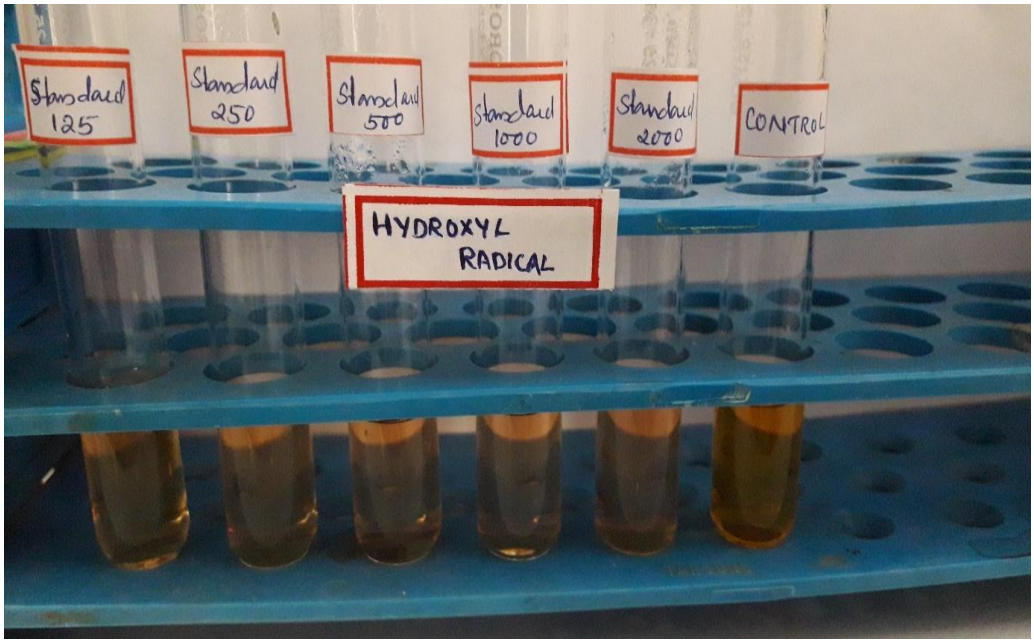
Procedure

Different concentration of sample such as 125-2000µg/mL from a stock concentration of 10mg/mL were mixed with 500µl reaction mixture ((2 deoxy 2 ribose (2.8mM), FeCl3 (100µm), EDTA (100µm), H_2O_2 (1.0mM), ascorbic acid (100µm) in KH_2PO_4 - KOH buffer (20 mM pH 7.4)) was made up to a final volume of 1 ml. A control without the test compound, but an equivalent amount of distilled water was taken. After incubation for 1 hour at 37°C, add 1ml of 2.8% TCA, then 1ml 1% aqueous TBA was added and the mixture was incubated at 90°C for 15 minutes to develop the colour. After cooling the absorbance was measured at 532nm against an appropriate blank solution.

CALCULATION

Percentage of inhibition = $\frac{control-test}{control} X100$





RESULTS

Concentration(µg/mL)	Absorbance	Percentage of inhibition
Control	0.3413	0.00
Standard: Gallic Acid		
125	0.2267	33.58
250	0.1190	65.13
500	0.0600	82.42
1000	0.0336	90.16
2000	0.0170	95.02

Concentration(µg/mL)	Absorbance	Percentage of inhibition
Control	0.3321	0.00
Sample code: Sample		
12.5	0.2907	12.47
25	0.2489	25.05
50	0.1982	40.32
100	0.1566	52.85
200	0.1269	61.79

IC 50 Value (Calculated using ED50 PLUS V1.0 Software) –

1. Gallic acid – 190.055µg/mL
2. Sample-1259.2 µg/mL

Dpph radical scavenging assay

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang et al [2001]. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

Principle

1, 1-diphenyl-2-picryl hydrazyl is a stable free radical with pink colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as, DPPH + [H-A] →DPPH-H + (A)

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Reagent preparation

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of methanol.

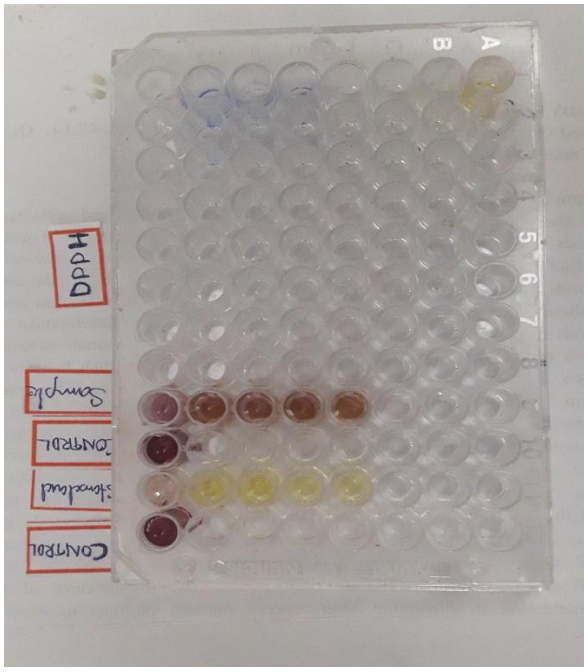
Procedure

Different concentrations of sample such as 12.5µg/mL- 200µg/mL from stock solution were made up to a final volume of 20µl with DMSO and 1.48ml DPPH (0.1mM) solution was added. A control without the test compound, but an equivalent amount of distilled water was taken. The reaction mixture incubated in dark condition at room temperature for 20 minutes.

After 20 minutes, the absorbance of the mixture was read at 517nm. 3ml of DPPH was taken as control.

Calculation

Percentage of inhibition = $\frac{control-test}{control} X100$



RESULTS

Concentrations (µg/mL)	Absorbance	Percentage of inhibition
Control	0.4673	0.00
Standard: Ascorbic Acid		
12.5	0.2627	43.78
25	0.1860	60.20
50	0.1192	74.49
100	0.0694	85.15
200	0.0198	95.76

Concentrations (µg/mL)	Absorbance	Percentage of inhibition
Control	0.4526	0.00
Sample code: Sample		
12.5	0.4019	11.20
25	0.3762	16.88
50	0.2990	33.94
100	0.2084	53.95
200	0.1623	64.14

IC 50 Value (Calculated using ED 50 PLUS V1.0 Software)

1. Ascorbic Acid- 17.2351µg/mL
2. SAMPLE-127.318 µg/mL

CONCLUSION

The Hydroxyl radical scavenging activity of Kasthuri Mathirai showed highest percentage of zone of inhibition at 61.79 at 200 µg/mL when compared to standard Gallic acid. The DPPH Radical scavenging activity showed the high efficacy at 200 µg/mL is 64.14 with the standard Ascorbic acid. Thus the Kasthuri Mathirai showed a better antioxidant property.

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