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Research article

In-*vitro* Anti-Inflammatory activity of Siddha drug "*Dhrakshathy Mathirai*" by Protein (albumin) Denaturation Assay

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Abstract

Siddha system is one of the ancient system of medicine. This system cure many diseases purely on herbs. In this present study anti-inflammatory potential of the Siddha formulation "*Dhrakshathy Mathirai*" (DM) was investigated. Based on the resulted the extract of the drug showed the highest anti-inflammatory activity. The increments in absorbance of test samples with respect to control indicated stabilization of protein i.e., inhibition of heat induced protein (albumin) denaturation by DM and reference drug diclofenac sodium.

Keywords: Siddha, *Dhrakshathy mathirai* , anti-inflammatory activity, diclofenac sodium.

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Quick response code

Introduction

Asthma is a chronic reversible inflammatory destructive disease of the airways characterized by recurrent paroxysmal attacks of dyspnoea chiefly expiratory in nature accompanied by wheeze which may subside spontaneously or with treatment [1].Inflammation is a response of the immune system to an injury which is beneficial to the host under normal circumstances. However, an aberrant immune response to non-pathogenic stimuli in the asthmatic airway leads to a chronic inflammatory response relevant to the pathogenesis of the disease. Episodes of acute inflammatory reactions are often accompanied by an underlying chronic inflammation even in the absence of continuous allergen exposure. The complex interplay between the multi-cellular inflammatory infiltrate and parenchymal lung tissue cells is characterized by a broad network of self amplifying bioactive mediators, including cytokines, antibodies and growth factors. Inflammatory leukocyte recruitment is directed by small inflammatory soluble molecules known as chemokines. Inappropriate immune activation is thought to be, in part, is responsible for the chronicity of allergic asthma, however there is now increasing evidence that dysregulation of endogenous immune regulating processes are, in part, are responsible for the development of this disease [2].

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Drugs which are used presently for the management of pain and inflammatory conditions may produce adverse effects. On the other hand, polyherbal medicines which are safe, effective, time-tested and devoid of drastic side-effects are the need of the hour. Siddha system of medicine has many herbal formulations indicated for the treatment of inflammations.

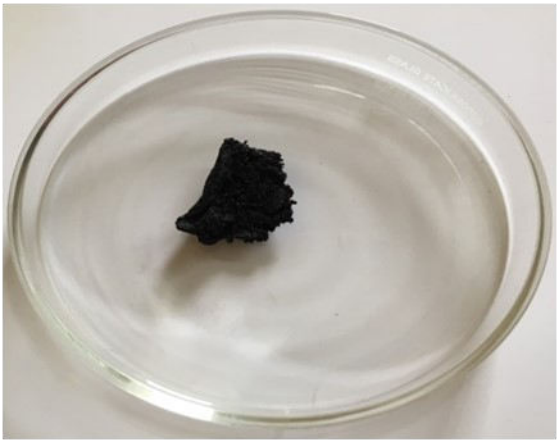
"Dhrakshathy Mathirai" mentioned in Siddha book *Anuboga Vaithiya Navaneetham-Part VIII* contains polyherbal drugs is indicated for bronchial asthma.

MATERIALS AND METHODS

The test drug DM was prepared as per the Standard Operative Procedure (SOP) based on the Siddha literature, *Anuboga Vaithiya Naveetham.* The ingredients of the test drugs includes.

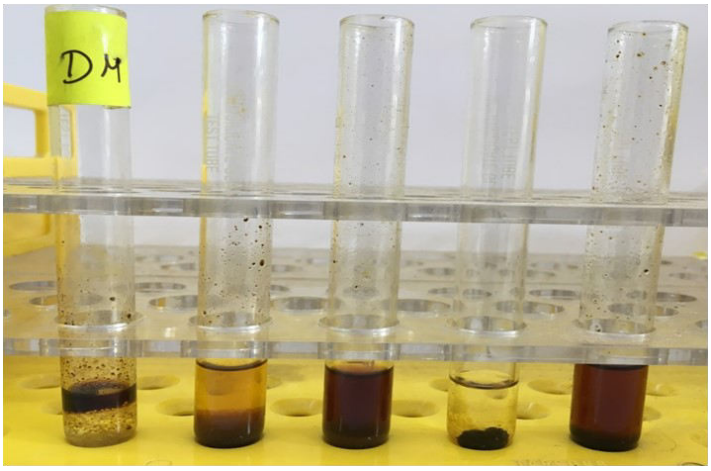
TAMIL NAME	BOTANICAL NAME
Dhrakshai Pazham	*Vitis vinifera,Linn.*
Chukku	*Zingiber officinale,Roscoe.*
Chittrarathai	*Alpinia officinarum,Hance.*
Thesavaram	*Piper longum,Linn.*
Sathikkai	*Myristica fragrans,Gronov.*
Sathipathiri	*Myristica fragrans,Gronov.*
Lavangam	*Syzygium aromaticum,Linn.*
Valmilagu	*Piper cubeba,Linn.*
Lavanga Pattai	*Cinnamomum verum,J.Presl.*
Athimathuram	*Glycyrrhiza glabra,Linn.*
Elam	*Elettaria cardamomum,Linn.*
Kungumapoo	*Crocus sativus,Linn.*
Karkandu	*Borassus flabellifer,Linn.*

In-vitro Anti-Inflammatory Activity by Protein (Albumin) denaturation Assay



State: Semi solid
Appearance: Dark brownish

Solubility Assay



Solvent Used	Solubility
Chloroform	Insoluble
Ethanol	Soluble
Water	Soluble
Ethyl acetate	Insoluble
DMSO	Soluble

Stock: 10mg/ml

Albumin Denaturation Assay Procedure

In-vitro anti-inflammatory activity DM was studied using albumin denaturation technique. The reaction mixture consisted of bovine serum albumin (5% aqueous solution) and test sample DM at varying concentration ranges from 100 to 500 µg/ml and standard Diclofenac sodium at the concentration of100 µg /ml of final volume. pH was adjusted by using a small amount of 1N Hydrochloric acid. The samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling the sample, 2.5 ml of phosphate buffer solution was added into each test tube. Turbidity developed was measured spectrophotometrically at 660 nm, for control distilled water was used instead of test sample while product control tests lacked bovine serum albumin. The experiment was performed in triplicate.

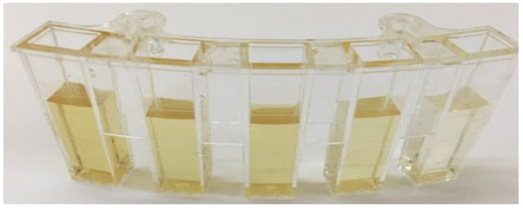
The Percentage protection from denaturation is calculated by using the formulae

$$\left[\frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}}\right] \times 100.$$

Statistical analysis

Results are expressed as Mean ± SD. The difference between experimental groups was compared by One-Way Analysis Of Variance (ANOVA) followed by Dunnet Multiple comparison test.

Absorbance of reaction mixture – Test Sample



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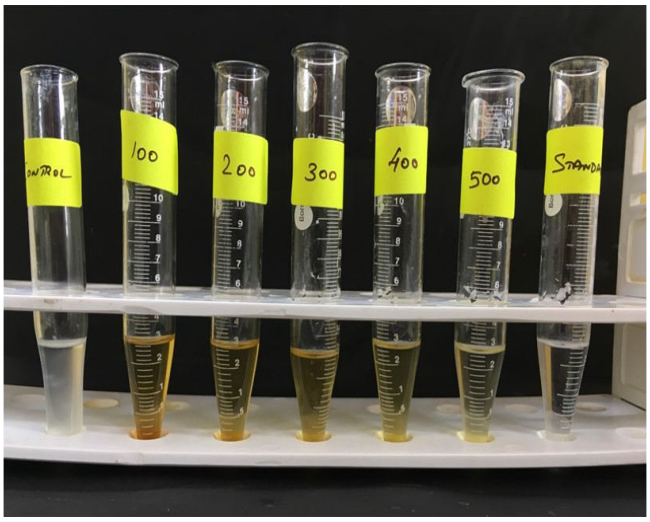
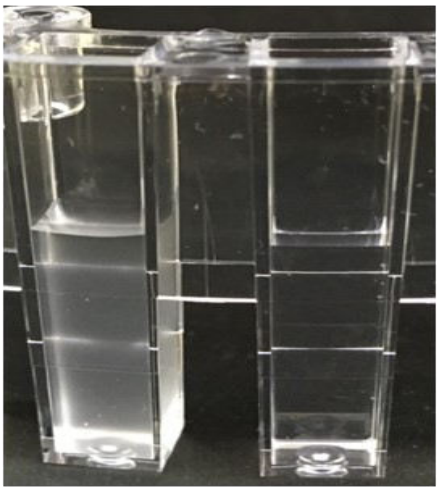
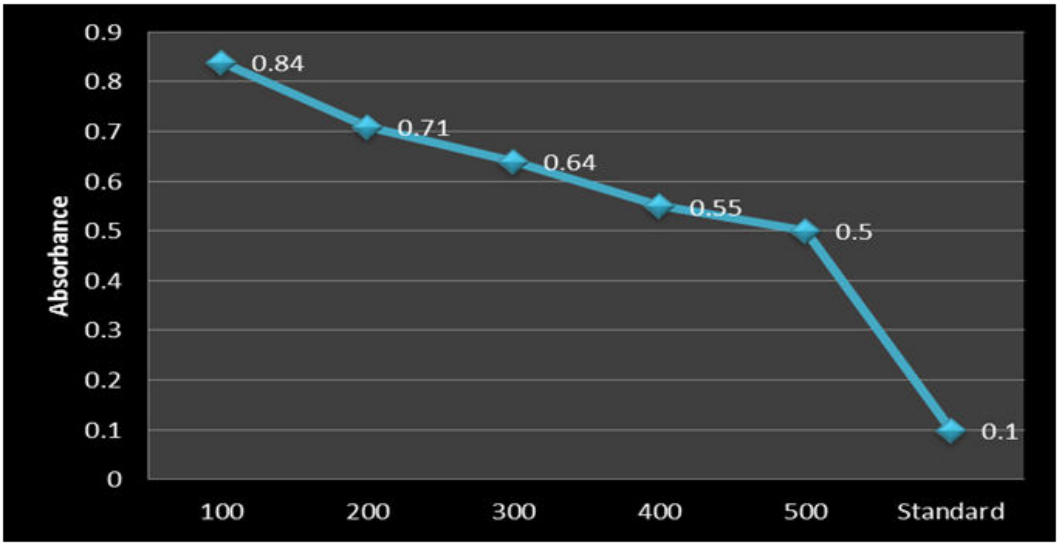


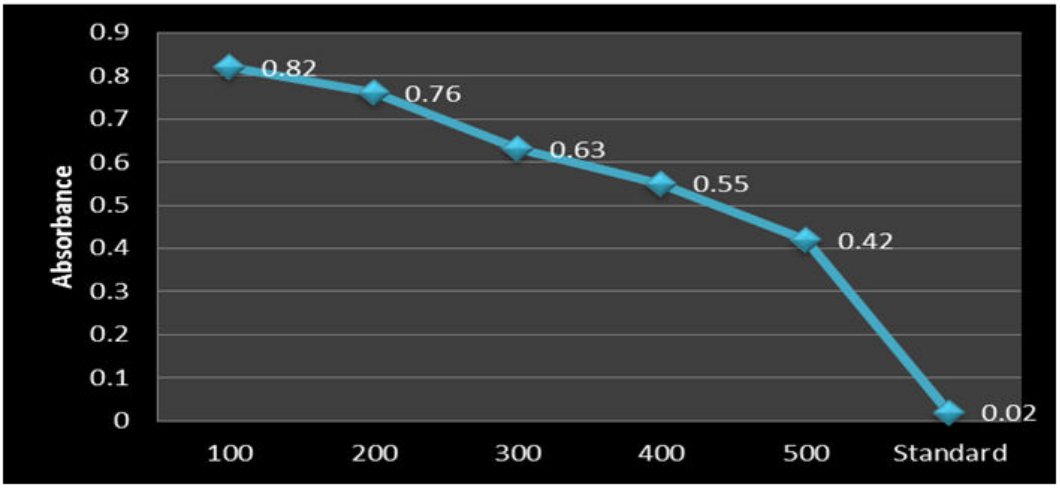
Absorbance of reaction mixture – Control and Stand- ard



Absorbance Range of test and standard at Trial 1



Absorbance Range of test and standard at Trial 2



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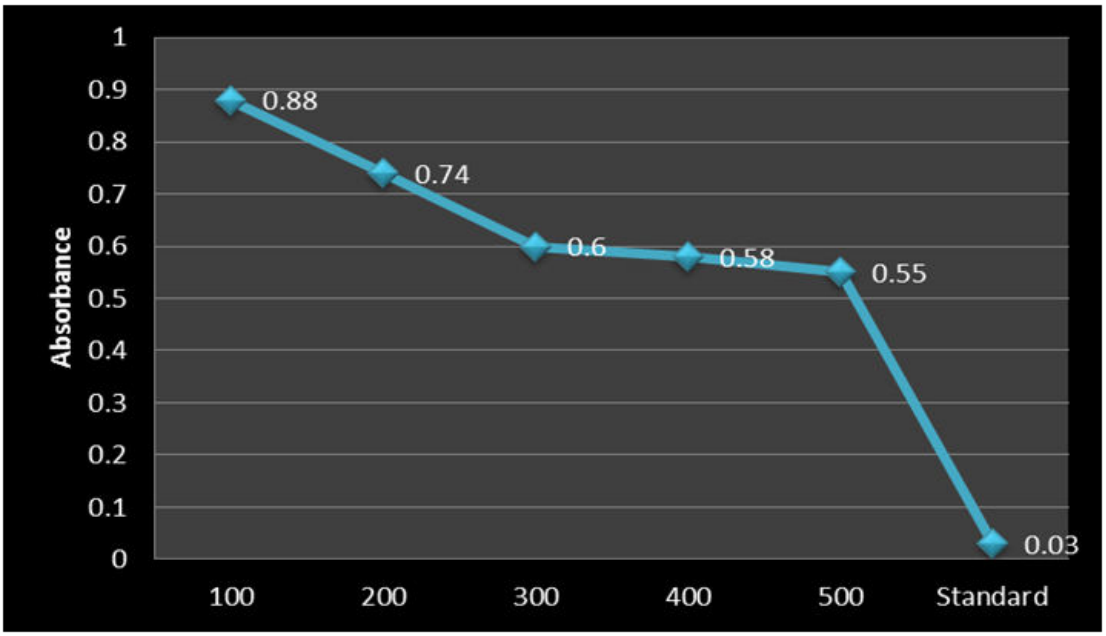
Absorbance Range of test and standard at Trial 3



Concentration in µg/ml	Absorbance
Control	1.016 ± 0.001
DM 100	0.84 ± 0.030
DM 200	0.73 ± 0.025
DM 300	0.62 ± 0.020
DM 400	0.56 ± 0.017
DM 500	0.49 ± 0.065
Diclofenac sodium (100 µg)	0.05 ± 0.043

Each value represents the mean ± SD. N=3

Concentration in µg/ml	Percentage Inhibition of Protein Denaturation
DM 100	18.21 ± 2.751
DM 200	29.03 ± 2.636
DM 300	40.19 ± 2.198
DM 400	46.43 ± 1.534
DM 500	53.33 ± 6.234
Diclofenac sodium (100 µg)	96.65 ± 4.246

Each value represents the mean ± SD. N=3

Result and Discussion:

The result obtained from the present clearly indicates that the test drug DM was effective in inhibiting heat induced albumin denaturation. Maximum percentage inhibition of about 53.33 % was observed at 500 µg/ml when compare to that of the Diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition 96.65 % at the concentration of 100 µg/ml. From the result of the study it was concluded that the test drug DM possess promising anti-inflammatory property in protein denaturation assay.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SOURCE OF FUNDING

Nil

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