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Research Article

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In-vitro anti-inflammatory screening of a Herbomineral Siddha medicine, "*Mandura Vajra Vadugangal*"

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Abstract

Siddha is considered as one of the oldest medicine system and very effective in treating most of the diseases. The challenges in treating such conditions with the currently available drugs (Non-Steroidal Anti-inflammatory Drugs – NSAID) often results in gastric irritation, renal damage, dependence etc. Traditional Siddha medicines offer a wide range of Anti-inflammatory drugs which are poly herbal in nature and devoid of the above said side-effects. This paper deals with Anti-inflammatory screening of such a medicine '*MANDURA VAJRA VADUGANGAL'* documented in Siddha text *"ANUBAVA VAITHIYA DEVA RAGASIYAM"* indicated for inflammation. Our results show a significant increase in the expression and activity with the Test drug in different concentrations compared with standard drug diclofenac sodium.

Keywords: Siddha Medicine, *In-vitro* studies, inflammation, anti - inflammatory activity, mvv, albumin denaturation assay

Introduction

Inflammation is a defence mechanism of the body immune system recognizes damaged cells ,irritants& pathogens and it begins the healing process[1]. Inflammation is a complicated and not fully understood communication between cellular and humoral elements. Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics, eg. Opiods or non-narcotics, salicylates, and corticosteroids, hydrocortisone. The usage of NSAID in the treatment of painful musculo-skeletal conditions often results in adverse effects such as gastric irritation, renal damage etc[2].

On the other hand, poly herbal medicines which are safe, effective, time-tested and devoid of drastic side-effects are the need of the hour.

Traditional Siddha Indian Medicine has many such herbal medicines indicated for the treatment of inflammation.

In siddha about inflammation:

In siddha, siddhars classified the diseases in to 4448. It includes many diseases based on vatha, pittham, kapham. Among these veekam (inflammation) is developed according to pittham in our body.If the ratio of pittham is increased or decreased it affect the udal thathukal. Based on that inflammation is produced [3].

Inflammation(veekam) is classified into 5 types[4].

1.Valiveekam
2.Alalveekam
3.Iya veekam
4.Irukuttramukutra kalapu veekam
5.Adipatta veekam.

This research paper deals with the in-vitro anti-inflammatory screening of such a medicine documented in Siddha text, *"Anubava vaithiya deva ragasiyam"* indicated for inflammation[5].

Materials and Methods

The test drug (mandura vajra vadugangal) was prepared as per the Standard Operative Procedure (SOP) based on the Siddha literature, '*Anubava vaithya deva ragasiyam'.* The ingredients of the test drug along with descriptions regarding their Botanical names,familynames in siddha medicine are given in **Table 1**.

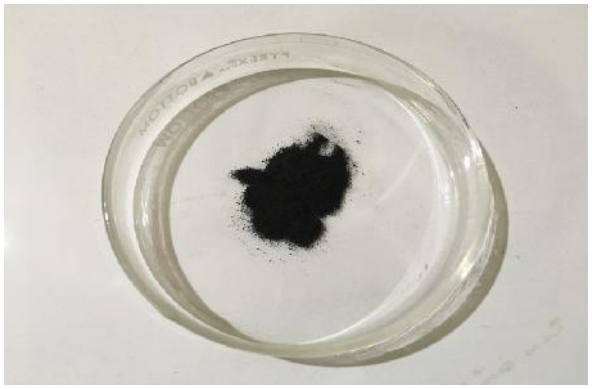
Ingredients of the test drug [6,7,8]:

- Chukku (*Zingiber officinale*)
- Milagu (*Piper nigrum*)
- Thippili(*Piper longum)*
- Thippilimoolam*(Piper longum*)
- Chevuiyam(*Piper nigrum*)
- Devadaru(*Cedrus deodara*)
- Chitramooalm*(Plumbago indica*)
- Kadukkai(*Terminalia chebula*)
- Nellikai*(Phyllanthus emblica*)
- Thandrikai(*Terminalia bellirica*)
- Vaividangam*(Embelia ribes*)
- Manduram(Ferroso ferric oxide)
- Koraikilanzhu(*Cyperus rotundus*)
- Komoothiram(cow's urine)

Table 1: Information about the ingredients of Mandura vajra vadugangal

S. No.	Common name Tamil/English	Botanical name/Family
1	Chukku/Dry ginger	*Zingiber officinale* / Zingiberaceae
2	Milagu / Pepper	*Piper nigrum*/ Piperaceae
3	Thippili/Long pepper	*Piper longum*/ Piperaceae
4	Thippilimoolam	*Piperlongum*/ Piperacace
5	Chevuviyam/Black pepper root	*Piper nigrum*/Piperaceae
6	Devadaru	*Cedrus deodara*/Pinaceae
7	Chitramoolam	*Plumbago zeylanica*/plumbaginaceac
8	Kadukkakai/ink nut	*Terminalia chebula*/Combretaceae
9	Nellikai /Indian gooseberry	*Phyllanthus emblica*/Euphorbiaceae
10	Thantrikai/belliric myrobalan	*Terminalia bellirica*/ Combretaceae
11	Vaivilangam	*Embelia ribes*/ Myrsinaceae
12	koraikilanzhu /nut grass	*cyperus rotundus*/Cyperaceae
13	Manduram	Ferroso ferric oxide
14.	Komoothiram	Cow's urine

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

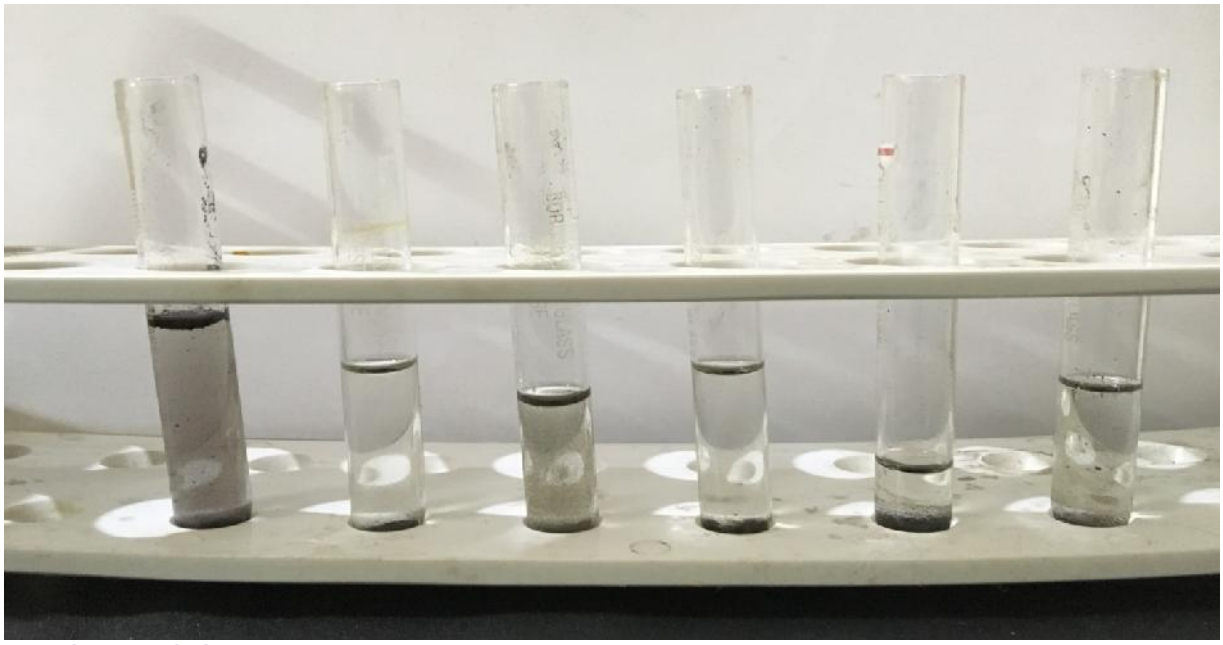


State	Solid
Appearance	Dark Blackish
Nature	Very fine powder

Solubility Assay

S.No	Solvent Used	Solubility
1.	Water	Highly Dispersible
2.	Ethanol	Moderately dispersible
3.	n- Hexane	Partly dispersible
4.	DMSO	Insoluble
5.	Ethyl acetate	Mildly dispersible
6.	Chloroform	Slightly dispersible

Water **Ethanol** **DMSO** **Ethyl acetate** **n-Hexane** **Chloroform**



Stock: 10mg/ml

Albumin Denaturation Assay Procedure[9]

In-vitro anti-inflammatory activity MVV was studied using albumin denaturation technique. The reaction mixture consisted of bovine serum albumin (5% aqueous solution) and test sample MVV 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.

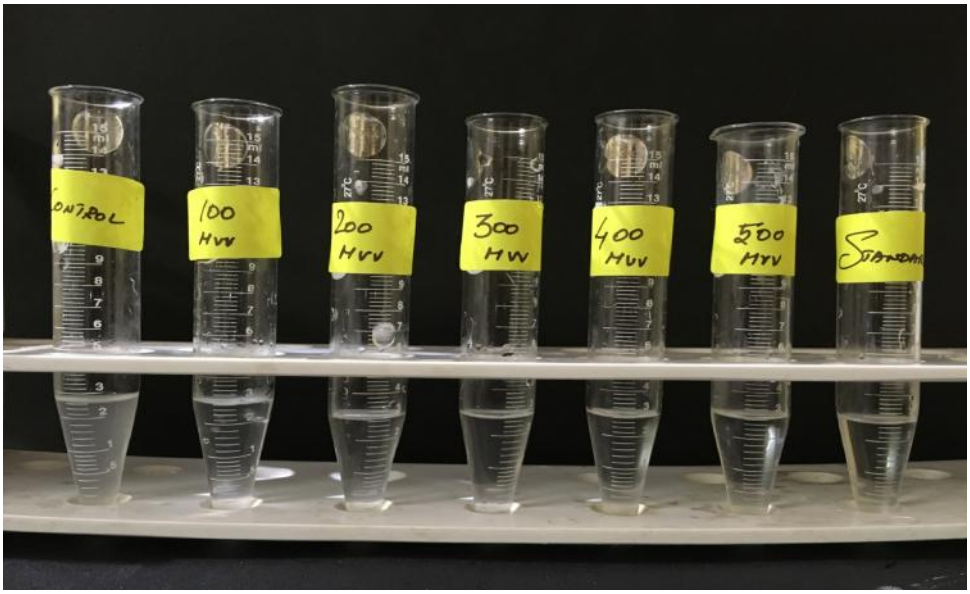
The Percentage protection from denaturation is calculated by using the formulae

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

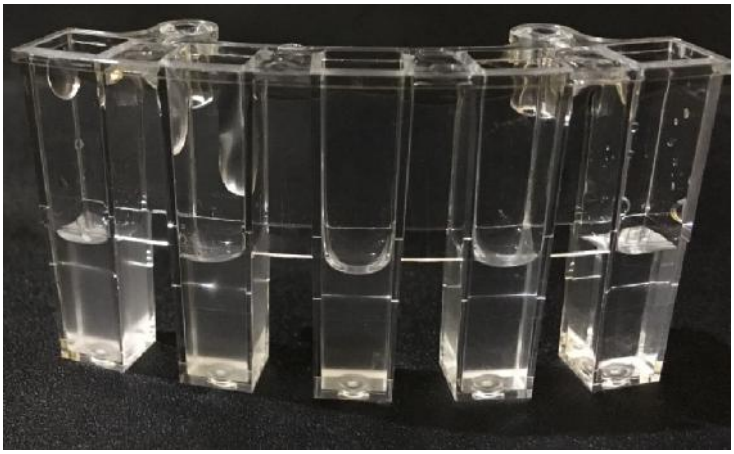
Statistical analysis

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.

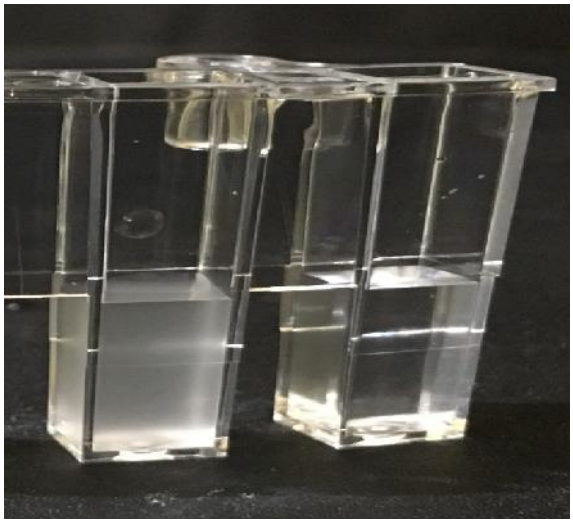
Preparation of Test and control



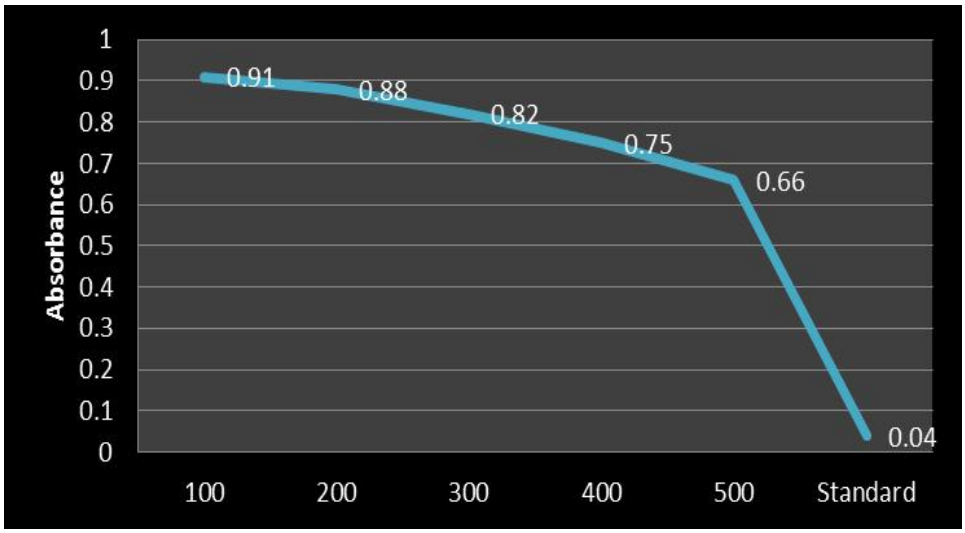
Absorbance of reaction mixture – Test Sample



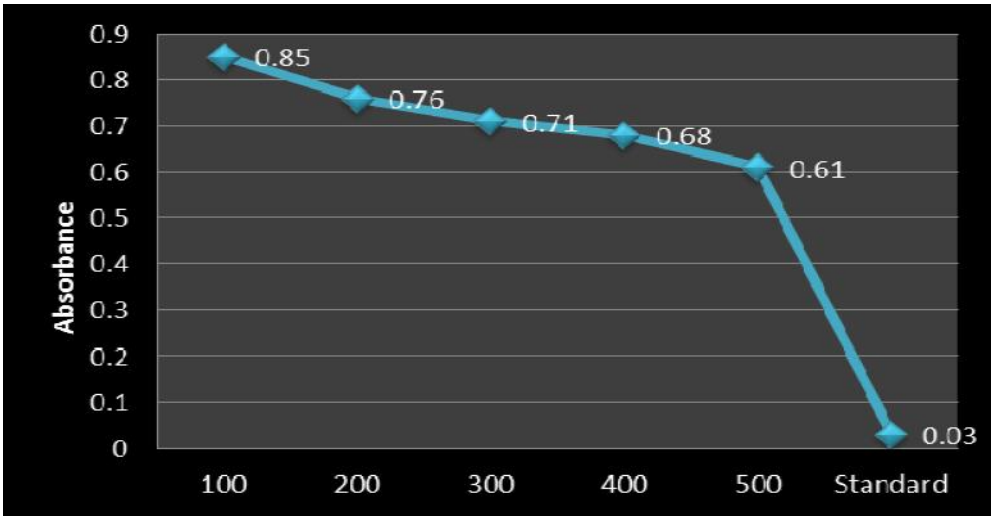
Absorbance of reaction mixture – Control and Standard



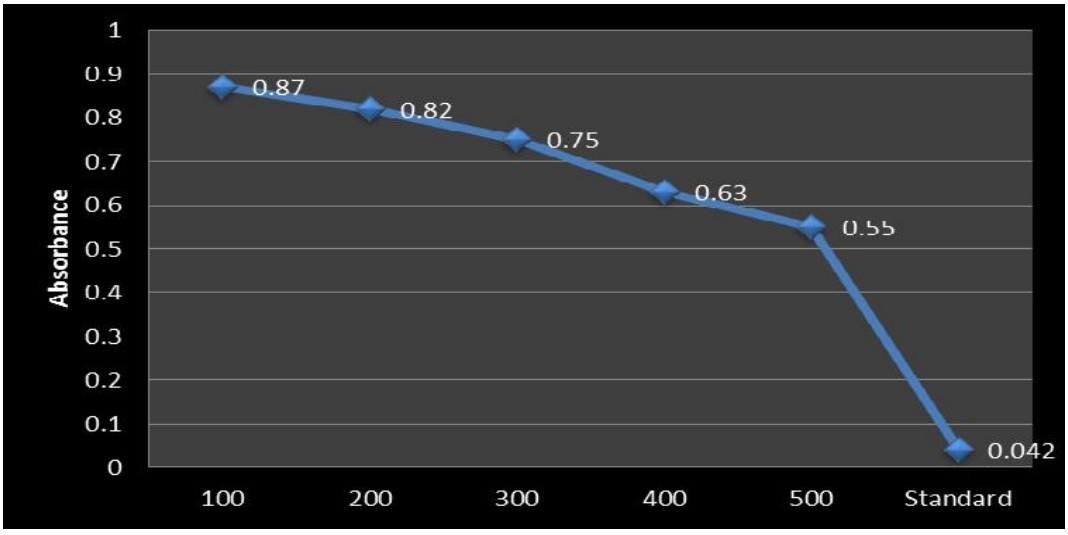
Absorbance Range of test and standard at Trial 1



Absorbance Range of test and standard at Trial 2



Absorbance Range of test and standard at Trial 3



Final Result

Concentration in µg/ml	Absorbance
Control	0.95 ± 0.01
MVV 100	0.87 ± 0.03
MVV 200	0.82 ± 0.06
MVV 300	0.76 ± 0.05
MVV 400	0.68 ± 0.06
MVV 500	0.60 ± 0.05
Diclofenac sodium (100 µg)	0.03 ± 0.09

Each value represents the mean ± SD. N=3

Concentration in µg/ml	Percentage Inhibition of Protein Denaturation
MVV 100	3.39 ± 1.23
MVV 200	9.35 ± 4.39
MVV 300	15.65 ± 3.98
MVV 400	23.33 ± 5.27
MVV 500	31.71 ± 5.03
Diclofenac sodium (100 µg)	91.96 ± 3.58

Each value represents the mean ± SD. N=3

Result Analysis

The result obtained from the present clearly indicates that the test drug MVV was effective in inhibiting heat induced albumin denaturation. Maximum percentage inhibition of about 31.71 % was observed at 500 µg/ml when compare to that of the Diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition 91.96 % at the concentration of 100 µg/ml.

Conclusion

From the result of the study it was concluded that the test drug MVV possess convincing anti-inflammatory property in protein denaturation assay.

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