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PRELIMINARY PHYTOCHEMICAL AND ANTI-MICROBIAL STUDIES ON A POLYHERBAL SIDDHA FORMULATION "DHURVAI CHOORANAM"

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ABSTRACT

Siddha system is the primitive system of medicine being practiced in South India for centuries. A great attention towards our system is being shown nowadays due to the potency of curing diseases with herbs, metals, minerals and animals sources. It is the concept of spreading a healthy soul in a healthy body. Fever in siddha is considered as a disease, rather than a symptom. It is of 64 types. Out of this 32 types is from the intrinsic causes and the other 32 types is from extrinsic causes. One among the 64 types is *kaduppu kaangai. Kaduppu* – intense with pain, *kaangai* – fever, raised temperature, pyrexia. The increase in temperature in hyperpyrexia is due to the involvement of brain which raises the baseline temperature against normal. The term hyperpyrexia differs from hyperthermia,which occurs without the involvement of brain. The incidence of hyperpyrexia in a large urban pediatric emergency department is 0.36 per 1000 visits or approximately one in 2,759 visits. Inorder to overcome from aftermath effects of hyperpyrexia, the siddha polyherbal formulation "*Dhurvai chooranam*" that is indicated for the treatment of *Kaduppu kaangai* which can be correlated with hyperpyrexia associated with the intense pain was subjected to the phytochemical analysis and anti-microbial testing. The phytochemical analysis showed the presence of certain components mainly phenol and quinones. The anti-microbial testing showed that it is active against the organisms such as *Staphylococcus aureus, Klebsiella pneumoniae,*etc., The study reveals it to be an highly effective medicine for *Kaduppu kaangai.*

KEYWORDS: *Kaduppu kaangai*, hyperpyrexia with pain, *Dhurvai chooranam*, polyherbal formulation, siddha,etc.,

INTRODUCTION

D*hurvai chooranam* is a siddha polyherbal formulation mainly consisting of Cynodon dactylon (arugan pul in tamil) referenced from *Agathiyar vaithiya rathna churukkam.* It is indicated for Kaduppu kaangai which can be correlated with hyperpyrexia.

Normal body temperature ranges from 97°F to 99ºF. Increased temperature of more than 106.7ºF is referred as hyperpyrexia. This is not an illness but an important symptom accompanied with various other symptoms such as muscle spasms, rapid breathing, seizures, loss of consciousness often leading to coma. If left untreated it may lead to organ damage and death may occur. It may often occur with infection caused by *Staphylococcus aureus, Staphylococcus pneumoniae, Haemophilus influenzae.*

Due to the existing shortcomings and adverse effect of current therapeutics, there is a search for an alternate, having its origin in nature, which is devoid of adverse effects.

The Siddha material medica mentions about the plant kingdom which been a lot of active ingredients, polyherbal combination for the treatment of challenging and chronic diseases. It not only mentions about herbs, but treatment which can be done through active principles obtained from metals, minerals, and salts.

The key ingredient used in this preparation is the root of *Cynodon dactylon*, which has a wide variety of traditional uses and is documented for febrifuge activity, anti-microbial activity, astringent, diuretic and styptic activities.

In siddha, fever (*suram*) is classified into 64 types. *Kaduppu* -intense, with pain *Kaangai*-fever, raised temperature, pyrexia can be corelated with hyperpyrexia. Based on the literature review and classic test evidence, our primary objective is to evaluate the phytochemical contents and anti-microbial activity of *Dhurvai chooranam* and the secondary objective is to correlate the ingredients with siddha principles such as *Suvai*(taste) and *Panchabootha thathuvam* (five elements theory).

MATERIALS AND METHODS

Drugs are identified in Department of Gunapaadam, Government Siddha Medical College, Palayamkottai.The specimen number is obtained.

COLLECTION OF RAW DRUGS

The table 1 and 2 used for preparation contains raw drugs obtained from a Traditional raw drug store, Tirunelveli, TamilNadu.

Table 1-Ingredients list of the test drug – Dhurvai chooranam

Herbs	**Parts used**	**Quantity**
Arugan pul (*Cynodon dactylon*)	Dried root	58g
Mathulai (*Punica granatum*)	Dried flower	35g
Milagu (*Piper nigrum*)	Dried fruit	35g
Athimathuram (*Glycyrrhiza glabra*)	Dried root	35g
Ilaneer (*Cocos nucifera*)	Tender coconut water	35g

Table 2-Classification of raw drugs based on Suvai(taste) and Panchabootha thathuvam theory (five elements theory)

Ingredients	***Suvai***	***Thanmai***	***Pirivu***
Cynodon dactylon	*Inippu*	*Thatpam*	*Kaarppu*
Punica granatum	*Kaippu, kaarppu*	*Veppam*	*kaarppu*
Piper nigrum	*Kaippu, Kaarppu*	*Veppam*	*Kaarppu*
Glycyrrhiza glabra	*Inippu*	*Thatpam*	*Inippu*

METHOD OF PREPARATION

The Siddha formulation Dhurvai *chooranam* is prepared as per SOP from Department of Gunapadam, Government siddha medical college, Palayamkottai. The fresh root of *Cynodon dactylon* and flowers of *Punica granatum* is collected and dried and used for preparation. *Piper nigrum* and *Glycyrrhiza glabra* are obtained as raw drugs and are purified. All these drugs are made into powder and then mixed with tender coconut water. It is indicated for *Kaduppu kaangai*(hyperpyrexia with intense pain).Dosage : 5g(kottai paakalavu) with butter.

PHYTOCHEMICAL ANALYSIS

ALKALOIDS

Mayer's test: (Ansari, 2006)

The extract was evaporated in a test tube. To the residue dilute HCL was added, shaken well and filtered.

Mayer's Test: To the 2-3 ml of filtrate Mayer's reagent was added. Formation of yellow precipitate showed the presence of alkaloids.

FLAVONOIDS

Shinoda Test: (Kokate, 1994)

To the extract, 5 ml of 95% ethanol and few drops of concentrated hydrochloric acid was added. To this solution 0.5 gm of magnesium turnings were added. Pink colouration indicated the presence of flavanoids.

GLYCOSIDES

Keller-Killiani Test: (Ansari, 2006)

To 2 ml of the extract, glacial acetic acid, one drop 5% $FeCl_3$ and conc. H_2SO_4 was added. Reddish brown colour appeared at junction of two liquid layers and upper layer turned bluish green indicating the presence of glycosides.

PHENOL:

Ferric chloride test: (Mukherjee, 2002)

The extract was diluted to 5 ml with distilled water. To that a few drop of neutral 5% ferric chloride solution was added. A dark green colour indicates the presences of phenolic compounds.

TANNINS

Lead Acetate Test: (Mukherjee, 2002)

On addition of 5% lead acetate solution to the extract white precipitate appeared.

TERPENOIDS (Horbone, 1984)

To the 5ml test solution 2ml chloroform was added with few drops of conc. Sulphuric acid (3ml) at the side of the test tube. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

STEROIDS

Salkowski Test: (IP, 1996)

To 2 ml of extract, 2 ml of chloroform and 2 ml of conc. H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

SAPONINS

Foam Test: (Ansari, 2006)

Sample extract was added with distilled water and shaken vigorously. Observe the stable foam formation.

QUINONES

1ml sample was mixed with 1ml of concentrated sulfuric acid and stand for few minutes. Observe the formation red color indicates the presence of quinines in the sample.

ANTHOCYANIN

2ml of 2N Hcl was mixed with 2ml of test sample. Observe the pink red color turns into blue violet color indicates the presence of anthocyanin in sample.

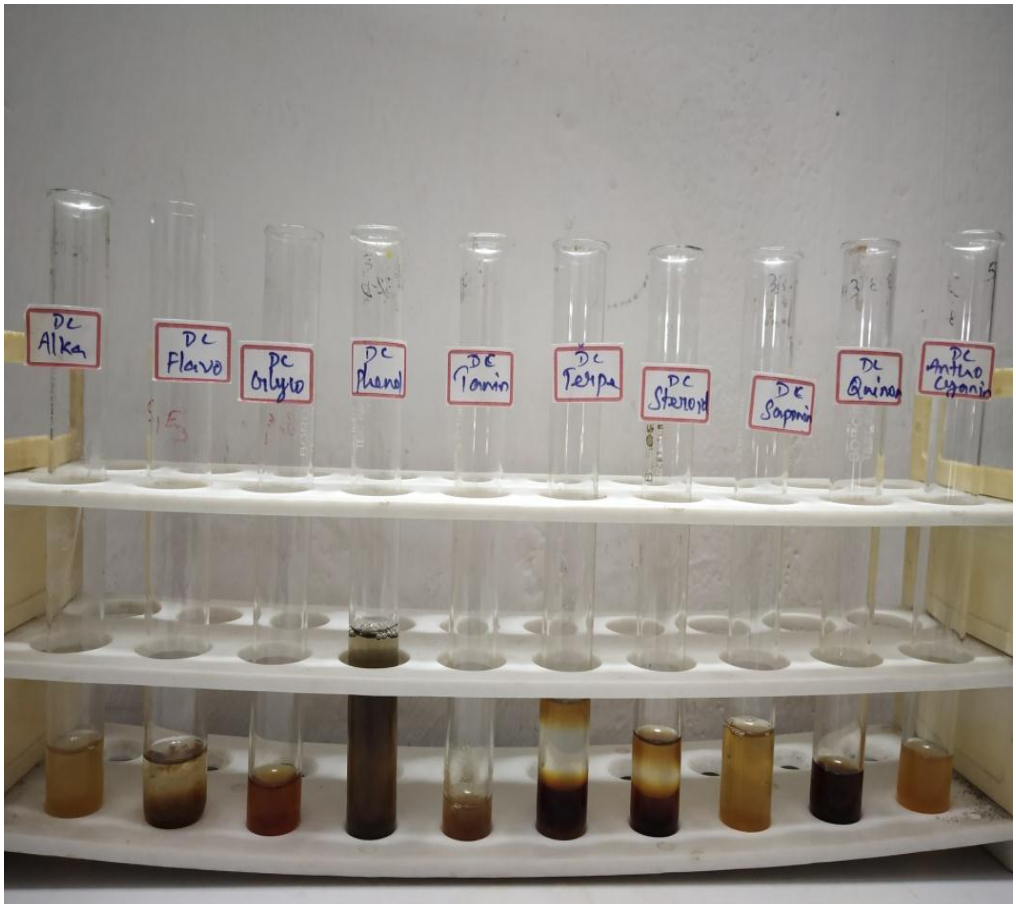
PHYTOCHEMICAL RESULTS

Table 3: Qualitative Phytochemical analysis of plant samples

TEST NAME	Aqueous DC
Alkaloids	Absent
Flavonoids	Absent

Glycosides	**Absent**
Phenol	**Present**
Tannin	**Present**
Terpenoids	**Present**
Steroids	**Present**
Saponin	**Present**
Quinones	**Present**
Anthocyanins	**Absent**

Figures: Qualitative Phytochemical analysis of plant samples



ANTIMICROBIAL ACTIVITY PROCEDURE

Antibacterial Activity Procedure

Dilution: 0.1g in 1ml

Test Organism

The test microorganisms used for antimicrobial analysis *Microorganism Name* were purchased from Microbial Type Culture Collection and Gene Bank (***MTCC***) Chandigarh. The bacterial strains were maintained on Nutrient Agar (NA).

Nutrient Broth Preparation

Pure culture from the plate were inoculated into Nutrient Agar plate and sub cultured at 37ºC for 24 h. Inoculum was prepared by aseptically adding the fresh culture into 2 ml of sterile 0.145 mol/L saline tube and the cell density was adjusted to 0.5 McFarland turbidity standard to yield a bacterial suspension of 1.5×108 cfu/ml. Standardized inoculum Used for Antimicrobial test.

Antimicrobial Test

The medium was prepared by dissolving 38 g of Muller Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 Lbs pressure at 121^0C for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured petriplates (25 ml/plate) the plates were swabbed with Pathogenic Bacteria culture viz. ***Microorganism name*** Finally, The Sample or Sample loaded Disc was then placed on the surface of Mullar-Hinton medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimetres. The size of the zone of inhibition (including disc) was measured in millimetres. The absence of zone inhibition was interpreted as the absence of activity (Kohner *et al*., 1994; Mathabe *et al*., 2006). The activities are expressed as resistant, if the zone of inhibition was less than 7 mm, intermediate (8-10 mm) and sensitive if more than 11 mm (Assam *et al*., 2010)

ANTIMICROBIAL RESULTS

Samples	Strains				
	Staphylococcus aureus (G+) MTCC 916	**Bacillus subtilis** MTCC 1134 (G+)	**E- coli** (G-) MTCC 1671	**Klebsiella pneumonia** (G-) MTCC 503	**Proteus vulgaris**(G-) MTCC 426
DC .Aq 25 µl	8	12	10	10	9
DC.Aq 50 µl	10	14	12	16	11
DC.Aq 75 µl	12	16	14	17	12
DC.Aq 100 µl	14	17	16	18	14
PC	13	21	27	19	24
NC	-	-	-	-	-

Table 4:

Keys

PC (Bacteria) - ***Positive control (Streptomycin- S 25)***

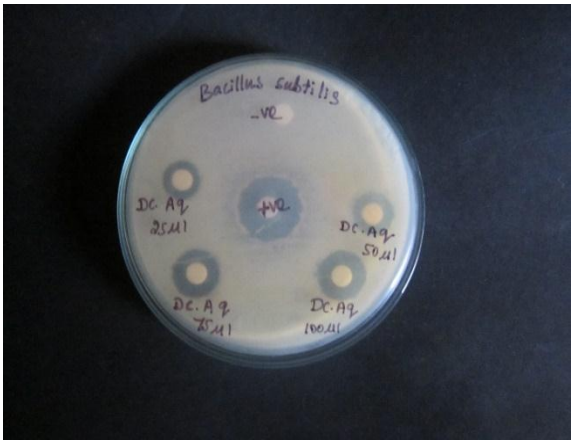
NC - ***Negative (plain disc)***

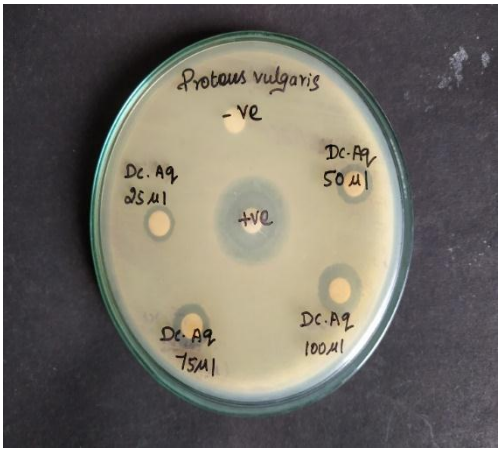
- - ***No Zone***

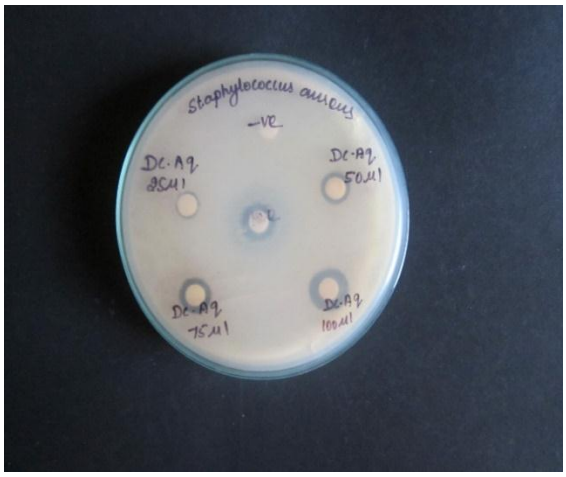
Mm - ***Millimetre***

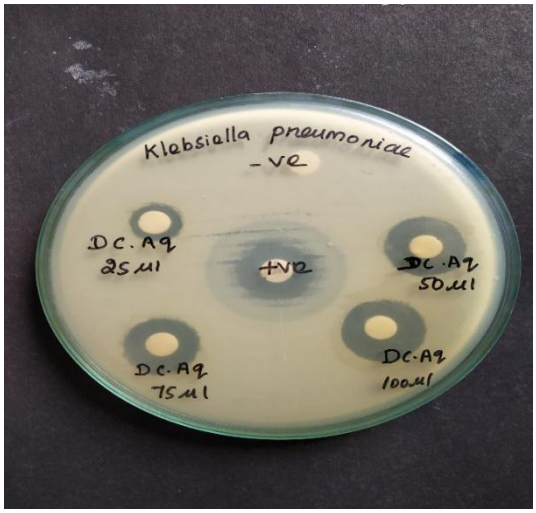
G+ - ***Gram Positive organism***

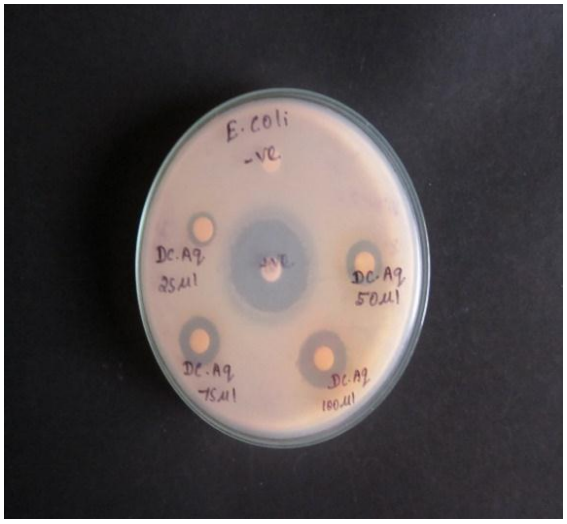
G- - ***Gram Negative organism***


Bacillus subtilis
-ve
Dc.Aq 25µl
+ve
Dc.Aq 50µl
Dc.Aq 75µl
Dc.Aq 100µl


Proteus vulgaris
-ve
Dc.Aq 25µl
Dc.Aq 50µl
+ve
Dc.Aq 75µl
Dc.Aq 100µl


Staphylococcus aureus
-ve
Dc.Aq 25µl
Dc.Aq 50µl
Dc.Aq 75µl
Dc.Aq 100µl


Klebsialla pneumoniae
-ve
Dc.Aq 25µl
+ve
Dc.Aq 50µl
Dc.Aq 75µl
Dc.Aq 100µl


E.Coli
-ve
Dc.Aq 25µl
+ve
Dc.Aq 50µl
Dc.Aq 75µl
Dc.Aq 100µl

MIC PROCEDURE

Two fold dilutions of the antibiotic solution in Mueller Hinton broth were prepared and describe below: Ten sterile tubes were placed in a rack and were labeled each 1 through 8 and last 9th labeled as antibiotic control and 10th was labeled as growth control. 10 ml of Mueller Hinton broth was added in each test tube(1to 8) 2mg of Sample was added to test tube no 1, 1mg of Sample was added to test tube no 2, 0.5 mg of Sample was added to test tube no 3, 0.25 mg of Sample was added to test tube no 4 , 0.12 mg of Sample was added to test tube no 5, 0.06mg of Sample was added to test tube no 6 , 0.03 mg of Sample was added to test tube no 7 and 0.01 mg of Sample was added to test tube no 8. The 9th received no antimicrobial agent and was served as a growth control. 10^{th} labeled test tube has only antimicrobial agent was served as a positive control. Each tube was inoculated (including the growth control except antibiotic control) with 0.1 ml of the culture of respective organism. The tubes were incubated at 37^0C for 24 hours. The tubes were examined for growth and were determined the MIC of tested antibiotics, which is bacteriostatic for the test organism. The tubes were examined for visible growth (cloudy) and was recorded growth as (+) and no growth as (-).

Minimum Inhibitory Concentration (MIC)

Sample Concentration	
2.0	-
1.0	-
0.5	-
0.25	-
0.12	+
0.06	+
0.03	+
0.01	+
PC	+
NC	-

Result

Minimum Inhibitory Concentration is 0.25.

Keys

\+ = Growth

\- = No Growth

PC = Bacteria only

NC = Sample only

RESULTS AND DISCUSSIONS

Based on Suvai concept, Hyperpyrexia is mainly due to elevation of *pitham* accompanied with *kabham* in the body. Each ingredient is analysed in table 2 that showed various quantities of ingredients with *thuvarppu, inippu, kaippu*, that mitigate dominant *pitham* as a result of which disease burden can be reduced. The symptoms of hyperpyrexia are also related to *pitham* and it can be mitigate by *neer*(water) and *prithvi*(earth) bootham. By comparing these basic principles the treatment selection pattern can be done based upon the combination of the drugs.

The phytochemical analysis of this formulation reveals the presence of phenol, tannin, saponin, terpenoids, steroids and quinones. Among these, quinones which has the property of anti-pyretic action that aids in rehabilitation of *Kaduppu kaangai.*

Anti-microbial activity of the *Dhurvai chooranam* mentioned in Table 4 shows significant antibacterial engulfing potency. It can help in evading the microbial infections especially *Klebsiella pneumoniae.*

CONCLUSIONS

Evaluation of traditional medicine can be done through proper documentation of the literary evidence and scientific validations through universally accepted parameters. The results from the phytochemical analysis and anti-microbial activity testing of *Dhurvai chooranam* proves it to be an effective medicine in the management of *Kaduppu kaangai* as mentioned in the literature. This is only a preliminary study done with available infrastructure, more work is to be done to explore the

efficacy of this safe, economical, herbal, non invasive drug for hyperpyrexia associated with intense pain.

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