

IN VITRO EVALUATION OF ANTI INFLAMMATORY AND ANTI MICROBIAL ACTIVITY OF SIDDHA FORMULATION ADAKKI KASAYAM

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Abstract- Inflammation represents an adaptive response generated by injuries or harmful stimuli. Natural remedies represent an interesting alternative to traditional therapies, involving several biochemical pathways. Besides, the valorization of agrochemical wastes nowadays seems to be a feasible way to reduce the health spending and improve the accessibility at bioactive natural compounds.

The emergence of pandrug resistant bacteria has become a major problem. There is a continuous effort by the pharmaceutical industries to develop new antimicrobial agents for the treatment of the infections. Due to the increase of resistance to antibiotics, there is a pressing need to develop new and innovative antimicrobial agents. One approach is to screen local medicinal plants which represent a rich source of novel antimicrobial agents. Athimadhuram (*Glycyrrhiza glabra*) and seeragam(*cuminum cyminum*) are such plants which has shown remarkable activity against large number of organisms, such as bacteria, fungi, viruses, parasites etc.

This review has tried to give an account of multifold activity of various bioactive molecules of ADAKKI KASAYAM that mentioned in madhalai noi thoguthi ,ingredients are Athimadhuram (*Glycyrrhiza glabra*) and seeragam(*cuminum cyminum*) against various Microorganisms and inflammatory mediators.

Key words: Adakki kasayam, Anti inflammatory, Anti microbial, Athimadhuram, Seeragam

INTRODUCTION

The control of hospital-acquired infection caused by multi-drug resistant bacilli has proved to be a problem over the last 20 years. Such infections are often extremely difficult for the clinician to treat because of the widespread resistance of these bacteria to the major groups of antibiotics. So there is a continuous effort by the pharmaceutical industries to develop new antimicrobial agents for the treatment of the infections. The use of herbal medicines by traditional healers for the treatment of diseases remains the main stay of health care system. It is gaining increasing popularity especially among the rural population in the developing countries since it is an effective and cheap source of medical care.

The World Health Organization (WHO) estimated that about an 80% population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs. Particularly in rural India, uses of raw plant products as well as some concoction of siddha medicines are sought after to a great proportion, because of cheap availability, and in urban areas too those are popular.

India is blessed with a great heritage of traditional knowledge on medicines. Siddha, being the most ancient and still successfully practiced science amongst all these systems, needs a special attention while searching for solutions to the unresolved health problems. There exists a serious need to validate the uses of different therapeutic modalities which are in practice and can serve as a potent, safer and cost effective solution to many global disorders.

Athimadhuram (*Glycyrrhiza glabra*) and seeragam(*cuminum cyminum*) are such plants which symbolizes all that is wondrous in nature because, the whole plants has been used as traditional medicine for household remedy against various human ailments from antiquity. It has been a mainstay of Siddha and other traditional medicines. In ancient siddha System, more than 1250 preparations are described containing athimathuram and seeragam as one of its constituents, most of them pediatric medicines, Many amongst these preparations have been successfully used in various infectious and inflammatory conditions.

DRUG REVIEW**ADAKI KASAYAM**

- 1)Glycyrrhiza glabra-அதிமதுரம்
- 2)Cuminum cyminium-சீரகம்

PREPARATION:

Above the drugs are partially grinded take 5.1g of the kashaya chooranam added into 120ml of water,then it has been shrunken into the 30ml of decoction.

USAGE: Internal

INDICATION:SURA PEENISAM(Pediatric Sinusitis)

REFERENCE:madhalai noi thoguthi part-1 pg no 372,
Dr.T.Mohan raj.Bsc,BSM.

ANTI-MICROBIAL ACTIVITY OF ADAKKI KASAYAM**PROCEDURE:**

Test drug were screened for antimicrobial activities at the concentration of 50 , 100 and 200 microgram by paper disc diffusion technique. Compounds are screened in vitro for their anti-microbial activity against *E. coli*, *S. aureus*, *P. aeruginosa* and *B. subtilis* are compared with standard drug ciprofloxacin (std disc). The zones of inhibition formed for the compounds against bacteria were calculated. Test compound showed certain degree of anti-bacterial activities. Zone of inhibition was observed for 24 hours and also for 48 hrs. There is no significant change was observed between the 24 and 48 hr data.

Detail of the organism used for the study.

Grams strain	Name of the organism	Std Code
Gram-negative rod	<i>Pseudomonas aeruginosa</i>	(ATCC-2853)
Gram negative	<i>Escherichia coli</i>	(ATCC-25922)
Gram- positive bacterium	<i>Bacillus subtilis</i>	(ATCC-6051)
Gram-positive spherical bacteria	<i>Staphylococcus aureus</i>	(ATCC-9144)

Disc-diffusion assay

The antibacterial activities of all test compounds were carried out by disc diffusion method .The concentrations of the test compounds were used in the concentration of 50, 100, 200 µg /ml diluted with DMSO.

The target microorganisms were cultured in Mueller– Hinton broth (MHB). After 24 h the suspensions were adjusted to standard sub culture dilution. The Petri dishes containing Muller Hinton Agar (MHA) medium were cultured with diluted bacterial strain. Disc made of Whatman No.1, diameter 6 mm was pre sterilized and was maintained in aseptic chamber. Each concentration was injected to the sterile disc papers (Whatman No.1, diameter 6 mm). Then the prepared discs were placed on the culture medium. Standard drug Ciprofloxacin (10 µg) was used as a positive reference standard to determine the sensitivity of each microbial species tested. Then the inoculated plates were incubated at 37 °C for 24 h for the tested microorganisms. The diameter of the clear zone around the disc was measured and expressed in millimeters as its anti-microbial activity. Four discs per plate were used.

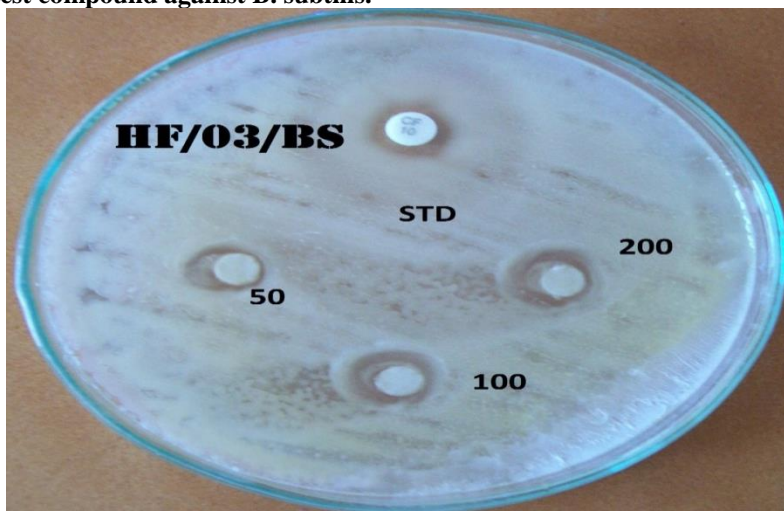
Anti-bacterial activity of test compound against the *E. coli*, *S. aureus*, *P. aeruginosa* and *B. subtilis*.

Results and Interpretation

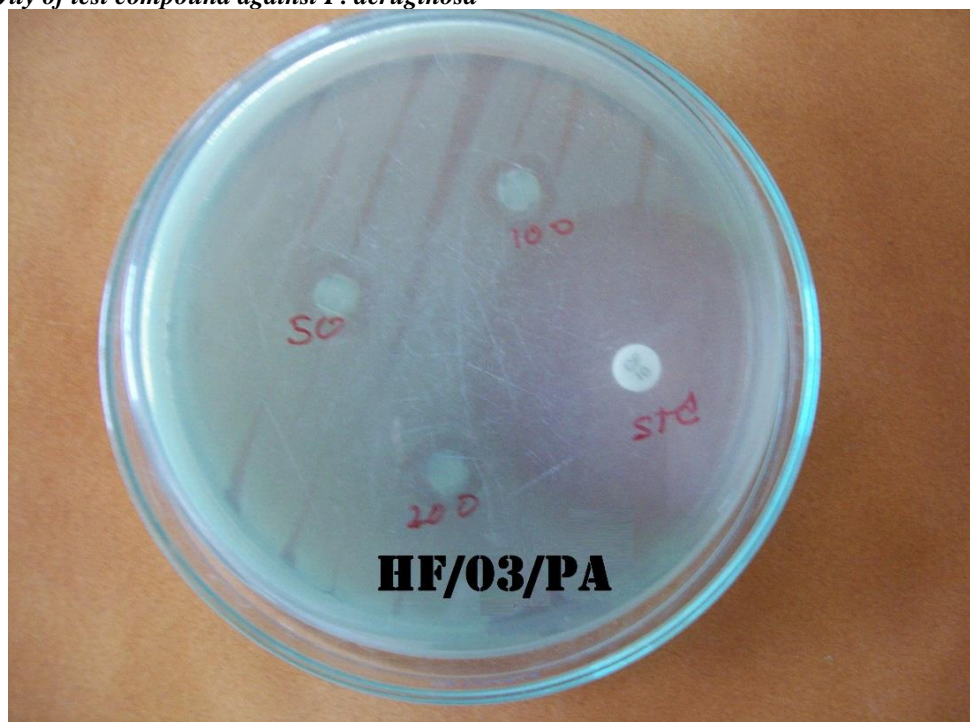
Zone of Inhibition in mm				
CODE	50 µ g	100 µ g	200 µ g	Std Ciprofloxacin (10µg)
HF/03/EC <i>E. coli</i>	9	12	15	26
HF/03/SA <i>S. aureus</i>	5	08	10	17

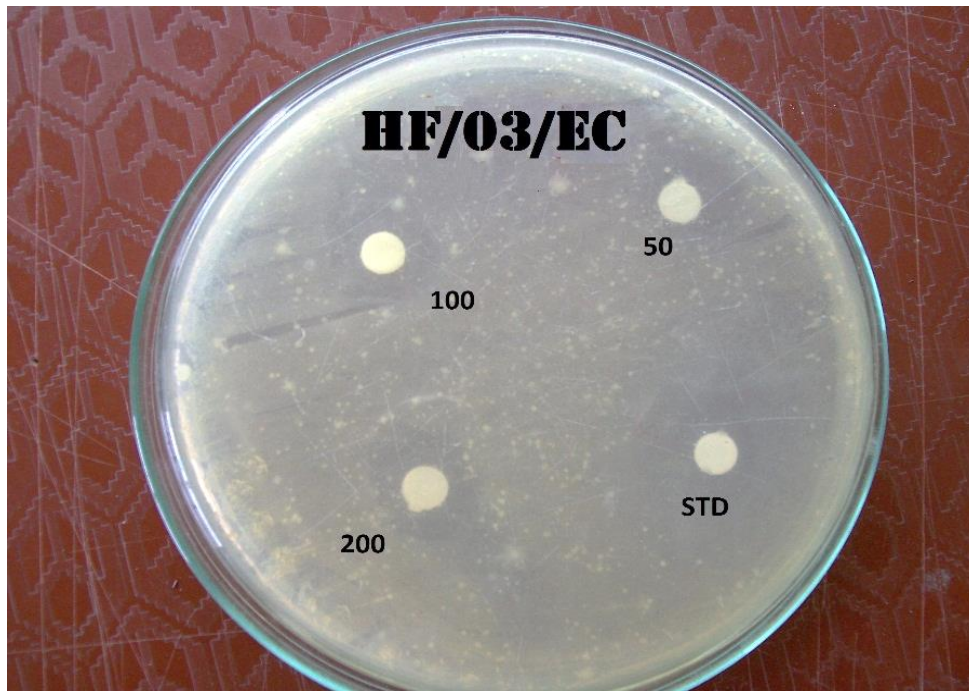
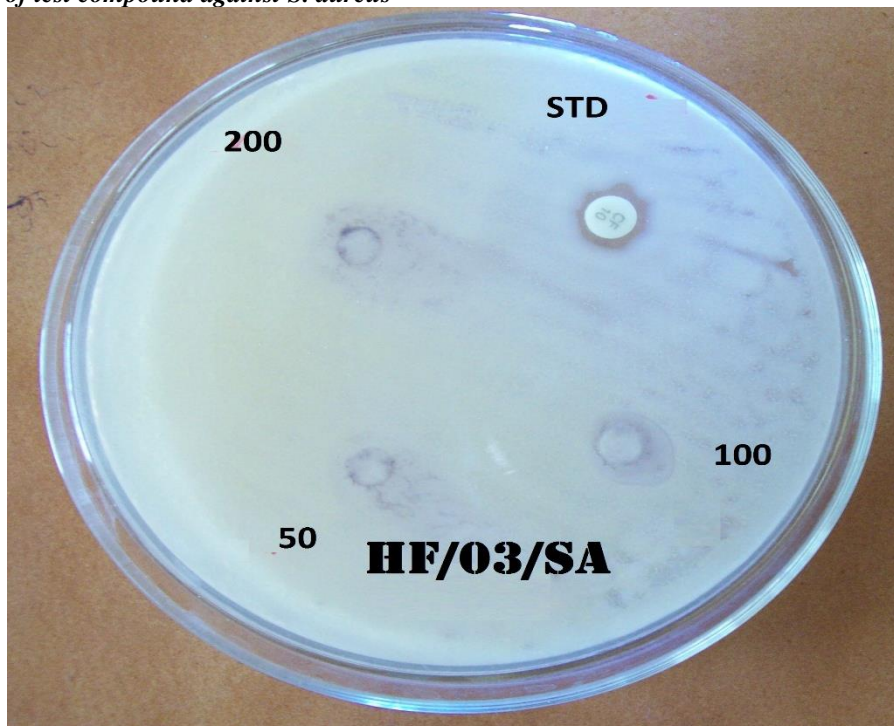
ssHF/03/BS <i>B. subtilis</i>	4	6	8	22
HF/03/PA <i>P. aeruginosa</i>	5	7	7	31

Anti-bacterial activity of test compound against *B. subtilis*.



Anti-bacterial activity of test compound against *P. aeruginosa*



Anti-bacterial activity of test compound against E. coli*Anti-bacterial activity of test compound against S. aureus***RESULT:**

From the observation of zone of inhibition it was concluded that the test compound was highly effective against E-coli with maximum zone of inhibition of 15 mm at the concentration of 200 μg and also markedly effective against S. aureus with zone of inhibition of 10 mm at the concentration of 200 μg .

Test compound shows least activity against inhibitory zones against B. subtilis and P. aeruginosa

ANTI-INFLAMMATORY STUDIES USING ADAKKI KASAYAM (AK)

For the experiment, the animals were divided into 5 groups with 6 animals in each group.

- Group-I (control) received 3% gum acacia 10 ml/kg p.o.
- Group-II (Carageenan) received 0.1ml of 1% w/v suspension of carrageenan S.C
- Group-III (standard) received Indomethacin 40 mg/kg p.o.

- Group-IV(Test-1) received AK 20ml /kg p.o. (Low dose)
- Group-V(Test-2) received AK 40ml /kg p.o. (High dose)

All the drugs were administered orally and the volume of medicaments kept constant at 20ml/kg body weight of the animals it was administered orally to rats 1 hr before subcutaneous injection of carrageenan. After 1 hr 0.1ml of 1% w/v suspension of carrageenan was injected into sub-plantar region of the left hind paw to all the groups. The paw volume was measured at 1, 2, 3, 4, and 5 hr using Plethysmometer (Model 7150 UGO Basile, Italy) Edema was expressed as the mean increase in paw volume relative to control animals.

PAW EDEMA VOLUME

Group	Dose	Initial paw volume	Change in paw edema mm at different time intervals				
			0hr	1 hr	2hr	3hr	4hr
I	Control	1.29 ± 0.14	1.29±0.14	1.29±0.14	1.29±0.14	1.29±.14	1.29±0.14
II	Carrageenan	1.29± 0.17	1.98 ± 0.21	2.31 ± 0.02	2.39 ± 0.14	2.40 ± 0.18	2.71 ± 0.17
III	Indomethacin	1.01± 0.06	2.32 ± 0.26	1.59 ± 0.15	1.49 ± 0.05	1.33 ± 0.18	1.14 ± 0.16
IV	Low dose	1.44 ± 0.13	1.54 ± 0.32	1.64 ± 0.36	1.72 ± 0.64	1.53 ± 0.22	1.59 ± 0.32
V	High dose	1.22 ± 0.44	1.92 ± 0.42	1.86 ± 0.54	1.66 ± 0.64	1.60 ± 0.28	1.30 ± 0.12

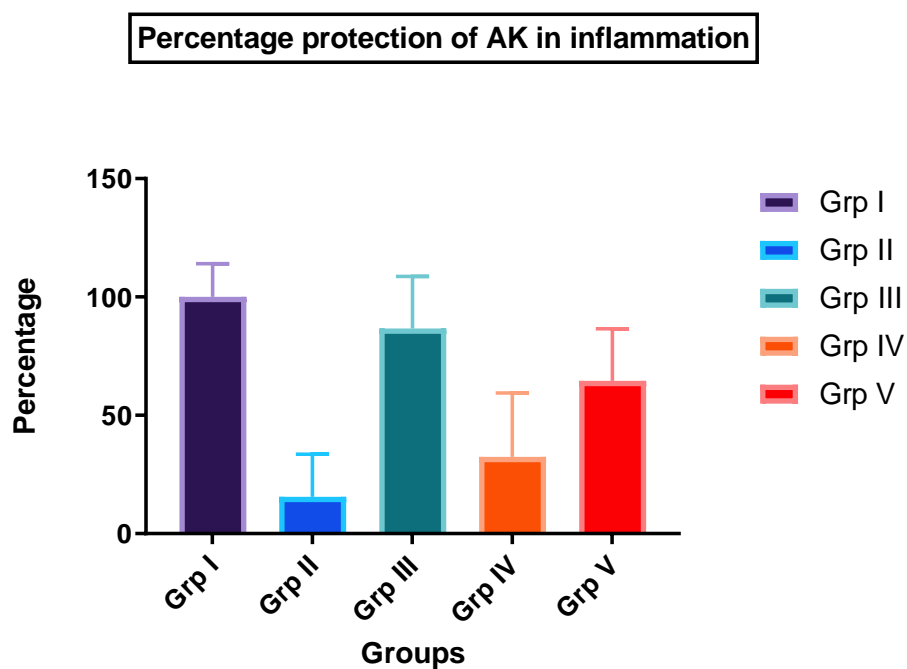
The paw volume up to the tibiotaral articulation was measured at 0, 1, 2, 3, 4, 5 hrs

Group	Initial paw volume	5 hr in mm	Difference in paw volume	Percentage protection
I	1.20 ± 0.14	1.20±0.14	0.00	100
II	1.21± 0.17	2.62 ± 0.17	1.41	15.59
III	1.01± 0.06	1.15 ± 0.16	0.24	86.67
IV	1.44 ± 0.13	1.59 ± 0.32	0.15	32.42
V	1.32 ± 0.44	1.30 ± 0.12	0.08	64.54

Percentage protection is calculated by the formulae: $(T_2 - T_1 / T_2) \times 100$

T₁----normal control

T₂----drug treated test

**RESULT:**

From the observation of paw edema volume and percentage protection of AK in inflammation it was concluded that the test compound was highly effective in Inflammatory cells.

CONCLUSION:

Based on these results, It can be concluded that **ADAKKI KASAYAM** has potential **Anti inflammatory** and **Anti microbial** activity.

This study results confirmed the validating of traditional indication adakki kasayam in microbial and inflammatory conditions. so more clinical trails and in vivo studies should be done for standard drug development.