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In vitro Anti – inflammatory and Anti microbial activity Profiling by diffusion method for Siddha Formulation drug- Vali Kana Kudineer (VKK)

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Abstract

This study aimed to reveal the anti – inflammatory and anti - microbial activities of Vali kana kudineer. From the very beginning of the civilization, there is an extreme relationship between human beings and plants. In ancient period, the system of treatment was not enriched like today. The ancient people used to utilize several parts of plants in different treatment purposes. An antibacterial agent that either kills microorganism or suppresses its growth is often termed as antibiotic. The term antibiotic covers a broad range of agents like antimicrobials, including antifungal and other compounds.

The anti - microbial activities of the test sample were carried out by well diffusion method. In-vitro anti-inflammatory activity of VKK was studied using albumin denaturation technique.

From the result of the study it was concluded that the test drug Vali kana kudineer possess promising higher Anti inflammatory property than Anti microbial by protein denaturation assay method. In addition the sample Vali kana kudineer also help in URT diseases.

Keywords: Vali kana kudineer, Anti – inflammatory, Antimicrobial, Siddha drug

Introduction

Pharyngitis is being 1/3 of the primary system of the upper respiratory tract infection in children. Acute pharyngitis inflammation of mucous membranes and underlying structures of the throat, characterized by fever, sore throat and pharyngeal exudates. Most common infectious agents are Group A Streptococcus and various viral agents. Every year, 10% of children are affected by Acute pharyngitis. Peak prevalence of Acute Pharyngitis in children is aged between 5-10 years.

In siddha literature, valikanam is one of the 24 types of ‘‘kanam’’ that occurs in children. From the very beginning of the civilization, there is an extreme relationship between human beings and plants. In ancient period the system of treatment was not enriched like today.The ancient people used to utilize several parts of plants in different treatment purposes. An antibacterial agent that either kills microorganism or suppresses its growth is often termed as antibiotic. The term antibiotic covers a broad range of agents like antimicrobials, including antifungal and other compounds.

The medicine chosen for the treatment and management of the valikanam was valikanakudineer, 15-30ml internally, twice a day after food as referenced in pillaipinimaruthuvam. In this study, valikanakudineer was selected and standardization technique as per procedures were done. Ingredients of vali kana kudineerare *Trigonella foenum graecum, Allium cepa, Aegle marmelos.* Vali kana kudineer considerably has promising Anti inflammatory activity against pathogenic organisms. In the same way, the anti-microbial activity tested was found ineffective against the respective pathogens.

Materials and Methods

In-vitro Anti-Inflammatory Activity

Albumin Denaturation Assay Procedure

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

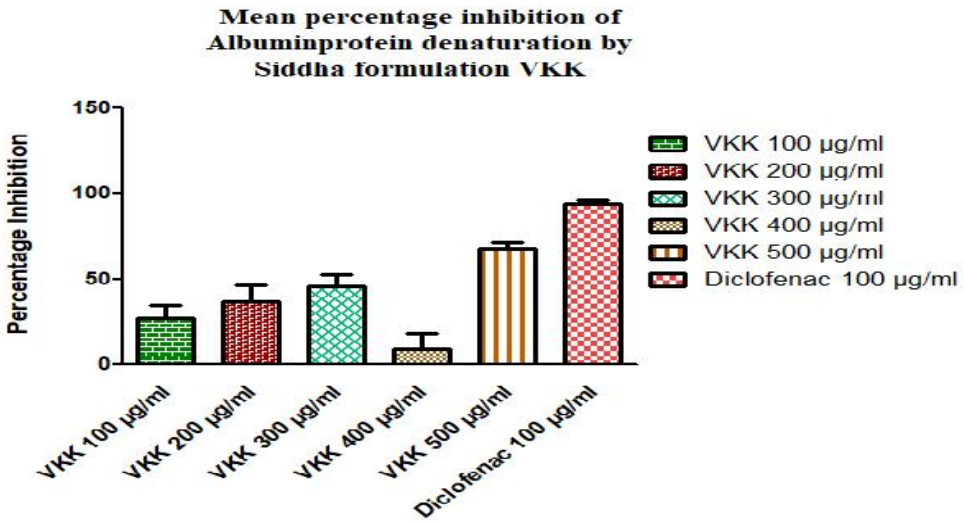
Results are expressed as Mean ± SD. The difference between experimental groups was compared by One-Way Analysis of Variance (ANOVA) followed by Dunnet Multiple comparison test.

Results

Concentration in µg/ml	Percentage Inhibition of Protein Denaturation
VKK 100	26.93 ± 7.639
VKK 200	36.65 ± 9.61
VKK 300	46.07 ± 6.549
VKK 400	57.73 ± 9.417
VKK 500	68.95 ± 8.564
Diclofenac sodium (100 µg)	94.81 ± 3.029

Each value represents the mean ± SD. N=3

Percentage Inhibition of Protein Denaturation by VKK and Standard



Result Analysis

The result obtained from the present clearly indicates that the test drug VKK was effective in inhibiting heat induced albumin denaturation. Maximum percentage inhibition of about 68.95 ± 8.564 % was observed at 500 µg/ml when compare to that of the Diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition 94.81 ± 3.029at the concentration of 100 µg/ml.

Anti - microbial Disc-diffusion method:

The antibacterial activities of the test sample were carried out by well diffusion method. The concentrations of the test compounds were used at the concentration of 250, 500, 1000 and 2000 µg. 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. Well made with the diameter of 6 mm was in aseptic chamber. Each concentration was injected to the well cut on the surface of the culture medium. Standard drug Streptomycin (20µg) was used as a positive reference standard to determine the sensitivity of each microbial species tested. Then the inoculated plates were incubated at 37^{o} C for 24 h. The diameter of the clear zone around the disc was measured and expressed in millimeters as its anti-bacterial property. The results were depicted in the table.

Organisms used for Anti-Bacterial Activity

S.no	Organisms	Type
1	*E. coli*	Gram - Negative
2	*Staphylococcus aureus*	Gram - Positive
3	*Klebsiella pneumoniae*	Gram - Negative
4	*Pseudomonas aeruginosa*	Gram - Negative

Code sequence of well Expansion

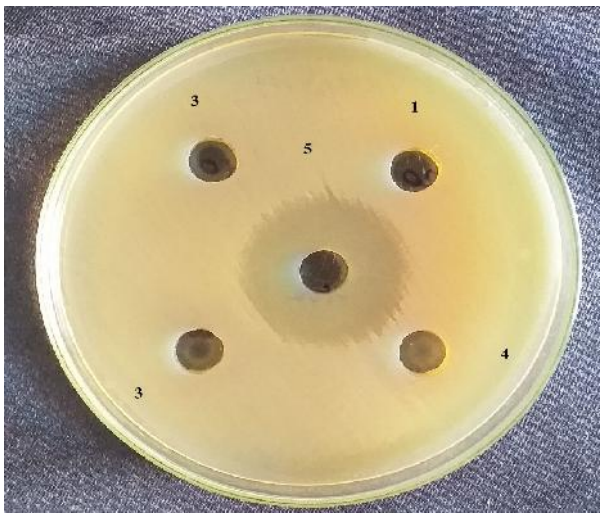
Well No	1	2	3	4	5
Drug Concentration	VKK- 250 µg	VKK -500 µg	VKK -1000 µg	VKK -2000 µg	Streptomycin 20 µg

Zone of Inhibition data of Anti-bacterial activity

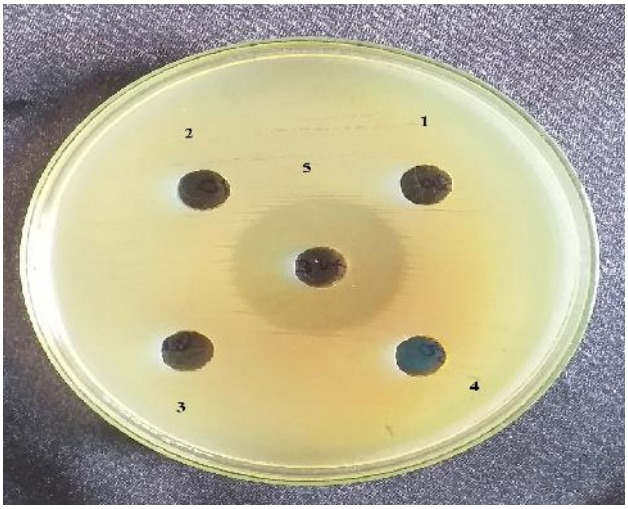
Microorganisms/Sample	**Zone of Inhibition in mm**				
	250 µg (Well no 1)	**500 µg (Well no 2)**	**1000 µg (Well no 3)**	**2000 µg (Well no 4)**	**Streptomycin 20µg (Well no 5)**
E. coli	-	-	-	-	21
Staphylococcus aureus	-	-	-	-	30
Klebsiella pneumoniae	-	-	-	-	27
Pseudomonas aeruginosa	-	-	-	-	29

Anti- Microbial Effect of Sample VKK against E. coli

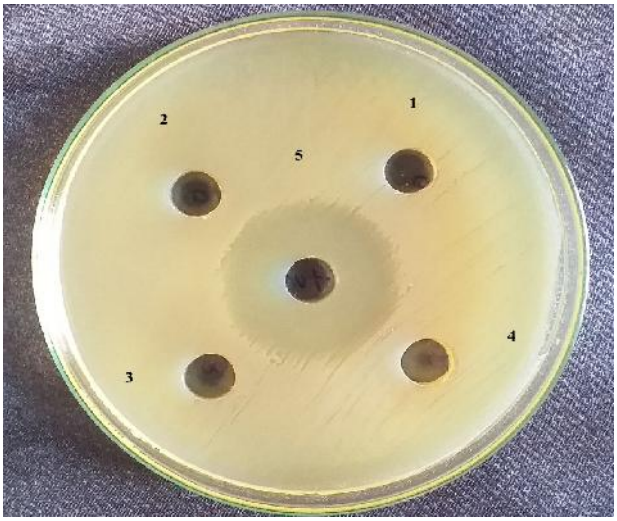
Anti- Microbial Effect of Sample VKK against Staphylococcus aureus



Anti- Microbial Effect of Sample VKK against Klebsiella pneumoniae



Anti- Microbial Effect of Sample VKK against Pseudomonas aeruginosa



Conclusion

From the result of the study it was concluded that the test drug Vali kana kudineer possess promising higher anti-inflammatory property than anti microbial by protein denaturation assay method. In addition the sample vali kana kudineer also helps in URT diseases. It can also be a fruitful study in future for well – being of mankind.

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