

**EVALUATION OF ANTI-MICROBIAL POTENTIAL OF POLYHERBAL SIDDHA  
FORMULATION AGHIL KATTAI CHOORANAM (AKC) AGAINST GARDNERELLA  
VAGINALIS AND CANDIDA ALBICANS**

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**ABSTRACT**

Bacterial vaginosis (BV) is a common vaginal infection associated with numerous gynecological and obstetric complications. This condition is characterized by the presence of thick adherent vaginal biofilms, composed mainly of *Gardnerella vaginalis*. This organism is thought to be the primary etiological cause of the infection. Vaginal candidiasis (VC) is another most common gynecological problem seen in primary care. *Candida albicans* account for 90% of the infection. Antibiotic therapy for the clinical management of BV and VC often ends with failure due to resistance offered by the pathogen towards such drugs. Hence there is a need of developing antimicrobial lead from alternate origin which is tend to act my multiple mechanism .In recent years, there has been a growing interest in the field of drug discovery for developing new antimicrobial agents from herbal origin to combat microbial resistance. Siddha system of medicine have identified numerous medicinal plants and its applications in strengthening human health care system and the formulations based on such medicinal plants play an important role in alleviating most pathogenic infection occurs in humans. One such formulation is Aghil kattai Chooranam (AKC) used in the traditional practice for clinical management of several infectious diseases. The main objective of the present investigation is to evaluate the anti-microbial potential of AKC against vaginal pathogens *Gardenerella vaginalis* and *Candida albicans* by agar disc diffusion method. The result of the study signifies that siddha formulation AKC offers significant activity against *Gardenerella vaginalis* with zone of inhibition varying from 7mm to 12 mm when compare to standard Metronidazole with maximum zone of 25 mm at a same time the formulation AKC is not effective against *Candida albicans*. In conclusion the Siddha formulation AKC may use for the clinical management of bacterial vaginosis and leucorrhoea associated with *Gardenerella vaginalis* infection.

**KEYWORDS:** Bacterial vaginosis, Vaginal candidiasis, Aghil kattai Chooranam , Antimicrobial, *Gardenerella vaginalis* , *Candida albicans*.

**INTRODUCTION**

Siddha system of medicine is practiced in south Indian region especially in the state of Tamil Nadu. It has close affinity to Ayurveda yet it maintains a distinctive identity of its own. This system has come to be closely identified with Tamil civilization. The term 'Siddha' has come from 'Siddhi'- which means achievement. *Siddhars* were the men who achieved supreme knowledge in the field of medicine, yoga or *tapa* (meditation).<sup>[1]</sup>

Siddha system of medicine is believed as a brilliant achievement and symbol of Tamil culture which

originated in Southern parts of India. Siddha medicine invented from Dravidian culture and is grown in the time of Indus valley civilization. Chinese alchemy, Taoism, and Taoist Patrology are considered as a main source of inspiration for Siddha alchemy.<sup>[2]</sup> It is believed that in ancient time, the system was developed by eighteen siddhar (a class of Tamil sages). Though Siddha system of medicine resembles with Ayurveda in many aspects it has own philosophy and concept, holistic approach, and lifestyle oriented measures.<sup>[3,4]</sup>

In Indian scenario women's of childbearing age are often suffered with clinical infectious condition called Bacterial vaginosis (BV)<sup>[5,6]</sup> This condition is characterized by the replacement of vaginal lactobacilli with a variety of predominantly-anaerobic pathogens, such as Gardnerella vaginalis, Prevotella, Peptostreptococcus, and Bacteroides spp., with total bacterial numbers often rising 100 to 1000 fold compared to the normal levels in the vagina.<sup>[7-12]</sup> These changes within the vaginal microbiota are frequently (but not always) accompanied by an elevation in vaginal pH and by an abundance of vaginal secretions that have a typical amine odour.

Bacterial vaginosis (BV) is the most common vaginal infection in women of childbearing age.<sup>[1,2]</sup> Aside from being a major nuisance due to its symptoms, BV (even in its asymptomatic form) has been associated with serious gynecological and obstetric complications.<sup>[13-16]</sup>

Based on the literature it was evident that in most of the cases BV ends in preterm birth in pregnant women, a major risk factor for perinatal mortality and morbidity.<sup>[17,18]</sup> Further there is a growing evidence that BV increases the chance of transmission and acquisition of sexually-transmitted infections, such as HIV<sup>[19,20]</sup> and HSV-2.<sup>[21]</sup> Gardnerella Vaginitis is an infection of the female genital tract by bacteria of the *Gardnerella vaginalis* strain, often in combination with various anaerobic bacteria. Also called bacterial vaginosis. *Gardnerella vaginalis* is the most common cause of bacterial vaginitis in the sexually active mature patient. The patient complains of a malodorous, nonirritating discharge, and examinations reveal homogenous, gray-white secretions.

Vaginal candidiasis is one of the most common gynecological problems seen in primary care. *Candida albicans* account for 90% of the infection. It is reported that all women will suffer at least one episode of vaginal candidiasis during their life time and up to 40-55% of them having recurrent vaginal candidiasis. There are many treatment options for recurrent vaginal candidiasis. Whichever regime is chosen, treatment must always be individualized. The treating physician must consider cost-effectiveness, convenience, possible side effects and the availability of the medication. Fluconazole the most commonly prescribed antibiotic for candida infection which is often reported to have dizziness, drowsiness, stomach or abdominal pain, upset stomach, diarrhea etc.<sup>[22]</sup>

According to many scientific literature it was known fact that many human pathogenic bacteria have developed resistance against several synthetic drugs.<sup>[23-25]</sup> There are several reports on antimicrobial activity of crude extracts prepared from plants that inhibit various bacterial pathogens, but a limited numbers of *in vitro* studies on herbal preparations have been published. It is need of the hour to identify alternate antibacterial lead from herbal

origin for clinical management of leucorrhoea associated with *G. vaginalis* infection. Hence the present investigation aimed at evaluating the antibacterial potential of polyherbal Siddha preparation *Aghil kattai Chooranam* (AKC) against *Gardenerella vaginalis* and *Candida albicans* by agar disc diffusion assay.

## MATERIALS AND METHODS

The formulation AKC comprises of the following herbs as its ingredients

### Ingredients

1. Chandanam ( <i>Santalum album</i> )	-17.5g
2. Agil kattai ( <i>Aquilaria agallocha Roxb</i> )	-17.5g
3. Elam ( <i>Elettaria cardamomm</i> )	-17.5g
4. Lavangappattai ( <i>Cinnamomum verum</i> )	-17.5g
5. Kirambu ( <i>Syzygium aromaticum</i> )	-17.5g
6. Sombu ( <i>Pimpinella anisum</i> )	-17.5g
7. Ati-maduram ( <i>Glycyrrhiza glabra</i> )	-17.5g
8. Karboki vitthu ( <i>Psoralea corylifolia</i> )	-17.5g
9. Vetpalai arisi ( <i>Wrightia tinctoria</i> )	-17.5g
10. Thettran vithai ( <i>Strychnos potatorum</i> )	-17.5g
11. Arugam veer ( <i>Cynodon dactylon</i> )	-17.5g
12. Chitramutti ( <i>Pavonia Zeylanica</i> )	-17.5g
13. Karkandu	- Quantity Sufficient

**Indication:** Leucorrhoea.

### Source of raw drugs

The required raw drugs are procured from a well reputed indigenous drug shop. The raw drugs taken for study will be authenticated by the Botanist of Medicinal botany department, Govt. Siddha Medical College, Chennai.

### Preparation<sup>[26]</sup>

Take each ingredient about 17.5 gm. and made it to dry in the sun light, after that it was ground and powdered. Then equal Quantity of *karkandu* was added and bottled up.

### Drug Storage

The trial drug is stored in clean dry air tight container and it is dispensed to the patients in air tight bottle.

<b>Vehicle</b>	:	Butter
<b>Dose</b>	:	1 gm, twice a day
<b>Duration</b>	:	48 Days

### Stock preparation

The stock solution of test formulation AKC were prepared at the concentration of 2gm/ml.

### Test Organism

Organisms used for Anti-Bacterial Activity.

1. *Gardenerella vaginalis*

Organisms used for Anti-Fungal Activity.

1. *Candida albicans*

### Invitro Anti- Bacterial Activity by Disc-diffusion method<sup>[27]</sup>

The antibacterial activities of the sample AKC were carried out by disc diffusion method. The concentrations of the test compounds were used at the concentration of 500, 100, 2000 µg. The target microorganisms were cultured in HBT Medium. After 24 h the cultured streaks were swabbed for study purpose. The Petri dishes containing HBT medium were cultured with *Gardenerella vaginalis*. 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. Then the prepared discs were placed on the culture medium. Standard drug Metronidazole (5µg) was used as a positive reference standard to determine the sensitivity of each microbial species tested. Then the inoculated plates were incubated at 35° C for 48 hrs. The diameter of the clear zone around the disc was measured and expressed in millimeters as its anti-microbial property.

### Invitro Anti-fungal activity by Disc diffusion method<sup>[28]</sup>

The anti-fungal activities of the sample AKC were carried out by disc diffusion method. The concentrations of the test compounds were used at the concentration of

500, 1000, 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. 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. Then the prepared discs were placed on the culture medium. Standard drug Fluconazole (25µg) was used as a positive reference standard to determine the sensitivity of the species tested. Then the inoculated plates were incubated at 37° C for 72 hr (Fungal). The diameter of the clear zone around the disc was measured and expressed in millimeters as its anti-fungal property.

## RESULTS

### Effect of AKC on *Gardenerella vaginalis*

From the results of the present investigation it was evident that the Siddha formulation AKC offers significant anti-microbial activity by offering zone of inhibition of about 7mm at 500µg dose, 9 mm at 1000 µg dose and maximum of 12 mm at 2000 µg dose when compare to that of the standard Metronidazole (5µg) which offers maximum zone of inhibition of about 25 mm. The results were tabulated in Table 1 and Figure 1.

**Table 1: Zone of Inhibition of AKC on Anti-Microbial Activity.**

Sample code	<i>Gardnerella vaginalis</i>			<i>Candida albicans</i>		
	500 µg	1000 µg	2000 µg	500 µg	1000 µg	2000 µg
MC	7	9	12	-	-	-
Metronidazole (5µg)	25			-		
Fluconazole (25µg)	NA			24		

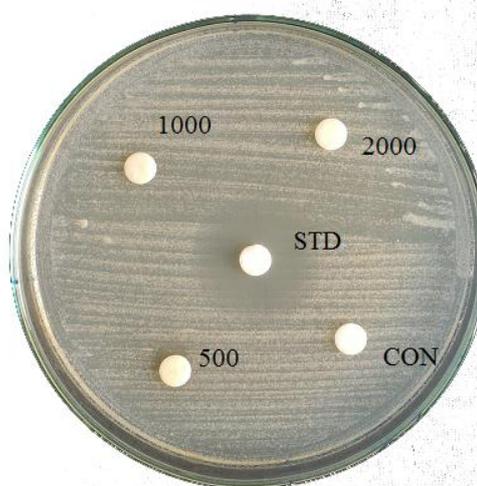
- = Not active NA = Not Applicable



**Figure 1: Anti- Bacterial Effect of AKC against *Gardenerella vaginalis*.**

### Effect of AKC on *Candida albicans*

From the results of the anti-fungal evaluation it was evident that the Siddha formulation AKC has no significant anti-fungal activity at any of the dose level viz 500 to 2000 µg. The results were tabulated in Table 1 and Figure 2.



**Figure 2: Anti-Fungal Effect of AKC against *Candida albicans*.**

## DISCUSSION

Lactobacilli were considered to be the dominant among the entire microbial flora found in vagina. Lactobacilli maintain an acidic pH by producing H<sub>2</sub>O<sub>2</sub> and lactic acid. Alterations in this ecosystem can lead to bacterial vaginosis (BV) and *Candida* vaginitis, which account for

90% of vaginal infections.<sup>[29-31]</sup> *G. vaginalis* is a facultative anaerobic, non-motile, pleomorphic gram negative organism which is well recognized colonizer of female genital tract. Due to change in the vaginal flora the *G.vaginalis* tend to cause infection associated with discharge<sup>[32]</sup>

Currently, various oral and topical antibiotic agents such as metronidazole, clindamycin are effectively utilized in the treatment of Gardnerella Vaginitis (GV). However, associated adverse events such as Agitation, back pain, blindness, blurred vision, hallucinations, headache, irritability, nausea, Abdominal or stomach cramps, joint and muscle pain limit their widespread and long-term use.<sup>[33]</sup> The major problem affecting antibiotic therapy of GV has also been the increasing bacterial resistance to standard drugs.

The therapy of bacterial vaginosis (BV) now days has become a topic of debate with research pointing at the complexity of the issue and probable new pathogens associated with the clinical condition. The primary recommendation for treatment of BV has been metronidazole, (oral or intra-vaginal) given 500 mg twice daily for a week. However, with patients showing recurrence of symptoms within a short period of the completion of the therapy, the causative pathogen and/or its resistance to antibiotics is under focus. Antibiotic resistance is being increasingly reported around the world. Goldstein *et al.*, in 1993 had demonstrated a 20% resistance of *G. vaginalis* to metronidazole and the same group reported 29% resistance to metronidazole in 2002.<sup>[34,35]</sup>

Plants and other natural sources can provide a huge range of complex and structurally diverse compounds. Recently, many researchers have focused on the investigation of plant and microbial extracts, essential oils, pure secondary metabolites and new synthesized molecules as potential antimicrobial agents.<sup>[36-38]</sup> Traditional siddha practitioners also identified a number of herbal preparations like Chooranam for curing various ailments and diseases.<sup>[39]</sup>

The primary healthcare benefits of using such plant-derived siddha formulations are relatively safer when compared to allopathic drugs and offer profound therapeutic benefits.<sup>[40]</sup> Single and polyherbal preparations have diverse range of bioactive molecules and play a dominant role in the maintenance of human health since ancient times.<sup>[41]</sup> More than 1500 herbal preparations are sold as dietary supplements or ethnic traditional medicines.<sup>[42]</sup> The most frequently used type of herbal preparations are choornas. Choornas are preparations comprising of fine powders of medicinal plants and may be single or in combination.

The major advantage of using polyherbal formulations are its acts as a unique blend of versatile bioactive phytocomponents may render multiple action and also

act by synergetic mean. Combinations of medicinal plants may increase the antimicrobial spectrum and potency of the preparations. A variety of laboratory methods can be used to evaluate or screen the in vitro antimicrobial activity of an extract or a pure compound. The most known and basic methods are the disk-diffusion and broth or agar dilution methods. From the results of the present investigation it was evident that the siddha formulation AKC offers significant anti-microbial activity by offering zone of inhibition of about 7mm at 500µg dose, 9 mm at 1000 µg dose and maximum of 12 mm at 2000 µg dose when compare to that of the standard Metronidazole (5µg) which offers maximum zone of inhibition of about 25 mm. From the results of the anti-fungal evaluation it was evident that the siddha formulation AKC has no significant anti-fungal activity at any of the dose level viz 500 to 2000 µg.

## CONCLUSION

From the data's of the present investigation it was concluded that the Siddha formulation *Aghil kattai Chooranam* is polyherbal preparation which comprises of 12 potential herbs with numerous bioactive phytocomponents present with in it. Results of the Invitro anti-microbial study revealed evidence based data with respect to the anti-bacterial potential of the formulation AKC. Siddha formulation AKC offers significant activity against *Gardenerella vaginalis* and at a same time the formulation is not effective against *Candida albicans* at the tested dose level and hence it may use for the clinical management of bacterial vaginosis and leucorrhoea associated with *Gardenerella vaginalis* infection. Further studies have to be carried with special emphasis on molecular biology aspect of the drug and its target receptor in the biological system in near future.

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