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# SCREENING OF ANTIMICROBIAL ACTIVITY OF SIDDHA HERBAL DRUG MILAGARANAI VER CHOORANAM (ROOT OF *TODDALIA ASIATICA*)

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**ABSTRACT**

Milagaranai Ver Chooranam (root of *Toddalia asiatica*) is one of the Siddha herbal drugs; which has been indicated for its anti-microbial properties. The aim of the present study was to validate anti-bacterial and anti-fungal activity of Milagaranai Ver Chooranam (MVC) extract against various microorganisms. The microorganisms used in the present study include *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*, *Xanthomonas maltophilia*, *Proteus vulgaris, Chromobacterium violaceum, Streptococcus pyogens, Staphylococcus aureus, Staphylococcus albus* and *Candida albicans*. The anti-microbial study was conducted according to the agar diffusion method. Ciprofloxacin (10 µg) and Ketoconazole (10 µg) were employed as standard drugs for anti-bacterial and anti-fungal studies respectively. It was observed that MVC extract exerted effective anti-bacterial activity against almost all the organisms tested, when compared to the standard drugs which was evident from the zone of inhibition. The drug Milagaranai Ver Chooranam showed the minimum inhibition of the growth of microorganism at 100 µg/ml concentration for all the organisms. This bioactivity is mainly due to the presence of alkaloids, flavonoids, glycosides, tannins, saponins and coumarins known for its effectiveness against microbial agents. Our results confirmed the traditional use of Milagaranai Ver Chooranam as an anti-microbial agent.

**Keywords:** Anti-bacterial, Anti-fungal, Anti-microbial, Milagaranai Ver Chooranam, Siddha.

## INTRODUCTION

Siddha system of medicine described various treatments by the Siddhars in the struggle to preserve human health and combat diseases for healthy human life resources from nature, and many of their findings confirmed by the modern scientific research. Medicinal plants have been used for centuries and are appreciated for their multiple effects in a wide variety of ailments. In recent decades concentration on medicinal plants has increased dramatically not only in our country but also globally. For this reason, the World Health Organization (WHO) encourages and promotes drugs from natural resources. In this context, some of the pharmacology laboratories have focused on obtaining antimicrobials from microbial resources and medicinal plants.[1-3] Effort has been devoted over the years to the search for new antimicrobial materials from natural sources for food preservation[4-5]. Naturally derived compounds and other natural products may have applications in controlling bacteria in foods[6-7]. More than half of the pharmaceutical products are derived from natural sources [8]. Infections caused by bacteria have become one of the most difficult and costly diseases to treat. Currently infectious diseases caused by different microorganisms are a major cause of death worldwide. Many antibiotics are being developed to treat, but their misuse is causing the so-called drug resistance. So that, the search for new antimicrobial agents has become indispensable and natural products have been one of the potential sources for antimicrobial compounds because they help to fight against a wide range of pathogens such as bacteria, fungi and viruses. According to the World Health Organization (WHO), in 2008, 80 % of the world population relies on traditional medicine for primary health care[9]. In the background of chemical plants, essential oils, alkaloids and other phenolic compounds represent molecules of strong values, used in the pharmaceutical, cosmetics and food. The antibacterial activities of these products have been reported in numerous studies[10]. Anti-microbial therapeutics based on medicinal plants has a huge advantage over chemical treatments. In plants the active ingredients are biologically balanced by the presence of additional substances, so that, in general, do not accumulate in the body, and its undesirable effects are limited. Even though there are increased research and scientific studies of medicinal plants, the active ingredients of the many plants and their extraordinary qualities were not studied scientifically. Near 1340 plants are known to be potential sources of antibacterial agents[11]. But only few of them have been studied scientifically[12-13]. Milagaranai (*Toddalia asiatica*) is one of the most widely used herbs in the Siddha system of medicine for centuries against various ailments. *Toddalia asiatica* (L) Lam is a monotypic genus of flowering plant belonging to the family Rutaceae[14]. It is commonly known as orange climber native of Asia and many countries. The root bark is bitter, astringent and acrid and has been reported in Siddha classical literature as expectorant, anti-bacterial, diaphoretic, anti-pyretic, analgesic and anti-inflammatory[15]. Many studies have been made on this

plant and a number of active constituents are identified such as alkaloids, coumarins, flavonoids and essential oils[16]. The root of the plant contains coumarins which have antiplasmodial activity[17]. Extracts of this plant have demonstrated for their antiviral activity against $H_1N_1$ influenza in the laboratory[18]. Some of the compounds and their derivatives such as alkaloids, flavonoids, isoflavonoids, tannins, coumarins, glycosides, terpenes and phenolic compounds have antimicrobial properties[19-20]. Keeping in view, an attempt was made in the present study to validate the anti-bacterial and antifungal activity of Milagaranai Ver Chooranam (Root of *Toddalia asiatica*) against gram- positive, gram-negative bacterial strains and anti-fungal (*Candida albicans*) by the agar diffusion method. Determination of antimicrobial susceptibility *in vitro* is one of the most important functions of clinical microbiology laboratories. This activity allows monitoring the level of resistance of microorganisms to antimicrobial substances which will help to establish the appropriate therapeutic policies and assess the future development of new drugs.

## MATERIALS AND METHODS

### Plant materials

The fresh roots of *Toddalia asiatica* (Milagaranai Ver) were collected from in and around Courtalam, Tamil Nadu, India. Specimen of the root was identified and authenticated by the botanist and experts of Gunapadam from Government Siddha Medical College, Palayamkottai, Tamil Nadu, India. A sample specimen (AK/M09/2011) was deposited in the Post graduate department. The roots of *Toddalia asiatica* were cleaned well and allowed for complete drying in a shady place. Then the roots were cut into pieces and made into powder form by using stone mortar. This powder was sieved by thin white cloth and purified by the method mentioned in the Siddha classical text[21]. After purification, the powder form of root was named as Milagaranai Ver Chooranam (MVC) and was preserved in an airtight container.

### Preparation of extract

10 g of Milagaranai Ver Chooranam was taken and then100 ml of distilled water was added and kept in a boiling water bath for 20 minutes and then filtered through a Whatmann filter paper no.1, autoclaved at $121^0$C for 15 minutes. Then the extract was kept in clean and sterilized test tubes at $4^0$C. The extract of the drug was tested with the microorganisms.

### Test microorganisms

Strains, including fungi and bacteria were obtained from Persian Type Culture Collection (PTCC). The microorganisms used in the present study include *Escherichia coli, Salmonella typhi, Shigella flexneri, Xanthomonas maltophilia, Proteus vulgaris, Chromobacterium violaceum, Streptococcus pyogens, Staphylococcus aureus, staphylococcus albus* and *Candida albicans*.

### Anti-Bacterial and Anti-Fungal Screening

Anti-bacterial and antifungal activity of Milagaranai Ver Chooranam extract (50 µg and 100 µg/disc in concentration) was evaluated using agar disc diffusion assay method according to the method of Bauer *et al*[22]. The nine bacterial strains of Gram positive bacteria such as *Streptococci pyogens, Staphylococci aureus* and *Staphylococci albus* and Gram negative bacteria which includes *Escherichia coli, Salmonella typhi, Shigella flexneri, Proteus vulgaris, Chromobacterium violaceum* and *Xanthomonas maltophilia* were selected for anti-microbial study. *Candida albicans* was selected for the evaluation of antifungal activity. The strains of microorganisms obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at $37^0$C for 24 hours and were referred to as seeded broth. Media were prepared using Mullar Hinton agar provided by Himedia, Mumbai, India poured on petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. Sterile discs of 6 millimeter width had been impregnated with 20 ml of test extract and introduced into the upper layer of the seeded agar plate. The plates were incubated over night at $37^0$C. Anti-bacterial activity was assigned by measuring the inhibition zone formed around the discs. Ciprofloxacin (10 µg/disc) was used as a standard drug for anti-bacterial screening. For evaluating the anti-fungal activity, Sabouaud's dextrose agar medium was prepared. 100 µl of *Candida albicans* was gently spread on the medium plate. Well was made using well cutter. 50 µg and 100 µg/disc in concentration of samples were loaded and incubated at $37^0$C for 48 hours. Zone of inhibition was measured using Himedia zone of inhibition scale results. Ketoconazole (10 µg /disc) was used as a standard drug for anti-fungal screening. The inhibition zones with diameter less than 14 mm for 50 µg and 16 mm for 100 µg were considered as having no anti-microbial activity. For an accurate analysis; all the assays were done in triplicate to avoid any error.

## RESULTS AND DISCUSSION

Minimum Inhibitory Concentration (MIC) values obtained for the anti-bacterial and anti-fungal activity of MVC are reported in Table 1 and Table 2 respectively. The anti-microbial activity of MVC was evaluated on ten microbial strains (nine strains of bacteria and one fungal strain). The result of the percentage inhibition of the two doses of MVC extract (50 µg/ml and 100 µg/ml concentration) determined from the diameters of the inhibition zone (Figure 1). Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents[23]. It is also documented that the method of diffusion from wells on agar is more appropriate study to evaluate the anti-microbial activity of aqueous extract of medicinal plants[24].

**Table 1: The antibacterial activity of Milagaranai Ver Chooranam (MVC) against various bacterial strains**

| Organism used | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | Different concentrations of MVC | | Positive control (Ciprofloxacin) 10 µg | Negative Control |
| | 50 µg | 100 µg | | |
| *Escherichia coli* | 16 | 18 | 22 | R |
| *Salmonella typhi* | 16 | 18 | 22 | R |
| *Shigella flexneri* | 17 | 18 | 22 | R |
| *Xanthomonas maltophilia* | 15 | 17 | 22 | R |
| *Proteus vulgaris* | 15 | 17 | 22 | R |
| *Chromobacterium violaecum* | 15 | 18 | 20 | R |
| *Streptococcus pyogens* | 15 | 17 | 20 | R |
| *Staphylococcus aureus* | 17 | 18 | 22 | R |
| *Staphylococcus albus* | 16 | 18 | 22 | R |

The inhibitory diameter was measured by Himedia zone of inhibition scale

**Table 2: The antifungal activity of MVC against *Candida albicans***

| Organism used | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | Different concentrations of MVC | | Positive Control (Ketoconazole) 10 µg | Negative Control |
| | 50 µg | 100 µg | | |
| *Candida albicans* | 10 | 12 | 16 | R |

The inhibitory diameter was measured by Himedia zone of inhibition scale

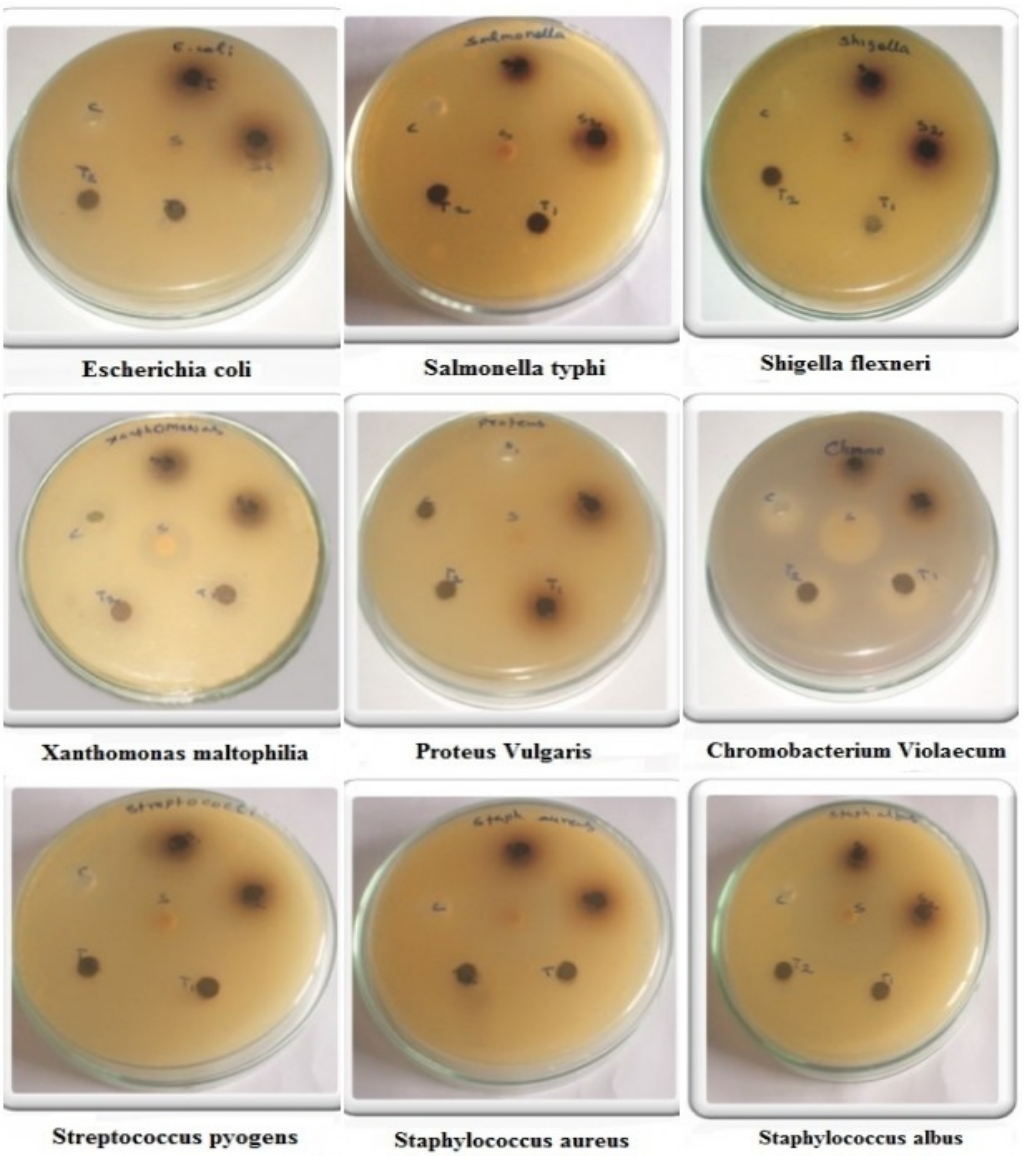


**Figure 1: Zone of inhibition of MVC extract and Standard drug against different microorganisms**

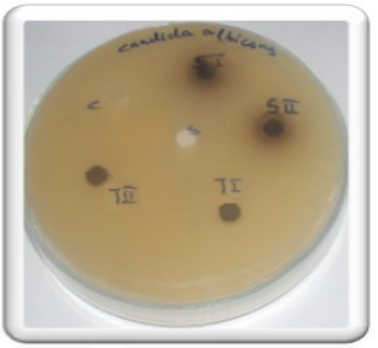


**Figure 2: Zone of inhibition of MVC extract and Standard drug against *Candida albicans***

**Anti-bacterial activity**

In this study three gram +ve organisms and six gram negative organisms were used. It was observed that MVC extract exerted effective anti-bacterial activity against almost all the organism tested, when compared to the standard drug (Ciprofloxacin 10 µg/ml) which was evident from the zone of inhibition (Figure 1). The drug showed the minimum inhibition of the growth of microorganism at 100 µg/ml concentration for all the organisms (*Escherichia coli* = 18, *Salmonella typhi* = 18, *Shigella flexneri* = 18, *Xanthomonas maltophilia* = 17, *Proteus vulgaris* = 17, *Chromobacterium violaceum* = 18, *Streptococcus pyogens* = 17, *Staphylococcus aureus* = 18, *staphylococcus albus* = 18). However, the inhibition of the test drugs was lower than that of standard drug, which showed a very strong inhibition of bacterial growth inhibition zones (*Escherichia coli* = 22, *Salmonella typhi* = 22, *Shigella flexneri* = 22, *Xanthomonas maltophilia* = 22, *Proteus vulgaris* = 22, *Chromobacterium violaceum* = 20, *Streptococcus pyogens* = 20, *Staphylococcus aureus* = 22, *Staphylococcus albus* = 22).

**Anti-fungal activity**

In this MIC study the effective dose for exerting anti-fungal effect of the test drug MVC extract was found to be 100 µg (Figure 2). The maximum concentration 100 µg/ml of the test extract having a zone of inhibition of 12 mm was comparable to that of the standard drug Ketoconazole at 10 µg concentrations (zone of inhibition = 16). Based on the results, the efficiency of MVC assessed in vitro anti-microbial study have shown effective inhibitory action against almost all the microbes strains used in this study particularly gram negative organisms with zone of inhibition of 18 mm. This may be due to the presence of anti-microbial substances such as alkaloids, flavonoids, isoflavonoids, tannins, coumarins, glycosides, terpenes and phenolic compounds.

## CONCLUSION

From this present study, it can be concluded that the Siddha herbal drug Milagaranai Ver Chooranam exerted effective anti-microbial activity against gram positive, gram negative bacterial and anti-fungal activity against *Candida albicans*. In Siddha system of medicine, various parts of the Milagaranai (*Toddalia asiatica*) are used alone or in combination with other herbal formulations as anti-microbial agent. Additional research on the chemical composition and also *in-vivo* studies of Milagaranai Ver (root of *Toddalia asiatica*) is desirable to understand the mechanism of action.

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