Research Article

In vitro study to determine antimicrobial activity of selected Ayurvedic preparations against bacteria and fungi causing superficial skin infections

PLR Gomes[1], S Hewageegana[2], J Kottahachchi[1], GIDDAD Athukorala[1], MM Weerasekera[1], TDCP Gunasekera[1], DMBT Dissanayake[1], DFD Meedin[1], SSN Fernando[1]

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Abstract

The increasing antimicrobial resistance exhibited by microorganisms causing superficial skin infections has led to extensive research on the therapeutic potential of Ayurvedic preparations. Medicinal plants contain many types of naturally occurring and side effects-free anti microbial compounds that can be effectively used against microbial infections. We tested the antimicrobial activity of twenty-eight Ayurvedic preparations used to treat superficial infections in a local Ayurvedic healthcare institution. They were tested against *Trichophyton rubrum, Microsporum gypseum, Candida albicans, Malassezia furfur, Staphylococcus aureus* and *Streptococcus pyogenes*. Twelve preparations showed significant antimicrobial activity and gave inhibition zones >10 mm. Two Ayurvedic preparations (Mixture *containing Terminella chebula, Terminella bellerica* and *Emblica officinalis* and one of *Terminella chebula* only) showed antimicrobial activity against all the microorganisms tested. We suggest that these two decoctions could be effectively used against all the above tested strains.

Introduction

Superficial skin infections are very common in general practice and are caused by bacteria, fungi and viruses. Bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes* are responsible for the majority of superficial infections.[1] Fungal infections of the skin, nails and hair are commonly caused by dermatophytes, *Candida albicans* and *Malassezia furfur*.[2]

[1] *Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura*
[2]. *Department of Kaya Chikitsa, Institute of Indigenous Medicine, University of Colombo.*

Address for correspondence: Gomes PLR, Department of Microbiology Faculty of Medical Sciences, University of Sri Jayewardenepura. E mail. laksiri79@yahoo.com

Treatment of superficial skin infections is mainly based on the use of topical antibacterial and antifungal agents. However, the indiscriminate use of antimicrobial agents has led to antimicrobial resistance. Development of antimicrobial resistance has been found in both bacteria and fungi that cause superficial skin infections.[3-6]

The increasing antimicrobial resistance exhibited by microorganisms causing superficial skin infections has led to extensive research on the therapeutic potential of Ayurvedic preparations. Medicinal plants contain many types of naturally occurring anti microbial compounds that can be effectively used against microbial infections.[7] Alkaloids, flavones (flavonoids, flavonols, Quinones), essential oils, lectins, polypeptides, phenolics, polyphenols, tannins and terpenoids are the major groups of naturally occurring antimicrobial agents that can be extracted from medicinal plants.[8]

Currently there are about 29 Ayurvedic preparations used to treat superficial skin infections. In vitro testing has been sparsely carried out to evaluate the effectiveness of these preparations against standard strains. A comprehensive in vitro study will provide valuable scientific information towards the development of more effective topical Ayurvedic applications for superficial infections. Therefore, in order to determine the antimicrobial activity, we investigated 28 Ayurvedic preparations against six common microorganisms causing superficial skin infections including *Trichophyton rubrum*, *Microsporum gypsum, Candida albicans*, *Malassezia furfur*, *Staphylococcus aureus* and *Streptococcus pyogenes*.

Methodology

Ayurvedic preparations

Twenty-eight Ayurvedic preparations used to treat superficial skin infections were kindly provided by a local Ayurvedic healthcare institution. The medicinal plants used for the preparations are listed in table 1.

Antimicrobial activity against bacteria

The antibacterial activity of each Ayurvedic preparation was tested against *Staphylococcus aureus* ATCC 25923 and *Streptococcus pyogenes* using the agar well method.[9,10] The diameter of a well was 7mm and had a height of 4mm. For each strain, a suspension was prepared in sterile normal saline and turbidity adjusted to 0.5 McFarland standard. The suspension of organisms was used to inoculate blood agar plates to obtain a confluent growth. Wells were cut in the agar surface with the help of a cork borer. Each well was filled with 100µl of the Ayurvedic preparation. At the same time, cefoxitin 30 µg and penicillin 10 µg discs were tested against Staphylococcus aureus and Streptococcus pyogenes respectively. All the plates were incubated 24 hours at 37°C before measuring the inhibition zone sizes. Any zone of inhibition around the decoction containing wells was considered as sensitive.

Table 1: **Medicinal plants used for the Ayurvedic preparations included in the study**

No	Herbs used for the Ayurvedic preparation	Part of the plant used	Sinhala name of the herb	English name of the herb
1	*Ficus religiosa* Linn.	Bark	Bo	Sacred fig
2	*Thespesia populnea* (L.) Sol.	Leaves/Bark	Gansuriya	Portia tree
3	*Ficus racemosa* Linn.	Bark	Attikka	Cluster fig
4	*Coscinium fenestratum* (Gaertn) Colb	Stem	Wenival-geta	Columbo weed
5	*Azadirachta indica* A. Juss.*Curcuma longa* Linn.	Leaves Rhizome	Kohomba Amu kaha	Margosa Turmeric
6	*Azadirachta indica* A. Juss.	Bark	Kohomba	Margosa
7	*Glycyrrhiza glabra* Linn.	Stem	Valmee	Licorice
8	*Emblica officinalis* Linn.	Fruit	Nelli	Indian gooseberry
9	*Hemidesmus indicus* **L.R.Br**	Root	Iramusu	Indian sarsaparilla
10	*Rubia cordifolia* Linn.	Root	Welmadata	Indian madder
11	*Terminella chebula* Retz. *Terminella belerica* Roxb. *Emblica officinalis* Linn.	Fruit Fruit Fruit	Aralu Bulu Nelli	Black myrobalan Beleric myrobalan Indian gooseberry
12	*Garcinia cambogia* Pierre.	Fruit	Goraka	Malabar tamarind
13	*Mimosa pudica* Linn.	Root	Nidikumba	Sensitive plant
14	*Terminella chebula* Retz.	Fruit	Aralu	Black myrobalan
15	*Curcuma longa* Linn. *Mimosa pudica* Linn.	Rhizome Root	Kaha Nidikumba	Turmeric Sensitive plant
16	*Bacopa monnieri* (L.) Pennell	Whole plant	Lunuwila	Water hyssop
17	*Achyranthes aspera* Linn.	Whole plant	Karal sebo	Prickly Chaff-flower
18	*Acasia chundra* (Roxb)DC.	Stem	Rat khiriya	Red cutch
19	*Cassia fistula* Linn.	Bark	Ahala	Golden shower tree
20	*Holarrhena antidysenterica* (Linn.) wall.	Bark	Kelinda	Bitter oleander
21	*Bauhinia acuminata* Linn.	Bark	Koboleela	Dwarf White Bauhinia
22	*Cyperus rotundus* Linn.	Rhizome	Kalanduru	Nut grass
23	*Syzygium cumini* (Linn.) Skeels.	Bark	Ma-dam	India Jamun
24	*Curcuma zedoaria* (Christm.) Roscoe.	Rhizome	Harankaha	White turmeric
25	*Symplocos racemosa* Roxb.	Bark	Lothsumbul	Lodh tree
26	*Nardostachys jatamansi* DC.	Root	Jatamangsha	Muskroot
27	*Mangifera indica* Linn.	Bark	Amba	Mango
28	*Carissa congesta* Wight.	Bark	Karanda	Christ's Thorn

Antimicrobial activity against dermatophytes and Candida

Using the same method, the Ayurvedic preparations and ketoconazole 10µg disc were tested against standard strains of *Trichophyton rubrum*, *Microsporum gypseum* and *Candida albicans* on Sabouraud agar.[11,12] For dermatophytes, the plates were incubated at 30°C for 7 days. Zones for *Candida albicans* were interpreted after incubating the plates for 24 hours at 37°C.

Antimicrobial activity against *Malassezia furfur*

Similarly the activity of the Ayurvedic preparations and ketoconazole 10µg disc against a standard strain of *Malassezia furfur* was tested on Sabouraud agar supplemented with 3% tween 20. The results were interpreted after an incubation period of 48 hours at 37°C.

The effect of freezing on the antimicrobial activity

An aliquot of each Ayurvedic preparation was frozen for 7 days at -80°C and tested simultaneously for all the microorganisms along with the normal solution. The inhibition zones were compared in order to determine any effect of freezing on the antimicrobial activity of the preparations.

The potency of the *Ayurvedic* preparations

For each strain, a suspension was prepared in sterile normal saline and the turbidity was adjusted to 0.5 McFarland standard. The suspension (100 µl) was mixed separately with 900 µl of each decoction that showed antimicrobial activity. The mixture was incubated at 37°C (30 °C for dermatophytes) and plated on appropriate media at 30 minutes and 60 minutes intervals. The plates were incubated overnight at 37°C (30 °C for dermatophytes) and observed for complete inhibition of the microorganism. A mixture with 100 µl of suspension and 900 µl of sterile normal saline was used as the control. Five ten-fold dilutions were prepared from each decoction that inhibited growth after overnight incubation at 37°C (30 °C for dermatophytes) and observed for complete inhibition of the microorganism. The above experiment was performed for each dilution.

All experiments were performed in triplicates to ensure reproducibility of results.

Results

Out of the 28 Ayurvedic preparations, 12 showed antimicrobial activity against at least one microorganism tested. The antimicrobial activity of these 12 preparations is summarized in Table 2. All preparations that showed antimicrobial activity gave inhibition zones >10 mm. Two Ayurvedic preparations [the mixture of *T chebula, T belerica and E officinalis* and *T chebula*] showed antimicrobial activity against all the microorganisms tested. *Emblica officinalis* also showed activity against all the test organisms except *Candida albicans*. *Nardostachys jatamansi* was only effective against *Streptococcus pyogenes* and *Syzygium cumini* showed activity against *Staphylococcus aureus* only.

Table 2: Medicinal preparations that showed antimicrobial activity

No	Herbs used for the Ayurvedic preparation	*S.aur*	*S.pyo*	*C.alb*	*M.fur*	*T.rub*	*M.gyp*
01	*Coscinium fenestratum*(Gaertn) Colb	18 mm*	22 mm†	28 mm†	No ZOI	30 mm*	No ZOI
02	*Azadirachta indica* A. Juss.	12 mm†	15 mm†	No ZOI	No ZOI	15 mm†	15 mm†
03	*Glycyrrhiza glabra* Linn.	No ZOI	12 mm*	No ZOI	No ZOI	35 mm*	32 mm†
04	*Emblica officinalis* Linn.	14 mm†	15 mm*	No ZOI	24 mm†	30 mm*	41 mm*
05	*Rubia cordifolia* Linn.	No ZOI	16 mm*	No ZOI	No ZOI	25 mm*	36 mm*
06	*Terminella chebula* Retz. *Terminella belerica* Roxb. *Emblica officinalis* Linn.	11 mm†	30 mm*	15 mm†	22 mm*	42 mm*	35 mm*
07	*Terminella chebula* Retz.	13 mm†	25 mm*	22 mm†	20 mm†	40 mm†	40 mm*
08	*Cassia fistula* Linn.	15 mm†	15 mm*	No ZOI	No ZOI	14 mm*	15 mm*
09	*Syzygium cumini* (Linn.) Skeels.	No ZOI	No ZOI	No ZOI	No ZOI	No ZOI	No ZOI
10	*Nardostachys jatamansi* DC.	No ZOI	16 mm†	No ZOI	No ZOI	No ZOI	No ZOI
11	*Mangifera indica* Linn.	No ZOI	12 mm†	No ZOI	No ZOI	14 mm†	12 mm†

S.aur - Staphylococcus aureus, S.pyo - Streptococcus pyogenes, C.alb - Candida albicans, M.fur - Malassezia furfur, T.rub - Trichophyton rubrum, M.gyp - Microsporum gypseum

ZOI –zone of inhibition , * – exposure for 30 min to the 10^{-5} dilution of preparation inhibited growth, † – activity was observed in well-method only

Apart from the two preparations that showed antimicrobial activity against all the microorganisms tested, decoctions made from *A.indica, G glabra, E. officinalis, R cordifolia, C. fistula* and *M indica* were also effective against both dermatophytes tested. Only the decoction prepared from *T chebula, T belerica, E officinalis* mixture and *T chebula* were active against *C albicans* and *M furfur*. *C. fenestratum, A. indica, E. officinalis,* and *C. fistula* are the medicinal plants used for other decoctions that were effective against both *S aureus* and *S pyogenes*. We did not notice any effect of freezing (for 7 days at -80°C) on the microbial activity of the medicinal plant preparations.

The results show (Table 2) that even the 10^{-5} dilution of most Ayurvedic preparations could inhibit the growth of tested microorganisms in exposure intervals as low as 30 minutes. For instance, an exposure of 30 minutes to 10^{-5} dilution of *T chebula, T belerica, E. officinalis* mixture inhibited growth of all the tested microorganisms except *S aureus* and *C albicans*. When *T chebula* alone was used such an exposure was effective only against *S pyogenes* and *M gypsum*. An overnight exposure to non-diluted preparation was necessary for a broad-spectrum activity.

Discussion

The results show that a high percentage of the tested herbal plants have potent antimicrobial activity. Even the 10^{-5} dilution of most Ayurvedic preparations showed efficient inhibitory action. To determine whether the preparations can be effectively used in superficial skin infections, a case control study on humans should be done. Most of the plants used in the preparations are widely distributed and used in ethnomedical

preparations in Sri Lanka. We selected these 28 preparations since they are already used to treat superficial skin infections at the local Ayurvedic healthcare institution. *S aureus* and *S pyogenes* were selected as the main bacterial strains since they are responsible for the majority of superficial infections.[1] *T rubrum, M gypseum C albicans* and *M furfur* are the commonest fungal species responsible for skin infections.[2]

Decoctions made from *T. chebula, T. belerica, E. officinalis* mixture and *T. chebula* showed the widest antimicrobial activity in our study. The broad-spectrum activity of *T. chebula* has been reported previously.[13-16] Broad-spectrum antibacterial activity of *E. officinalis* and *T. belerica* has also been shown previously.[17, 18] According to our results (Table 2) several other preparations were also effective against specific groups of microorganisms when used un-diluted for longer durations. For instance, *C. fenestratum, A. indica,* and *C. fistula* decoctions showed inhibition zones against both *S aureus* and *S pyogenes*. Inhibition zones >10 mm indicate strong antimicrobial activity [19].

The activity of medicinal plant extracts has been intensely studied during recent times. However, varying results have also been observed in different studies. For instance, our results showed that *E. officinalis* extract is not effective against *Candida albicans*, which contradicts finding of several other researchers.[17, 18] The effectiveness of the preparation might be influenced by the extraction method. Antimicrobial activity of medicinal plants has been demonstrated to be higher in organic extracts than aqueous extracts [20, 21].

Increasing failure of chemotherapeutic antimicrobial agents has been reported due to development of antimicrobial resistance.[3-6] Since herbal plants contain natural antimicrobial substances such as alkaloids, flavones (flavonoids, flavonols, quinones), essential oils, lectins, polypeptides, phenolics, polyphenols, tannins and terpenoids,[8] they have very minimal side effects when compared with chemotherapeutic antimicrobial agents [22].

In conclusion, Ayurvedic preparations show promising antimicrobial activity against microorganisms causing superficial skin infections. We suggest the use of decoctions prepared from *T. chebula, T. belerica, E. officinalis* mixture and *T. chebula* as a universal treatment against microorganisms causing superficial skin infections including *T rubrum, M gypsum, C albicans, M furfur, S aureus* and *S pyogenes*.

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