



Anc Sci Life. 2017 Apr-Jun; 36(4): 187–190.

doi: [10.4103/asl.ASL_179_16](https://doi.org/10.4103/asl.ASL_179_16)

PMCID: PMC5726184

PMID: [29269969](https://pubmed.ncbi.nlm.nih.gov/29269969/)

Determination of Antimicrobial Potential of Five Herbs used in Ayurveda Practices against *Candida albicans*, *Candida parapsilosis* and Methicillin Resistant *Staphylococcus aureus*

[TDCP Gunasekara](#), [NDM Radhika](#),¹ [KK Ragunathan](#),¹ [DPP Gunathilaka](#),¹ [MM Weerasekera](#), [HGSP Hewageegana](#),² [L A D M Arawwawala](#),³ and [SSN Fernando](#)

Address for correspondence: Dr. TDCP Gunasekara, Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka. E-mail: kl.ca.pjs@akihitnihc

Received 2016 Sep; Accepted 2017 Oct.

[Copyright](#) : © 2017 Ancient Science of Life

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

Abstract

Background:

Aims:

Subjects and Methods:

Results:

Conclusions:

[Go to:](#)

Introduction

Nearly 2000 to 2500 species of Sri Lankan flora are being used in the Ayurvedic practices in Sri Lanka.[1] Studies on medicinal plants used in ayurvedic or traditional treatment have demonstrated their potential as antimicrobials.[2] Phytochemicals obtained from medicinal plants have been used widely in the development of novel therapeutic agents including antimicrobial agents. The antimicrobial activity of several medicinal herbs used in traditional and ayurvedic medicine in Sri Lanka have been reported.

Multidrug resistant microbes pose a major challenge in health care and therefore the demand for alternative antimicrobials are increasing. WHO reports that more than one quarter of *Staphylococcus aureus* infections are due to methicillin resistant *Staphylococcus aureus* (MRSA).[3] MRSA is emerging as an important nosocomial pathogen which can cause mild superficial skin infections to severe life threatening systemic infections among hospitalized patients and treatment is difficult due to β -lactam resistance.[3] MRSA is becoming one of the common nosocomial pathogens in Sri Lanka, creating a major challenge for clinicians and a heavy economic burden to the health sector.[4]

Candidiasis is the most common fungal infection in the world and may lead to superficial fungal infections or life threatening invasive infections.[5] Recently, antifungal resistance of *Candida* species has been increasingly reported requiring more potent antifungal agents.[5] Further the undesirable side effects such as hepatotoxicity, nephrotoxicity and neurotoxicity of most of the antibacterial and antifungal agents are important problems which need to be addressed. Therefore, new and potent antimicrobials are essential to face the challenge posed by multidrug resistant microorganisms. In this study, we have investigated the antimicrobial activity of five medicinal plants used in Ayurvedic preparations and decoctions against clinical isolates of MRSA and *Candida spp.* Among them *Berberis ceylanica* (Dāruharidrā) and *Garcinia zeylanica* (Ela Goraka) are endemic plants that merit investigation.

[Go to:](#)

Subjects and Methods

Plant materials

Dried stem bark of *P. pinnata*, dried stem of *R. cordifolia* Linn, dried stem of *B. ceylanica*, dried pericarp of *G. zeylanica* and tender leaves of *J. officinale* were collected, during the period of January to December 2014 and authenticated by Senior Scientist, Bandaranayaka Memorial Ayurveda Research Institute, Navinna, Maharagama, Sri Lanka.

Preparation of hot water extract

Raw materials were rinsed, dried and aqueous extract was prepared using 60 g of dried plant material (*P. pinnata*, *R. cordifolia*, *B. ceylanica*) and 120 g of fresh plant material (*J. officinale*) in 1440 ml water. This was boiled under low heat to reduce the volume to 240 ml according to Ayurvedic protocol.[6] The plant extracts were filtered using sterile Whatman No 1 large filter paper. Each filtrate was transferred to a sterile glass container and stored in refrigerator [4°C] up to 2 weeks.

Preparation of ethanol extracts

Plant materials (60 g from each plant) were refluxed separately with ethanol (1440 ml) for 4 h, filtered using a Whatman No 1 filter paper and evaporated to get the final volume of 240 ml under reduced pressure at 50°C.[3] Each filtrate was transferred to a sterile glass container and stored at 4°C up to two weeks.

Microorganisms

Five clinical isolates each of *C. albicans*, *C. parapsilosis* and two type strains of *Candida* species; *C. albicans* ATCC 10231, *C. parapsilosis* ATCC 22019, and six clinical isolates of Methicillin Resistant *Staphylococcus aureus* (MRSA) were obtained from the Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura. The methicillin resistant *Staphylococcus aureus* isolates were confirmed using Antibiotic sensitivity testing following the CLSI standards.

Well diffusion assay

Overnight cultures of *Candida spp.* and MRSA were used to make suspensions (0.5 Mac Farland) and spread on the surface of Mueller Hinton Agar (MHA) plates. Wells were cut using a 9 mm sterile cork borer. The bottom of the wells was sealed by adding a drop of molten agar. Ethanolic plant extracts were dissolved in Dimethyl sulfoxide (DMSO) (Sigma Aldrich) and brought to final (neat) concentration. Then 200µl of the aqueous or ethanolic plant extracts were loaded into the wells using micropipette. Fluconazole and Amphotericin B (0.05 mg/ml) were used as positive controls against *Candida* strains and Cloxacillin (Karnataka Antibiotics and Pharmaceuticals Limited) and Vancomycin (Karnataka Antibiotics and Pharmaceuticals Limited) (30 mg/ml) of IV antibacterial preparations were used as positive control against MRSA strains.

The inhibitory zone of microbial growth around the well was called as the zone of inhibition (ZOI), this was calculated by subtracting the measured diameter of the ZOI from the well diameter in aqueous extracts, and for the ethanolic extracts the ZOI was obtained by subtracting the diameter of ZOI from the DMSO control well ZOI. In the well diffusion assay any zone of inhibition observed was considered as significant. We included a cloxacillin containing well which shows no zone of inhibition as a negative control.

Well diffusion assay was done in triplicates for each individual isolate and average zone of inhibition was calculated.

Minimum inhibitory concentration

Minimum Inhibitory Concentration (MIC) was determined using the pour plate method as described previously.[7] Both aqueous and ethanolic extracts were prepared using 60 g of dried plant material and 120 g of fresh plant material boiled down from 1440 ml to 240 ml. Briefly, doubling dilutions of extracts (Neat, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64) were prepared in MHA using the aqueous extracts and ethanolic extracts. Molten MHA (20 ml) was cooled to 50°C, mixed well with each extract dilution and poured into sterile petri plates. The plates

were allowed to dry and a grid containing 12 squares were drawn on the back of the glass plate. Five microliters of each organism suspension (prepared to the 0.5 Mac Farland standard) were placed on the surface of the agar plate in each grid. The drop was allowed to stand for few minutes and the plate was incubated in the upright position at 37°C. Presence or absence of any growth of the organism was observed after 24 hours. The lowest concentration of the extract that inhibited the visible growth of a microorganism after overnight incubation was determined as MIC. Experiment was done in triplicates and the average MIC was calculated.

[Go to:](#)

Results

The aqueous extracts (250 mg/ml) of *B. ceylanica*, inhibited the clinical isolates of *C. albicans* (ZOI = 4.5 mm), while in the ethanolic extracts, both *C. albicans* ATCC 10231 (ZOI = 14 mm) and the *C. albicans* clinical isolates (ZOI = 15.8 mm) showed inhibition. Aqueous extract of *B. ceylanica* had a ZOI of 3 mm against *Candida parapsilosis* ATCC 22019 while a larger ZOI (13.7 mm) was obtained for the ethanolic extracts against the type strain and clinical isolates of *C. parapsilosis* [Table 1].

Aqueous extract of *G. zeylanica* exhibited antibacterial activity against MRSA with a ZOI of 4 mm. Dried stem of *R. cordifolia* gave a ZOI for two of the isolates but was resistant to the others. Zones of inhibition was observed with the ethanolic extracts of *P. pinnata* (4 mm), *R. cordifolia* (10.7 mm), *J. officinale* (4.6 mm) and *G. zeylanica* (14.2 mm) against MRSA [Table 1].

The results of the well diffusion assay showed sensitivity of *C. albicans* against aqueous extracts of *B. ceylanica* and the ethanolic extract of *R. cordifolia* while the MRSA strains were inhibited by aqueous and ethanolic extracts of *G. zeylanica*, ethanolic extracts of *P. pinnata* and *J. Officinale* [Figure 1].

Therefore, these extracts were further tested to determine their minimum inhibitory concentration against the isolates. The MIC of aqueous extract of *B. ceylanica* against *C. albicans* and MIC of aqueous extract of *G. zeylanica* against MRSA was at neat concentration. Both extracts were prepared according to the standard method described in Ayurvedic texts. The ethanolic extracts had a MIC of 1/8 dilution for *R. cordifolia* against *C. albicans*, while the MICs of ethanolic extracts of *P. pinnata*, *J. officinale* and *G. zeylanica* against MRSA was at the dilutions of 1/8, 1/16 and 1/16 respectively.

[Go to:](#)

Discussion

In this study, *G. zeylanica* exhibited an antibacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA). This is the first study to our knowledge which has investigated the antimicrobial activity of this plant. The pericarp of *G. zeylanica* is used as a

spice in Sri Lanka. It is an endemic plant with no reported studies on its antimicrobial activity.

MRSA is increasingly responsible for causing nosocomial infections, particularly in critically ill patients. Methicillin resistant *Staphylococcus aureus* show resistance to all β -lactam antibiotics, and is often resistant to antibiotics such as macrolides and aminoglycosides, thus being multi drug resistant.[8] According to a study conducted in Sri Lanka, MRSA was isolated from 12.9% patients on admission to a surgical unit in National Hospital, Sri Lanka, while 6% were nosocomially infected during the hospital stay.[9] It is also important to note that *G.gummi-gutta* which belong to the same genus *Garcinia* has antibacterial activity against *S. aureus*. *Garcinia gummi-gutta* is used for treatment of skin related diseases in Ayurvedic medicines in Sri Lanka.

Aqueous extracts of *B. ceylanica*, had exhibited an inhibitory activity against isolates of *C. albicans*. *Candida albicans* have been reported to be a major pathogen responsible for human Candidiasis. However, recently non-albicans *Candida* species such as *Candida parapsilosis*, *Candida glabrata*, *Candida tropicalis* and *Candida dubliniensis* have been frequently identified as both pathogens and commensals. Further the incidence of *C. parapsilosis* infections has increased in the past few decades and is reported as the second most frequently isolated *Candida spp* from blood cultures.[5] Prophylactic and empirical use of antibiotics and antifungals are an important factor contributing to the development of antimicrobial resistance among these pathogens.

In Sri Lanka *B. ceylanica* is used in Ayurveda medicine to treat variety of infections. However, no studies have been reported on the antimicrobial activity of *B. ceylanica* against these two pathogens to the best of our knowledge. In Ayurveda, *B. aristata* is used to treat trachoma, skin diseases and diarrhea in India. Therapeutic properties of *B. ceylanica* are very rarely validated except the effects of Vipadikahara Grita Taila in which *B. ceylanica* is a major ingredient.[10] The physico-chemical parameters, phytochemicals and some of the therapeutic properties are similar among both *B. aristata* and *B. ceylanica*.[11] In general, plants which have antimicrobial properties exhibit wound healing properties. Similarly, *J. officinale*,[12] *P. pinnata*[13] and *R. cordifolia*[14] have shown wound healing properties in animal models.

[Go to:](#)

Conclusion

Antimicrobial activity of two endemic plants *G. zeylanica* and *B. ceylanica* against MRSA and two *Candida spp* is reported for the first time in Sri Lanka. It is therefore important to investigate the potential of these plants as novel antimicrobial agents, targeting the multidrug resistant bacteria and fungi of clinical importance.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

[Go to:](#)

Acknowledgement

We acknowledge the kind assistance by Prof. G.J. Panagoda, Department of Oral Medicine and Periodontology, Dental Faculty, University of Peradeniya for his contribution.

[Go to:](#)

References

1. Ravishankar B, Shukla VJ. Indian systems of medicine: A brief profile. Afr J Tradit Complement Altern Med. 2007;4:319–37. [PMCID: PMC2816487][PubMed: 20161896]
2. Gomes P, Hewageegana S, Kottahachchi J, Athukorala G, Weerasekera M, Gunasekera T, et al. *In vitro* study to determine antimicrobial activity of selected ayurvedic preparations against bacteria and fungi causing superficial skin infections. Sri Lankan J Infect Dis. 2013;3:32–9.
3. WHO. Antimicrobial Resistance: Global Report on Surveillance 2014. Switzerland: World Health Organisation; 2014.
4. Song JH, Hsueh PR, Chung DR, Ko KS, Kang CI, Peck KR, et al. Spread of methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: An ANSORP study. J Antimicrob Chemother. 2011;66:1061–9. [PubMed: 21393157]
5. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J, et al. *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*: Biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiol Rev. 2012;36:288–305. [PubMed: 21569057]
6. Gangadhara P. Uttara Kandha. In: Sharangadhara Samhita., editor. Bombay: Pandurang Jawaji; 1920.
7. Abayasekara CL, Rangama BNLD, Panagoda GJ, Senanayake MRDM. Antimicrobial activity of *Tephrosia purpurea* (Linn.) Pers. and *Mimusops elengi* (Linn.) against some clinical bacterial isolates. J Natl Sci Found Sri Lanka. 2009;37:139–45.
8. Tosti R, Samuelsen BT, Bender S, Fowler JR, Gaughan J, Schaffer AA, et al. Emerging multidrug resistance of methicillin-resistant *Staphylococcus aureus* in hand infections. J Bone Joint Surg Am. 2014;96:1535–40. [PubMed: 25232077]
9. Corea E, de Silva T, Perera J. Methicillin-resistant *Staphylococcus aureus* Prevalence, incidence and risk factors associated with colonization in Sri Lanka. J Hosp Infect. 2003;55:145–8. [PubMed: 14529641]

10. Hewageegana HG, Arawwawala LDAM, Dhammaratana I, Ariyawansa HA, Tissera MH. Development of a substitute oil for vipadikahara grita taila and its evaluation against vipadika skin disease (A case study) *Am J Clin Exp Med.* 2015;3:306–9.
11. Hewageegana HG. A Study of Skin Diseases: Ksudra Kustha Depicted in Sanskrit Original Sources with Special Reference to Vipadika and its Management. Ph.D. Thesis. University of Kelaniya. 2015
12. Shekhar S, Prasad MP. Evaluation of antimicrobial activity of Jasminum species using solvent extracts against clinical pathogens. *World J Pharm Pharm Sci.* 2015;4:1247–56.
13. Bhandirge SK, Tripathi AS, Bhandirge RK, Chinchmalatpure TP, Desai HG, Chandewar AV, et al. Evaluation of wound healing activity of ethanolic extract of pongamia pinnata bark. *Drug Res (Stuttg)* 2015;65:296–9. [PubMed: 25607746]
14. Karodi R, Jadhav M, Rub R, Bafna A. Evaluation of the wound healing activity of a crude extract of *Rubia cordifolia* L. (Indian Madder) in mice. *Int J Appl Res Nat Prod.* 2009;2:12–8.

[Go to:](#)

Figures and Tables

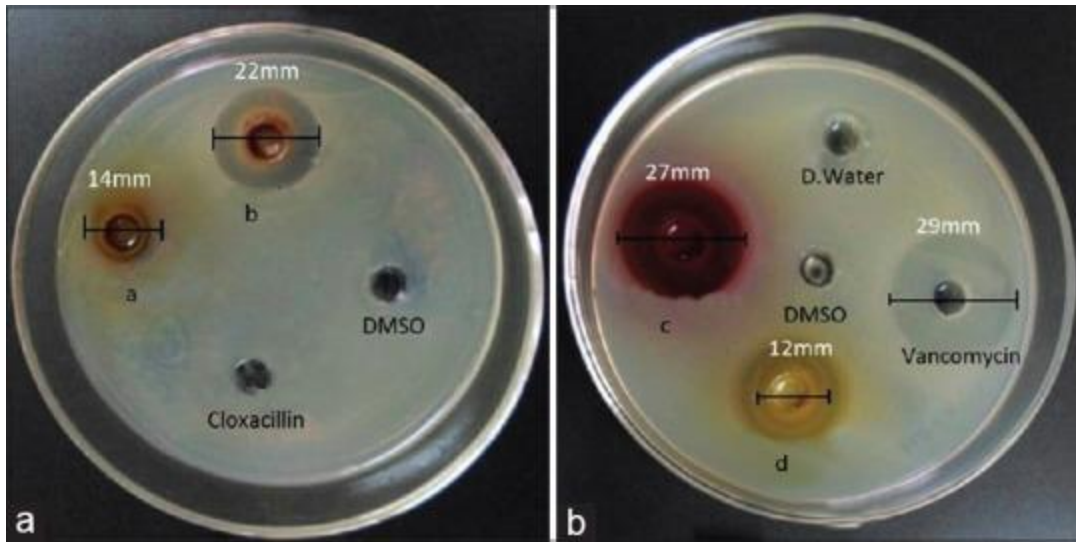
Table 1

Average zones of inhibition obtained for the aqueous and ethanolic extracts (neat concentration) of the plants

Plant	MRSA (mm)		<i>C. albicans</i> ATCC 10231 (mm)		<i>C. albicans</i> isolates (mm)		<i>C. parapsilosis</i> ATCC (mm)		<i>C. parapsilosis</i> isolates (mm)	
	A	E	A	E	A	E	A	E	A	E
<i>G. zeylanica</i>	4	14	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
<i>B. ceylanica</i>	NZ	-	NZ	14	4.56	15.88	3	13.7	NZ	13.7
<i>P. pinnata</i>	NZ	4	NZ	NZ	NZ	NZ	3	NZ	NZ	NZ
<i>R. cordifolia</i>	0.76	10.75	NZ	4	NZ	1.84	NZ	1.3	NZ	0.78
<i>J. officinale</i>	NZ	4.6	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Vancomycin	16.23	16.23	-	-	-	-	-	-	-	-
Flucanazole	-	-	18.6	18.6	NZ	NZ	16	22.4	22.48	22.4
Amphotericin B	-	-	17	17	15.5	15.5	19.6	19.6	19.42	19.4

Results are representative of triplicate experiments. A: Aqueous extract, E: Ethanolic extract, NZ: No zone of inhibition, MRSA: Methicillin resistant *Staphylococcus aureus*, *C. albicans*: *Candida albicans*, *C. parapsilosis*: *Candida parapsilosis*, *G. zeylanica*: *Garcinia zeylanica*, *B. ceylanica*: *Berberis ceylanica*, *P. pinnata*: *Pongamia pinnata*, *R. cordifolia*: *Rubia cordifolia*, *J. officinale*: *Jasminum officinale*

Figure 1



(a-d) Inhibition of methicillin resistant *Staphylococcus aureus* by the ethanolic plant extracts using the well diffusion assay (a) Tender leaves of *Jasminum officinale* Linn (Jasmine) (b) Dried fruit of *Garcinia zeylanica* (c) Dried stem of *Rubia cordifolia* Linn. (d) Dried stem bark of *Pongamia pinnata*. Vancomycin, Cloxacillin and Dimethyl sulfoxide were used as controls. Each assay was done in triplicate. A representative experiment is shown