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Original Article

Anticandidal activity of ten selected medicinal plants from Southern and North Central provinces of Sri Lanka.

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Abstract

Candida is a commensal yeast which normally resides on the human body causing mild to serious infections and is the most frequent cause of fungal infections worldwide. Antifungals prescribed for the treatment of candidiasis have side effects and have become incompetent due to emerging resistance of fungi. Therefore, effective novel antifungals are required to overcome this obstacle. This study aimed to determine the anticandidal activity of selected medicinal plants used in ayurveda, against *Candida albicans* ATCC12420 and *Candida glabrata* ATCC 90030. Plants for the study were selected from Southern and North-Central provinces of Sri Lanka. Methanol extractions prepared from each plant were subjected to antifungal susceptibility testing (AFST) against both *Candida* species, according to the NCCLS guidelines. Fluconazole and dichloromethane were used as positive and negative controls respectively. The minimum fungicidal concentration (MFC) was determined for each plant extract by broth microdilution method. The initial concentration of 200 mg/mL of plant extract showed no clear zone of inhibition thus fungicidal activity could not be determined by disc diffusion method, however there was a reduced density of the lawn of growth with *Citrus aurantiifolia*, *Cinnamomum verum*, *Phyllanthus emblica* and *Psidium guajava* against *C. glabrata* and for *C. verum* against C. *albicans*. MFC was determined using doubling dilution of plant extracts with concentrations ranging from (500 mg/mL – 15.62mg/mL). A MFC of 31.25mg/mL for *C. albicans* were given by the plant extracts *C. verum*, *C. longa* and *P. guajava.* While a MFC of 31.25mg/mL was observed for *C. glabrata* with the plants extracts of *S. grandiflora*, *C. verum*, *P. emblica* and *P. guajava*. Leaf extracts of both *C. verum and P. guajava* have good antifungal activity against *C. albicans* and *C. glabrata*

Key words **-** *Candida* species, Antifungal susceptibility testing, Medicinal plants, Antifungal drugs, Minimum fungicidal concentration.

Introduction

Candida species are commensal flora found in skin, gastrointestinal tract and mucous membranes. (Toya et al., 2007) *Candida* species can cause mild to serious fungal infections (candidiasis) in human beings. It is the most common cause of fungal infections worldwide (Manolakaki et al., 2010). Incidence of candidiasis has significantly increased in the past three decades mainly due to increasing elderly population, rise of AIDS epidemics and immunocompromised patients (Rüping et al., 2008). Main cause for the candidiasis is *Candida albicans,* however non-*C. albicans* species such as *Candida glabrata, Candida tropicalis* and *Candida parapsilosis* are now frequently identified as human pathogens (Silva et al., 2012). Reported study findings suggest that about 75% of women develop vulvovaginal candidiasis at least once in their lifetime (Denning et al., 2018). Invasive candidiasis occurs when *Candida* species enter the bloodstream and spread throughout the body when immunity is declined. Germ tube or pseudohyphae formation is the major virulence factor of *Candida albicans* (Rathod et al., 2016).

Candida glabrata is a species in genus *Candida* which was initially emphasized as a nonpathogenic commensal of human mucosal tissues (Silva et al., 2012). However, with the extended use of immunosuppressive agents, mucosal and systemic infections caused by *C. glabrata* have increased significantly (Toya et al., 2007).

Although plentiful antimicrobial agents have been discovered, microorganisms are constantly developing resistance to these agents (Sharanappa & Vidyasagar, 2013). Studies revealed that *Candida albicans* has developed resistance to azoles and polyenes like Amphotericin-B (Irshad et al., 2011). Further antifungals are expensive and have side effects including toxicity (Sharanappa & Vidyasagar, 2013). Therefore, it is necessary to search for more effective and less toxic novel antifungal agents that would overcome these disadvantages (Fan et al., 2008). Plants generally produce many secondary metabolites which have properties like microbiocidal, pesticidal and have been increasingly used in pharmaceutical industry (Rathod et al., 2016). The positive and negative effects of plant extracts on fungi have been studied vastly by researchers from different parts of the world (Hire & Dhale, 2012).

Psidium guajava is a small tree that has been used traditionally as a medicinal plant and leaf extracts of *P. guajava* have been reported to have analgesic, anti-inflammatory, anti-microbial, hepatoprotective and antioxidant activities (Ryu et al., 2012).

Senna alata is an ornamental bush and it has been identified that the phytochemical components such as alkaloids, flavonoids, saponins, tannins, terpenes, anthraquinones, steroids and carbohydrates present in *Senna alata* contain antifungal properties as reported from a study in Nigeria (Owoyale et al., 2006).

Curcuma longa commonly known as turmeric, is traditionally used as a spice in Indian and Sri Lankan cuisine (Luthra, et al., 2001). A study done by Upendra et al, demonstrated that the turmeric has appreciable inhibitory action against fungal contaminations at the concentration of 0.8 and 1.0 g/L (Upendra et al., 2011).

Various parts of *T. indica* tree such as seeds, root, leaves, bark and fruits are used in traditional medicine in India and Africa as antifungal agents (Gunasena & Hughes, 2000).

Cinnamon extract is active against *Candida albicans*, and *Helicobacter pylori* infections. The antimicrobial property of the cinnamon given by eugenol and a derivative of cinnamaldehyde (Devikatte, et al., 2005).

Phylanthus emblica commonly referred as "AmLa" fruit has been traditionally associated with numerous health benefits including anti-microbial properties (Hire & Dhale, 2012).

In Sri Lanka, studies conducted on natural anticandidal agents are comparatively low. Hence, this study evaluates the natural remedies which are currently used in Ayurveda and traditional medicine in Sri Lanka, for healing superficial fungal infections. Moreover, this study screened the anticandidal action of ten selected medicinal plants against *Candida albicans* ATCC 12420 and *Candida glabrata* ATCC90030.

Materials and methods

Identification of plants

Herbarium specimens selected and listed in Table 1 were sent to the National Herbarium of Royal Botanical Garden in Peradeniya for ethnobotanical identification.

Extraction of plants

The selected infection-free healthy plant materials were cleaned thoroughly with distilled water twice and dried in a shade until they achieved a constant mass of 250g. The dried plant materials were ground to obtain powder form and soaked in absolute methanol for two days. After two days, plant extracts were filtered through filter paper. The filtrate was then evaporated to dryness using a rotary evaporator (Temperature - 40ºC and pressure - 540mmHg). Dried extracts were stored in a freezer for further testing (Sánchez et al., 2016).

Determination of the anticandidal activity using disc diffusion method

Anticandidal activity of the plant extracts were determined according to the methodology proposed by Sánchez et al. (2016) with minor modifications.

Briefly a mass of 0.2g of dried extract was completely dissolved in 1mL of dichloromethane (DCM). Filter paper disks with 6 mm diameter were obtained (Whatman No: 1) and soaked in 10µl of each plant extract separately (2mg/disk). *Candida* suspensions of $1 - 2 \times 10^8$ CFU/mL were prepared in sterile normal saline for each isolate and compared with 0.5 McFarland turbidity standards. Sabouraud dextrose agar plates were inoculated separately with *C. albicans* and *C. glabrata*.

Each plant extract-soaked disc was separately placed 3 cm apart on the culture plates. All ten plants-soaked discs were placed in a 150mm SDA culture plate inoculated with 0.5 McFarland turbidity *Candida* suspensions according to NCCLS method and incubated 48 hours at 35±2°C (Pfaller et al., 2002).

Zones of inhibition were measured in millimeter. All tests were duplicated.

As the positive control, fluconazole (25µg) antifungal drug was used. As the negative control, DCM soaked 6mm diameter (Whatman No: 1) filter paper disc was used.

Scientific name	Common name	Part of the plant used in the study
Psidium guajava	Guava	Leaf
Senna alata	Aththora	Leaf
Curcuma longa	Turmeric	Rhizome
Ricinus communis	Castor	Leaf
Cymbopogon citratus	Lemongrass	Whole plant
Tamarindus indica	Tamarind	Leaf
Cinnamomum verum	Cinnamon	Leaf
Sesbania grandiflora	Kathurumurunga	Leaf
Phyllanthus emblica	Indian gooseberry	Fruit
Citrus aurantiifolia	Lime	Leaf

Table 1: Ethno botanical data of plant species

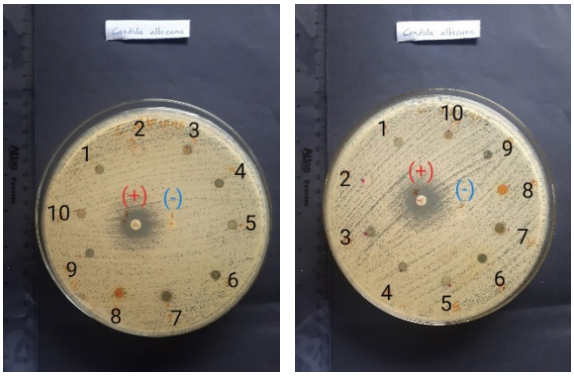

Figure 1: AFST for Candida albicans

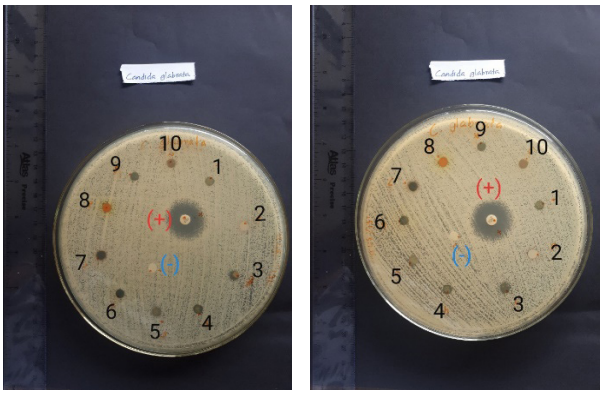

Figure 2: AFST for *Candida glabrata*

Sample number	Name of the plant extract
1	*Ricinus communis*
2	*Cymbopogon citratus*
3	*Tamarindus indica*
4	*Senna alata*
5	*Citrus aurantiifolia*
6	*Sesbania grandiflora*
7	*Cinnamomum verum*
8	*Curcuma longa*
9	*Phyllanthus emblica*
10	*Psidium guajava*
(-) control	DCM soaked disc
(+) control	Fluconazole (25µg)

Determination of minimum inhibitory concentration and minimum fungicidal concentration

All plant materials were further tested for minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). Dilution series of plant extracts were prepared using a microtiter plate ranging from 500mg/mL to 15.63mg/mL. As the positive control and negative control 2mg/mL of fluconazole drug and 100 µl of DCM were used respectively. *Candida* suspension of 1 - 2 × 10^8 CFU/mL (0.5 McFarland turbidity) was prepared using sterile normal saline for each isolate. Loopful of *Candida* suspension was inoculated into each well and incubated for 48 hours at 35±2°C to detect minimum inhibitory concentration. After 48 hours MIC was to be detected visually, but due to the colour of plant extracts visual detection was not possible.

MFC was determined by subculturing loopful of plant extract-*Candida* suspensions on SDA agar, from the dilution tubes (500mg/mL – 15.62 mg/mL). Sub cultured plates were incubated at 35±2°C for 48 hours to detect viability.

All the experiments were performed in duplicate. Results were expressed as means along with the standard deviation (SD) of two parallel measurements.

Results

Anticandidal activity of plant extracts

There was no clear zone of inhibition around the discs tested compared to the control (Figure 2). Zone of inhibition is affected by several factors like thickness of the agar media, incubation time, pH, environmental factors and diffusivity of product (Flanagan & Steck, 2017). Hence another method to determine antifungal activity was used. MFC was determined for the following plant extracts.

1. *Citrus aurantiifolia*
2. *Phyllanthus emblica*
3. *Senna alata*
4. *Ricinus communis*
5. *Cymbopogon citratus*
6. *Tamarindus indica*
7. *Cinnamomum verum*
8. *Curcuma longa*
9. *Psidium guajava*
10. *Sesbania grandiflora*

MFC of all plant extracts tested against the *C. albicans* and *C. glabrata* are shown in the figure 3.

Interestingly both *C. verum* and *P. guajava* showed a MFC of 31.25mg/mL for both *Candida* species tested.

MFC of 125 mg/mL was seen for both *R. communis* and *C. citratus* which was the highest inhibitory concentration for both *Candida* species tested. *C. verum* and *P. guajava* showed low fungicidal activity when compared to the above two plant extracts and gave a MFC of 31.25mg/mL.

Further, 125mg/mL MFC was observed with *S. grandiflora, P. emblica,* and *T. indica* against *Candida albicans*. Interestingly the MFC of 31.25 mg/mL was seen with both *S. grandiflora and P. emblica* against *C. glabrata* while 62.5 mg/mL was obtained for *T. indica* against *C. glabrata.*

A MFC of 62.5 mg/mL for *C. glabrata* was seen with *C. aurantifolia and C. longa,* while a MFC of 62.5mg/mL and 32.25mg/mL was seen with *C. aurantifolia* and *C. longa* respectively against *C. albicans*.

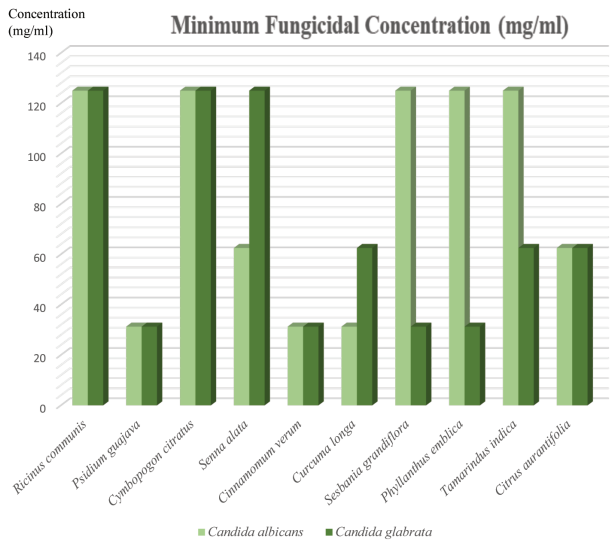

Figure 3: MFC of *Ricinus communis, Psidium guajava, Cymbopogon citratus, Senna alata, Cinnamomum verum, Curcuma longa, Sesbania grandiflora, Phyllanthus emblica, Tamarindus indica and Citrus aurantifolia*

Extract of *S. alata* showed a MFC of 62.5mg/mL and 125mg/mL respectively for *C. albicans and C. glabrata*.

However, *R. communis, C. citratus, T. indica, S. alata, S. grandiflora* and *C. aurantifolia* did not reveal any antifungal activity to the plants tested.

Discussion

Increasing resistance to antifungal agents is indeed a global problem. Further the toxicity and side effects to antifungal is yet another concern. Therefore, the use of natural products as alternative agents for the control of fungal disease is considered as an interesting alternative to synthetic fungicides.

As seen in this study both plant extracts taken from leaves of *P. guajava* and *C. verum* had good fungicidal activity (31.25 mg/mL) against both *Candida* species. Similarly studies from India also reported that the two concentrations (50mg/mL and 25mg/mL) of bark of the *C. verum* aqueous extracts had markedly inhibited several *Candida* species including *C. albicans* and *C. glabrata* (Vinitha & Ballal, 2008).

In keeping with the study findings, another study done in Northern Brazil from leaves of *P. guajava* had also shown to have a significant inhibitory activity against non- *Candida albicans* species when compared to *C. albicans* (Ferreira et al., 2013).

C. longa is another medicinal plant with known antibacterial and anticandidal activities. Current study revealed that the rhizome of the *C. longa* had potential anticandidal activity at low concentrations (31.25mg/mL) when tested against *C. albicans*. A previous study revealed that MIC of the DCM extracts of *C. longa* completely inhibited *C. albicans* at a concentration of 512 µg/mL (ÇıKrıkçı et al., 2008) showing that turmeric *(C. longa)* can be active at very low concentrations.

In the disc diffusion method, the inhibitory zones of respective plant extracts were not clear, and it could be due to poor diffusion of the plant extracts across the agar plate. Promising results have been seen when performing the MFC testing for *C. verum a*nd *P. guajava* against both *Candida* species tested.

In this study, part of the plant was only experimented for anticandidal effect.

Chemical composition of the plant differs according to the part of the plant, season of the year, climate, geographical variations and the age of the plant and thus could have varying activity. The selection of the parts of the plants for native treatment vary from geographical region to region. However as seen in this study the leaves of *C. verum*, *P. guajava, S. grandiflora, T. indica, S. alata* and rhizome of *C. longa* and fruit of *P. emblica* have seen to be effective and comparable with other reported studies (Gul & Bakht, 2013), (Vinitha & Ballal, 2008), (Kumar et al., 2021), (Zohrameena et al., 2017).

Further phytochemicals are antimicrobial in nature, but they also produce other biological activities in vivo resulting in induction of immunity, which can indirectly reduce the risk of infections (Packiyalakshmi et al., 2016).

According to the phytochemical analysis, previous studies revealed that *Cinnamomum verum is* rich with many phytochemical ingredients, such as cinnamic acid, cinnamaldehyde, cinnamate, and numerous polyphenols (Batiha et al., 2020).

Considering the phytochemical properties, leaves of *P. guajava* is rich with flavonoids such as quercetin, avicularin, apigenin, guaijaverin, kaempferol, hyperin, myricetin, gallic acid, catechin, epicatechin, chlorogenic acid, epigallocatechin gallate, and caffeic acid (Kumar et al., 2021). Hence these plants could prove to be useful alternatives to western medicines as they possess both antifungal and other properties which could curtail infection.

Conclusion and recommendations

Leaves of Sri Lankan cinnamon *(Cinnamomum verum)* and guava (*Psidium guajava)* have good antifungal activity against both *Candida* species tested. Thus, both plant species can be used as alternatives to traditional topical preparations for superficial *Candida* infections. Further investigations are needed to identify the active compounds from the active fractions of the extract.

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Conflicts of interests

There are no conflicts of interest.

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