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RESEARCH PAPER

The Efficacy of *Hibiscus Rosa-Sinensis* Leaf Extracts against *Candida Spp.* Causing Candidiasis

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Abstract

Over the years, the World Health Organization (WHO) has recommended traditional medicines as safe remedies for ailments of both microbial and non-microbial origins. The use of herbals has increased worldwide in recent years, as they are believed to be safer than modern medicines with lesser or no side effects. The present study has been designed to determine the role of *Hibiscus rosa-sinensis* leaves extract in-vitro antifungal activities against *Candida albicans, Candida tropicalis,* and *Candida glabrata* species. The leaves of *H. rosa-sinensis* were soaked in methanol and ethanol for solvent extraction. The extraction yield was diluted in DMSO (99.9%) to obtain 6 different concentrations (1000, 500, 250, 125, 62.5 and 31.25 mg/ml), and was then impregnated on blank discs. The disc diffusion method was used to test against 3 different candida species for sensitivity test. Basic phytochemical constituent's detection on phenol, carbohydrate, tannins and saponins were done on *H. rosa-sinensis***.** The extract that shows largest zone of inhibition of candida species is the leaf extract with ethanol. 1000 mg/ml of the leaf extract shows the highest inhibition zone against *C. albicans, C. tropicalis* and *C. glabrata* which are 9 mm, 8 mm and 9 mm respectively. Carbohydrate test showed both extractions highly positive and ethanol extract exhibited the highest saponin compared with methanol extract. It was found that ethanol extract of *H. rosa-sinensis* showed more potent anti-candidiasis compared with methanol extract.

Keywords: *Hibiscus rosa-sinensis*; Anti-candidiasis; *Candida albicans*; *Candida tropicalis*; *Candida glabrata*

INTRODUCTION

Candida albicans is an opportunistic fungal pathogen of humans. It is a yeast-type fungus that commonly causes cutaneous infections in humans. *C. albicans* is classified as opportunistic fungus because normally it only causes disease in people who are immunocompromised or those with altered natural flora. Lesions caused by *C. albicans* will appear as white patches on the skin or on the mucus membrane, therefore it is named as *Candida albicans.* It was observed that adherent *C. albicans* cells are more resistant to antimycotics than the stationary-phase cells and that resistance increases further with the formation of biofilms (Nicholls et al., 2011). *Candida tropicalis,* on the other hand, has become one of the most important *Candida* species. Studies had shown that it is currently considered the second most virulent *Candida* species, after *C. albicans*. *C. tropicalis* is a clinically common species and is the second or third etiological agent of candidemia, especially in Latin American countries and Asia (Dixon et al., 2004). It has been identified that *C. tropicalis* has become resistant to some of the antifungals

such as to azoles, polyenes, and echinocandins. Next, *Candida glabrata* is another species of *Candida* can be found as a part of the natural microflora in the body. It can present in the gastrointestinal tract, mouth, and also at the genital area. *C. glabrata* is typically well-controlled or usually harmless in healthy people. No matter what, it can lead to a medical problem in those with immunosuppressed condition (Komshian et al., 1999). *C. glabrata* indeed has a high resistance to some antifungal medications, which makes it hard to treat. Prolonged and prophylactic treatment with antifungals such as azoles and echinocandins, has also shown to results in the clinical resistant of *C. glabrata*. *Hibiscus rosa-sinensis*, a flowering plant in the *Hibisceae* tribe of the family *Malvaceae*, native to East Asia, is a species of tropical hibiscus. *H. rosa-sinensis* flowers and leaves are found to possess antioxidant, antifungal, anti-infectious, antimicrobial and antipyretic properties (Pamplona-Roger, 1999). This study was conducted to determine the efficacy of *Hibiscus rosa-sinensis* leaves extract against *Candida albicans*, *Candida tropicalis* and *Candida glabrata*.

MATERIALS AND METHODS

Collection of samples

Hibiscus rosa-sinensis leaves were collected from Klang, Selangor.

Test microorganism

Microorganisms that were used in this research are the *Candida albicans* (ATCC 10231), *Candida tropicalis* (ATCC 13803) and *Candida glabrata* (ATCC 60406). All organisms were cultured on Sabouraud dextrose agar (SDA).

Preparation of the plant extracts

Hibiscus rosa-sinensis leaves are separated from the main plant's body and rinsed with double-distilled water to remove the foreign material. The samples were evenly spread on a tray and placed in the oven for drying process. The samples were dried overnight at 50 °C. Then, the leaves are grounded into powder using commercial blender, weighed and stored in airtight bottle. A total of 250 g of dried leaves powder was soaked in 400 mL of organic solvents (methanol and ethanol respectively) in a Scott bottle for 24 hours. Then, it is filtered by using filter paper. The filtered extract was then placed in the rotary evaporator for approximately one and half hour until a thick extract was obtained. Then, it was placed in the oven at 50 °C for 24 hours to remove the remains solvent. The extraction yield was then stored at 4 °C for further studies (Anu-Kiruthika et al., 2011).

Extract concentrations

The extracts concentrations were diluted using the DMSO solution. 1g of the leaf extract was weigh for the respective solvent. 1000 µL of DMSO (99.9%) was mixed with the extract to obtain concentration of 1000 mg/ml. Two-fold dilution method was applied to produce 1000 mg/ml, 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml.

Disc diffusion method

Each of concentrations was impregnated on blank disc for sensitivity test on the tested organism. Then, these discs were placed directly on the surface of cultured media, which were swabbed with the test organism. The plates were incubated at 37°C for 24 hours. The zone of inhibition was measured using a ruler and the result was recorded (Kumar and Narain, 2010).

Estimation of phytochemical constituents
To estimate the phenol content, the extracts were treated with 3 drops of ferric chloride solution. Bluish black color indicates presence of phenols (McDonald et al., 2011).

Estimation of carbohydrates
The extracts were treated with Benedict's reagent. It is then heated on a water bath. Orange, reddish precipitate formation indicates the presence of reducing sugars (Chang et al., 2002).

Estimation of tannins
The extracts were treated with lead acetate solution. Yellow color precipitate indicates the presence of tannins (Chandha and Dave, 2009).

Estimation of saponins
A small amount of the extracts is shaken with little quantity of water. Formation of foam for approximately ten minutes it indicates the presence of saponins (Jayaraman, 2011).

RESULTS AND DISCUSSIONS

Table 1 presents the zone of inhibitions of the *Candida* species using ethanolic extraction. *C. albicans* and *C. glabrata* display the highest zone of inhibition at 1000 mg/ml concentration. *C.glabrata* also shows the lowest zone of inhibition when tested with 31.25 mg/ml of extract. While for methanolic extract showed 1000 mg/ml was the highest inhibition zone against *C. albicans* and the lowest inhibition for the concentration was the *C. glabrata* species. Furthermore, 62.5 mg/ml of methanol extracts shows no inhibition of *C. glabrata* and 31.25 mg/ml of the extract shows no inhibition for all three *Candida* species (Table 2). The anti-fungal activity was proportionate directly with the extract concentration. A previous study has indicated that *H. rosa-sinensis* possess bioactive properties and is recommended to be used as an herbal alternative to cure many diseases (Obi et al., 1998). From the study by David and Leonard on 1998, the flowers and leaves are found to possess antioxidant, anti-fungal, anti-infectious, antimicrobial, anti-inflammatory, anti-diarrheic and antipyretic activity (David and Leonard, 1998).

Phytochemicals such as tannin, phlobatannins, cardiac glycosides, flavonoids, terpenoids, and saponins are present in the leaves, stem, and root of the plant (Lalit et al., 2012). In present study, the highest extract concentration was showed the biggest inhibition zone in all tested organisms. This is due to presence of phytochemicals which this active compound can enhance the anti-candidiasis properties against the tested fungal species. Instead, the ability of the solvent to dissolve the secondary metabolites of the specific plant plays important role in the determination of antimicrobial activity of that particular plant. The presence of various organic compounds with different chemical characteristics and polarities may or may not be soluble in a particular solvent (Turkmen et al., 2006). Therefore, determination of the best solvent to obtain maximum anti-fungal properties of the particular plant is crucial. Conventional treatments for fungal infection currently are limited due to fungi morphology and metabolism. In current clinical setting, imidazoles, and triazoles drug group were commonly used for candidiasis treatment where azole drugs have been widely used as first-line treatments. A study by Chen in 2010 stated that azoles disrupt the biosynthesis of ergosterol, a fungal-specific sterol of cellular membranes (Chen et al., 2010). However, all *Candida* isolates have potential to develop resistance toward antifungal agents in vitro, especially azole group (Collin et al., 1999).

Table 1. Zone of inhibitions of *Candida* for ethanol leaf extracts (mm)

Concentration of extraction (mg/ml)	***Candida albicans* (mm)**	***Candida tropicalis* (mm)**	***Candida glabrata* (mm)**
1000	9.0	8.0	9.0
500	9.0	7.3	8.0
250	8.3	7.3	7.0
125	7.3	7.0	6.7
62.5	6.3	7.0	6.0
31.25	6.3	6.7	6.0
DMSO	0.0	0.0	0.0
Itraconazole	20	20	15

Table 2. Zone of inhibitions of *Candida* for methanolic extract (mm)

Concentration of extraction (mg/ml)	***Candida albicans* (mm)**	***Candida tropicalis* (mm)**	***Candida glabrata* (mm)**
1000	8.0	7.3	6.7
500	7.7	7.7	6.3
250	6.3	7.3	6.0
125	6.7	7.3	6.3
62.5	6.0	6.3	0.0
31.25	0.0	0.0	0.0
DMSO	0.0	0.0	0.0
Itraconazole	20	20	15

Table 3 revealed the phytochemical compound that *H. rosa-sinensis* leaf possesses in the present study. It shows that *H. rosa-sinensis* leaf possesses secondary metabolites such as phenol, carbohydrate, tannin, and saponins. This finding was supported by the phytochemical studies by Udita-Tiwari in 2015, which showed the presence of alkaloids, glycosides, flavonoids, saponins, tannin, and phenols in the hibiscus leaf extract, while hibiscus flower extract contains phytochemical like alkaloids, saponins, protein, phytosterols and carbohydrate (Udita-Tiwari et al., 2015).

Table 3. Phytochemical properties of *Hibiscus rosa-sinensis* leaf extract

Name of test		**Methanol leaf extract**	**Ethanol leaf extract**
Phenol	Ferric Chloride test	++	+
Carbohydrate	Benedict's test	++	++
Tannins	Lead acetate test	+	+
Saponins	Foam test	+	++

+ : Indicates positive for the phytochemical presence; ++ : Highly positive for the phytochemical presence

CONCLUSION

The role of *Hibiscus rosa-sinensis* leaf extract in vitro anti-candidiasis activities against *C. albicans, C. tropicalis,* and *C. glabrata* has been determined in the present study. There is a visible anti-candidiasis property by the ethanol leaf extract and methanol leaf extract, although ethanol leaf extract shows greater anti-candidiasis activities against the three *Candida* species. The efficacies of *H. rosa-sinensis* extraction with ethanol and methanol were depending on the presence of active compounds in the hibiscus leaf which enhanced the antimicrobial effect. Therefore, advance studies must be performed to test the other efficacies of *H. rosa-sinensis* leaf extract on the other opportunistic pathogens. Thus, the scientific medical evidence base

can be produced in order to come out with new potential anti-candidiasis drugs that can be used as complementary medicine in current clinical setting.

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REFERENCES

Anu-Kiruthika, K., Amutha Jaisheeba, A., Sornaraj, R. (2011). Evaluation of the antimicrobial property of selected flower extracts when exposed in a hospital environment. *International Journal of PharmTech Research*, 2, 769-774.

Chen, L.M., Xu, Y.H., Zhou, C.L., Zhao, J., Li, C.Y., Wang, R. (2010). Over expresion of CDR1 and CDR2 genes plays an important role in fluconazol resistance in Candida albicans with G487T and T916C mutations. *Journal of International Medical Research*, 38, 536-545.

Collin, B., Clancy, C.J., Hong Nguyen, M. (1999). Antifungal resistance in non-albicans *Candida* species. *Drug Resist Updates*, 2, 9-14.

Chandha, S., Dave, R. (2009). In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *African Journal of Microbiology Research*, 3, 981-986.

Chang, C., Wen, H., Yang, M. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10, 178-182.

David, B., Leonard, L.A. (1998). Healing plants of the Hawaiian kingdom-*Hibiscus rosa sinensis* (Aloalo), Roast Duck Production.

Dixon, T.C., Steinbach, W.J., Benjamin, D.K., Williams, L.W., Myers, L.A. (2004). Disseminated *Candida tropicalis* in a patient with chronic mucocutaneous candidiasis. *South Medicinal Journal*, 97, 788-790.

Jayaraman, J. (2011) Estimation of chlorophyll and carotenoids. In Laboratory Manual in Biochemistry. New Age International, 141-142.

Komshian, S.V., Uwaydah, A.K., Sobel, J.D. (1999). Fungemia caused by *Candida* species and *Torulopsis glabrata* in the hospitalized patient: frequency, characteristics, and evaluation of factors influencing outcome. *Reviews of Infectious Diseases*, 11, 379-390.

Kumar, S., Narain, S. (2010). Herbal remedies of wetlands macrophytes in India. *International Journal of Pharma and Bio Sciences*, 1 (2), 1-12.

Lalit, K., Gunosindhu, C., Vjay, S., Avijit, M. (2012). *Hibiscus rosa-sinensis*: A review on divine herb. *Journal of Advanced Pharmaceutical Technology & Research*, 2 (4), 9-18.

McDonald, S., Prenzler, P., Robards, K. (2001). Phenolic content and antioxidant activity of olive extracts. *Food Chemistry*, 73, 73-84.

Nicholls, S., MacCallum, D.M., Kaffarnik, F.A., Selway, L., Peck, S.C., Brown, A.J. (2011). Activation of the heat shock transcription factor Hsf1 is essential for the full virulence of the fungal pathogen *Candida albicans. Fungal Genetics and Biology*, 48, 297-305.

Obi, F.O., Usenu, I.A., Osayande, J.O. (1998). Prevention of carbon tetrachloride-induced hepatotoxicity in the rat by *H. rosa sinensis* anthocyanin extract administered in ethanol. *Toxicology*, 131, 93-98.

Pamplona-Roger, M.D. (1999). Encyclopedia of medicinal plants. Madrid (Spain): Editorial Safeliz, S.L. Vol. 1, 781.

Turkmen, N., Sari, F., Velioglu, Y.S. (2006). Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. *Food Chemistry*, 99, 835-841.

Udita-Tiwari, Yadav, P., Nigam, D. (2015). Study on phytochemical screening and antibacterial potential of methanolic flower and leaf extracts of *Hibiscus rosa-sinensis*. *International Journal of Innovative Pharmaceutical Sciences and Research*, 3 (6), 9-14.