DETERMINATION OF ANTIFUNGAL ACTIVITY OF HERBAL OINTMENT PREPARED FROM LEAF EXTRACT OF *Cassia fistula* ON LABORATORY SPECIMEN OF *Candida albicans*

Fernando KAB*, Waliwita WALC**

[*]Department of Kawmarabruthya & Stree Roga, Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya, Sri Lanka

[**]Department of Kayacikitsa, Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya, Sri Lanka

baddrafernando@gmail.com

DOI: 10.29322/IJSRP.10.03.2020.p99100
http://dx.doi.org/10.29322/IJSRP.10.03.2020.p99100

Abstract: Plants produce variety of medicinal components that can inhibit the growth of pathogens. Current advancement in drug discovery has intensified the efforts for exploring novel medicines from Ayurveda Medicine. In Ayurveda system of medicine, every part of *Cassia fistula* is used for the treatment of many diseases. Leaves extract of *Cassia fistula*, which is used as an external application in indigenous medicine in Sri Lanka to cure many skin diseases such as *Kustha, Dadru, Visarpa* and *Vruna*. *Candida albicans* is the most common fungi that cause skin infections. This study was conducted to determine the antifungal activity of *Cassia fistula* leaf extract against the laboratory specimens of *Candida albicans*. 6 mm diameter wells on Sabouraud Dextrose Agar were used in the application of well diffusion method. Fluconazole 2.5 mg/ml was used as positive control. Supernatant solution of leaf extract of *Cassia fistula* and fresh leaf extract of *Cassia fistula* were used comparatively to determine the antifungal activity. The mean inhibitory zone diameter of fresh leaf extract of *Cassia fistula*, supernatant solution and positive control were 23±1.0 mm, 0±0.0 mm and 22.66±0.5 mm respectively. Fresh leaf extract of *Cassia fistula* showed more inhibitory effect than the effect of supernatant solution of leaf extract of *Cassia fistula* and positive control. Antifungal (22±0.5 mm) ointment was prepared by using fresh leaf extract of *Cassia fistula* on the basis of previous results. The mean inhibitory zone diameter of the positive control was 23.3±0.5 mm. According to the results obtained, it could be concluded that herbal ointment of *Cassia fistula* fresh leaf extract has potential effect in the management of infections caused by *Candida albicans*. Further studies are needed to determine the antifungal effect of isolated active compounds present in fresh *Cassia fistula* leaf extract.

Keywords: *Cassia fistula*; Leaf extract; Antifungal effect

Introduction

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide. Large number of antimicrobial agents have been discovered; pathogenic microorganisms are constantly developing resistance to these agents. Therefore, it is necessary to search for more effective and less toxic novel antifungal agents. An important group of the skin pathogens are the fungi, among which dermatophytes and *Candida* spp. are prominent. *Candida albicans* (sometimes referred to as monilia) is a fungus that is normally present on the skin and in mucous membranes such as the vagina, mouth, or rectum. When an overgrowth of *Candida* develops on the skin, an infection can occur that called as candidiasis of the skin, or cutaneous candidiasis. Candidiasis of the skin often causes a red, itchy rash to form, most commonly in the folds of the skin which also spread to other areas of the body.

Cassia fistula Linn, belongs to family Caesalpiniaceae commonly known as *Ahala* in Sinhala has been widely used in different types of traditional medicines. *Cassia fistula* has been described to be useful against skin diseases, liver troubles, tuberculosis, hematemesis, pruritus, leukoderma, and diabetes. It contains various types of constituents such as rhein, triterpenes, sugar, and potassium. The antibacterial and antifungal activities of hydroalcohol extracts of leaves of *Cassia fistula* were tested against Gram-positive, Gram-negative, fungal strains. (Bhalodi and Shukla, 2011). In Ayurvedic medicine, the golden shower tree is known as *Aragvadha*, which meaning as "disease killer". Leaves extracts of *Cassia fistula* are medicine for external application which is used in Indigenous Medicine in Sri Lanka to cure disease such as *Kustha, Dadru, Visarpa, Vruna, Daha* and etc. (Department of Ayurveda, 1976). Application of the leaves pulp of *Rajavrksha* cures *Kustha*. (Sharma, 2004). Amoung the Eighteen types of *Kusthas*, *Dadru* is characterized by itching sensation, redness, pimples and circular patches with elevated edges (Sharma, 2004). Prepared *kalka* from the leaves of *Aragvadha* by titrating them along with *aranala* (a sour drink) used for external application to cures *Dadru* (Ringworm), *Kitima, Sidhma* and other varieties of *Kustha* like skin disease including Leprosy. (Lochan, 2006).

Objectives of this study were to identify the suitable method to prepare the Antifungal Ointment according to the antifungal activity of fresh leaf extracts of *Cassia fistula* and supernatant of *Cassia fistula* with fluconazole which is used as an antifungal drug in modern medicine. Also, to determine the Antifungal activity of prepared Ointment with fluconazole which is used as an antifungal drug in modern medicine.

Figure 1: *Cassia fistula* Plant

Figure 2: *Candida albicans*

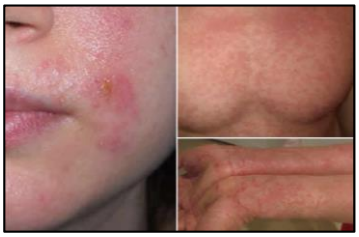


Figure 3: Candidiasis of the skin

Methodology

Fresh leaves of *Cassia fistula* were collected from the Gampaha Wickramarachchi Ayurveda Institute. All materials were cleaned by using hot water. 700g of fresh leaves were measured by using electronic precision balance. Fresh leaves were cleaned well by using water. Measured fresh leaves were blended well by using the wooden pestle and motor to form small particles. Fresh extraction was taking by filtering through autoclaved filter cloths. 500 ml of *Cassia fistula* fresh leaf extract was collected.

- Preparation of Supernatant of leaf extract of *Cassia fistula*

Cassia fistula fresh extraction was centrifuged by using centrifuger and 8 centrifuge tubes that filling of 12 ml for each tube. Fresh extraction was centrifuged in the rate of 3500 rpm for ½ hr. Precipitate was removed and reminder was collected to a conical plasque by using the pipet. 100 ml of above supernatant was collected to the beaker.

- Preparation of Inoculum

3-5 colonies of standard strain *Candida albicans* was suspended in 9 ml of distilled water. The turbidity was adjusted to be visually comparable with 0.5 Me Farland standards.

- Preparation of Sabouraud Agar

Sabouraud Agar was prepared from a commercially available dehydrated base according to the manufacturer's instructions and allowed to autoclave for 2 hrs, and then it allowed cooling. Poured medium into glass flat-bottomed petri dishes on a level; horizontal surface to give a uniform depth of approximately 4 mm. Tis corresponds to 25 ml to 30 ml for plates with a diameter of 100 mm. The plates were incubated at 37℃ for 1 hour then it allowed solidifying at room temperature.

- Preparation of positive control

Fluconazole (Pfizer-Roerig, Inc., New York, N.Y.) was prepared from 50-mg tablets suspended in distilled water to a final stock concentration of 2 mg/ml and filter sterilized.

- Preparation of Petridis

Inoculum was spread over the nutrient agar plate using a sterile cotton swab in order to obtain uniform microbial growth. Well was prepared by sterile 8 mm cork borer. Pour the 50 µl of Supernatant of *Cassia fistula*, 50 µl of prepared Fluconazole as the positive control and 50 µl of distilled water as the negative control into wells. Pour the 50 µl of Fresh leaf extract of *Cassia fistula*, 50 µl of prepared Fluconazole as the positive control and 50 µl of distilled water as the negative control into wells. The plates were incubated at 37℃ for 24 hours and measured the inhibition zone by using normal ruler.

- Interpretation of the zone sizes

Using the interpretative chart, the zones size of each antimicrobial, reporting the organisms as 'Resistant', Intermediate sensitivity', Sensitivity (susceptible).

Zone size for fluconazole 8 mcg: Resistant < 14mm, Susceptible dose dependent – 15mm-18mm, Sensitive > 19mm

- Preparation of an Herbal cream

White soft paraffin - 100g

Emulsifying wax - 60g

Liquid paraffin - 40 ml

Boiling distilled water – 40 ml

Leaves extraction of *Cassia fistula* – 160 ml

Emulsifying wax, White soft paraffin and Liquid paraffin were mixed in a beaker which immersed in a water bath at 70 °C while steering at 800-1200 rpm. Boiling distilled water was taken in another beaker and slowly added the water part into the container and kept steering. water bath was turned off and reduce the temperature up to 40°C. Leaves extraction was added to it and steered well to form the cream. It was steered until cool just above room temperature and transferred in to suitable containers.

Results

- Trial 01

(+) - Fluconazole

(-) - Distilled water

(P) - Supernatant of *Cassia fistula* leaf extract

Figure 4: Zone of inhibition in Trial 01

Disc number	1		2		3	
Type of Sample	P	Fluconazole 8mcg	P	Fluconazole 8mcg	P	Fluconazole 8mcg
Zone Diameter(mm)	0	23	0	23	0	22

Table 1: Mean inhibitory zone diameters between Sample P with Fluconazole

- Trial 02

(+) - Fluconazole

(-) - Distilled water

(A) - Fresh leaf extract of *Cassia fistula*

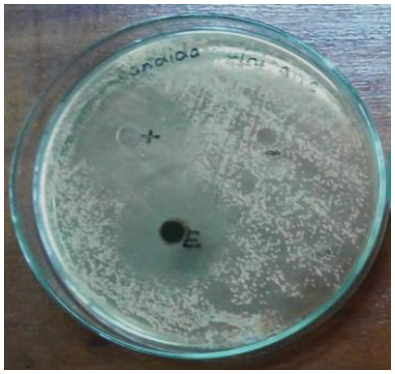
Figure 5: Zone of inhibition in Trial 02

Disc number	4		5		6	
Type of Sample	A	Fluconazole	A	Fluconazole	A	Fluconazole

		8mcg		8mcg		8mcg
Zone Diameter(mm)	24	22	22	23	23	23

Table 2: Mean inhibitory zone diameters between Sample A with Fluconazole

- Trial 03



(+) - Fluconazole

(-) - Distilled water

(E) - Prepared Ointment of Fresh leaf extract of *Cassia fistula*

Figure 6: Zone of inhibition in Trial 03

Disc number	7		8		9	
Type of Sample	E	Fluconazole 8mcg	E	Fluconazole 8mcg	E	Fluconazole 8mcg
Zone Diameter(mm)	22	23	22	23	22	24

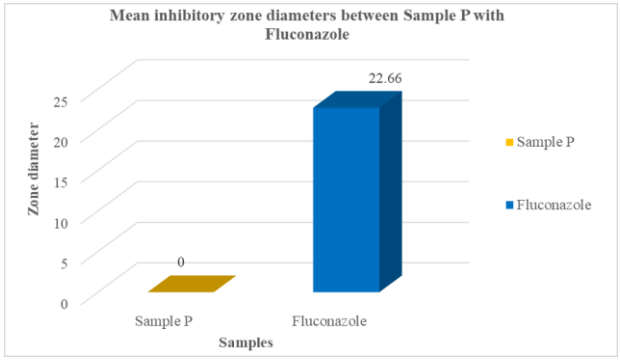
Table 3: Mean inhibitory zone diameters between Sample E with Fluconazole

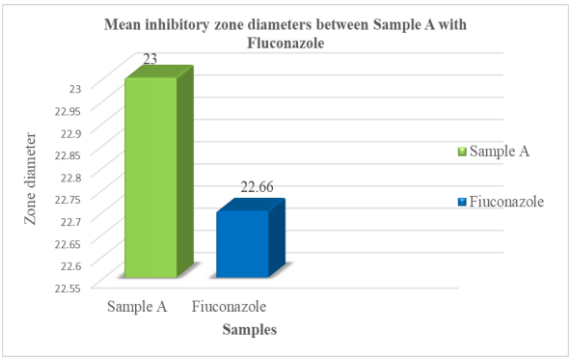
Type of Sample	P	Fluconazole 8mcg	A	Fluconazole 8mcg	E	Fluconazole 8mcg
Mean inhibitory Zone Diameter(mm)	0	23	23	22.7	22.4	23

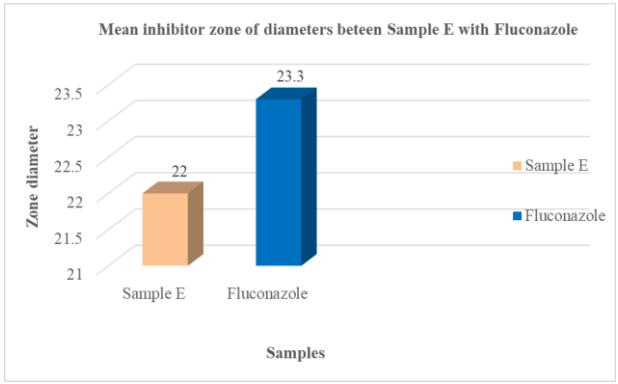
Table 4: Mean inhibitory zone diameters of Samples with Fluconazole

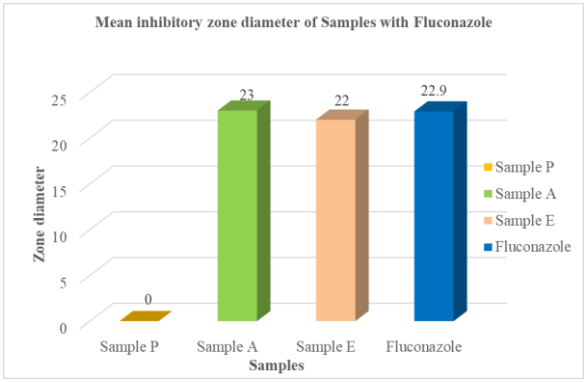
Trial	Samples	Mean inhibitory zone diameters	Std. Deviation
Trial 01	Sample P	0.0000	0.00000
	Fluconazole	22.6667	+0.57735
Trial 02	Sample A	23.0000	+1.00000
	Fluconazole	22.6667	+0.57735
Trial 03	Sample E	22.0000	+0.00000
	Fluconazole	23.3333	+0.57735

Table 5: Std. Deviation on Mean inhibitory zone diameters of Samples









Prepared Ointment of Fresh leaf extract of *Cassia fistula* was presented as 22.4 mm of Mean inhibitory zone diameter. Mean inhibitory zone diameter was higher than 19 mm. Therefor Sensitivity of *Candida albicans* against the Ointment was higher than the normal level of sensitivity (> 19 mm).

Discussion

Plant based antifungal compounds have enormous therapeutically potential as they can serve the purpose without any side effects that are often associated with synthetic antifungal. Antifungal activity of the *Cassia fistula* leaves measured by using Supernatant of leaf extract of *Cassia fistula* (sample P) and Fresh leaf extract of *Cassia fistula* (sample A). Suitable method was identified to prepare the antifungal cream according to the evaluated antifungal activity of those samples. Sample P was prepared by using the supernatant of leaf extract of *Cassia fistula* in trial 1 and Mean inhibitory zone diameter of sample P has given 0 mm that results showed no any inhibitory effect against *Candida albicans*. According to the sample P that antifungal effect on supernatant of leaf extract of *Cassia fistula* showed no inhibitory effect against *Candid albicans*. This showed that using the Supernatant of leaf extract of *Cassia fistula* was not the most suitable method to prepare the Antifungal cream. The trial 2 was done by using the Fresh leaf extract of *Cassia fistula* as sample A and Mean inhibitory zone diameter of sample A has given 23 mm. The Fluconazole 8 mcg were used as controls for comparison with the selected herbal extract. Mean inhibitory zone diameter of 8 mcg of Fluconazole has given 22.66 mm. These results were interpreted that the Fresh leaf extract of *Cassia fistula* has more inhibitory effect than the supernatant of *Cassia fistula*. This tends to show that the active ingredients were better extracted in Fresh leaf extract of *Cassia fistula* than Supernatant. The active ingredient of the *Cassia fistula* leaves was less extracted to the Supernatant and other part of active ingredient were extracted to the precipitate which was clear from the present results of sample A.

It means the efficacy of sample A is better than Fluconazole that treatment for *Candida albicans*. Therefore, Fresh leaf extract of *Cassia fistula* can use to treatment for *Candida albicans* infection. The Antifungal Ointment was prepared according to the above evaluated antifungal effect of Fresh leaf extract of *Cassia fistula.* Antifungal activity of the Ointment that made from Fresh leaf extract of *Cassia fistula* were evaluate and Mean inhibitory diameter was identified 22 mm as Sample E. Therefore, this antifungal Ointment that made from Fresh leaf extract of *Cassia fistula* can use to treat for *Candida albicans* infection.

Conclusion

Standing on above results it was concluded that *Cassia fistula* leaves has antifungal activity of *Candida albicans* infection. The efficacy of the Fresh leaf extract of *Cassia fistula* is higher than the Supernatant of leaf extract of *Cassia fistula*. The efficacy of the sample A was better than 8 mcg of Fluconazole. This antifungal Ointment that made from Fresh leaf extract of *Cassia fistula* can conclude to treat for *Candida albicans* infection.

Further research is necessary to determine the identity of the antifungal compounds from within the plants and also to determine their full spectrum of efficacy. Also, further research is necessary to determine the antifungal activity against fungal species of Human Ringworm Disease. However, the present study of in vitro antifungal evaluation of some plants forms a primary platform for further phytochemical and pharmacological studies to discover new antibiotic drugs.

References

1. Alam, M. M., Siddiqui, M. B., Hussian, W., 1990. Treatment of diabetes through herbal drugs in rural India. Fitoterpia 61, 240- 242.
2. Aslkar, L. V., Kakkar, K. K., Chakre, O. J., 1992. Second supplement to glossaryof Indian medicinal plant with active principals. Publication and Information Directorate, New Delhi, CSIR, I, p. 177.
3. Bhagawan Dash R.K. Sharma, (2004), CharakaSamhita,Vol iii, Chowkhamba, Krishnadas Academy, Chowkhamba press, Varnasi, pag. 322- 352.
4. Bhalodia, N. R., Shukla, V. J. (2011). Antibacterial and antifungal activities from leaf extracts of *Cassia fistula*: An ethno medicinal plant. Journal of Advanced Pharmaceutical Technology Research; 2 (2): 104-9.
5. Biggs WS, Williams R M. 2009, Common gynecologic infections. Prim care. Mar; 36 (1): 33-51.
6. Department of Ayurveda. (1985), Ayurveda Pharmacopoeia,Vol I, Department of Ayurveda, Sri Lanka, pag.49-50.
7. Department of Ayurveda. (1979), Ayurveda Pharmacopoeia, Vol ii, second edition, Department of Ayurveda, Sri Lanka, pag.24.
8. Jaipal, S., Sing, Z., Chauhan, R., 1983. Juvenile hormone like activity in extracts of some common Indian plants. Indian Journal of Agricultural science 53, 730-733.
9. Jayasinghe. D. M. (1990), Charma Roga Nidana Samgrahaya, Department of Ayurveda, pag.93.
10. Kasuko, I., Nagayo, O., 1951. Effects of vegitable drugs on pathogenic fungi I. Effect of anthraquinone, glucoside containing crude drugs upon the growth of pathogenic fungi. Buletin of Pharmaceutical Research Institute, Japan, 23-29.
11. Kirtikar, K. R., Basu, B. D., 1975. Indian Medicinal plants, vol. 4., second ed. Jayyed Press, New Delhi.
12. Kumar, V.P., Chauhan, N. S., Pabh, H., Rajani, M. (2006) Search for antibacterial and antifungal agents from selected Indian medicinal plants. Journal of Ethanopharmacology;107(2):182-8.
13. Liptak P et. al. A Cassia fistula Ber.Ungar.Pharm.Ges. 1937;13: 61-63.
14. Lochan Kanjiv, (2006), Bhaisajya Rathnavali of Govinda Dasji Bhisagratna, Vol iii, Chaukhamba Sanskrit Bhawan, CHOWK, Varnasi, pag.54.

15. Misra T.N. et. Al. A new diterpene from the pods of *Cassia fistula*; LXVII (58):375.
16. Nair, R., Kalariya, T., Sumithra, C., 2005. Antibacterial activity of some selected Indian Medicinal flora. Turkey Journal of Biology 29, 41-47.
17. Panda S.K et. al. Selective antifungal action of crude extracts of *Cassia fistula*,a preliminary study. Malaysian journal of microbiology. 2010; 6(1): 62-68
18. Patel, D., Karbhari, D., Gulati, D., Gokhale, D., 1965. Antipyretic and analgesic activities of Aconatum spicatum and Cassia fistula. Pharmaceutical Biology 157, 22-27.
19. Perry, L. M., 1980. Medicinal plants of East and South East Asia. MIT Press, Cambridge.
20. Perumal Samya, R., Ignacinuthu, S., Scn, A, 1998. Screaning of 34 medicinal plants for antibacterial properties. Journal of Ethnopharmacology 62, 173-182.
21. Prashnth Kumar, V., Chauhan, N. S., Padh, H., Rajani, M., 2006. Search for antibacterial antifungal agents from selectrd Indian medicinal plants. Journal of Etmopharmacology 107, 182-188.
22. Rajagopal et al. Phytochemical and Pharmacological Review on *Cassia fistula* IJPCBS 2013, 3(3), 672-679.
23. Raja, S., Baburaja, D. S., Srthuraman, M., Parimala, S., 2001. Stem and stem bark used medicinally by the Tribals Irulas and Paniyas of Nilgiri Districk, Tamilnadu, Ethnobotany 6, 19-24.
24. Souwalak Phongpaichitet. al. Antifungal activity from the leaf extract of *Cassia fistula*. J.Sci.Technol. 2004; 26 (5):741-748.
25. Sukanya.S,L,J. Sudesha,P. Hariprasad, S. R. Niranjana H. S. Prakash and S. K. Fathima, 2010, Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. Department of studies in Applied Botany, Seedpathology and Biotechnology, University of Mysore, Manasagangotri, Kamataka India- 570006.
26. Timothy et al. Antifungal Activity of Aqueous and Ethanolic Leaf Extracts of *Cassia alata* Linn. Jurnal of Applied Pharmaceutical Science 02 (07); 2012:182-185

- First Author & Correspondence Author – Dr. K. A. B. Fernando, Demonstrator (Temporary), Department of Kawmarabruthya & Stree Roga, Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya, Sri Lanka.

  E mail - baddrafernando@gmail.com Contact number – +94 719304763 / +94 715997966

- Second Author – Dr. WALC Waliwita, Senior Lecturer, Department of Kayacikitsa, Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya, Sri Lanka. E mail - rpugwai@yahoo.com