JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue

JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)

An International Scholarly Open Access, Peer-reviewed, Refereed Journal

IN VITRO ANTIFUNGAL SCREENING OF *HARIDRA (CURCUMA LONGA), APAMARGA* (ACHYRANTHES ASPERA) AND *KADALI* (MUSA PARADISIACA) EXTRACTS

[1]Ravi Shankar Khatri, [1]B M Singh, [2]Nidhi Pandey, [2]Dharmendra Kumar, [2]Ragini Tilak

[1]Department of Kaumarbhritya, Faculty of Ayurveda, I.M.S, B.H.U, Varanasi, Uttar Pradesh

[1]Department of Kaumarbhritya, Faculty of Ayurveda, I.M.S, B.H.U, Varanasi, Uttar Pradesh

[2]Department of Microbiology, I.M.S, B.H.U, Varanasi, Uttar Pradesh

[2]Department of Microbiology, I.M.S, B.H.U, Varanasi, Uttar Pradesh

[2] Professor, Department of Microbiology, I.M.S, B.H.U, Varanasi, Uttar Pradesh

***Corresponding author E-mail address: drrskhatri@gmail.com**

Abstract

Background: Different parts of medicinal plants have been traditionally used for many kinds of ailments including infectious diseases by their phytochemical properties. **Objective:** To compare and investigate the *in vitro* Antifungal activity of Haridra (*Curcuma longa*) rhizome, whole plant of Apamarga (*Achyranthes aspera*) and Kadali (*Musa paradisiaca*) stem. **Methods:** petroleum ether, ethanol and aqueous extracts were obtained with Soxhlet extraction. Antifungal activity was evaluated by disc diffusion method as per standard protocol. **Result:** These extracts showed antifungal activities against *Candida albicans, Trichophyton mentagrophytes* and *Cryptococcus neoformans* with varying magnitudes. **Discussion:** The present study justified that the petroleum ether, ethanolic and aqueous extracts of Apamarg, Kadali and Haridra exhibited good inhibitory activity against *Candida albicans*, *T. mentagrophytes* and *Cryptococcus neoformans* while Kadali displayed Resistant against *Cryptococcus neoformans*. **Conclusion:** The antimicrobial activity showed by the plant was due to the presence of phytochemicals. Further comprehensive studies are highly needed for rational clinical use as antifungal drugs.

Key words: Antifungal, Herbal, Ayurveda, Candida Trichophyton.

Introduction: Fungal infections of the skin and nails are a common global problem. The high prevalence of superficial mycotic infections shows that 20-25% of the world's population has skin mycoses, making these one of the most frequent forms of infection.[1]

Healthy children have natural immunity against fungal infections, but now a day's fungal infection among children are increasing very fast. Virtually not all fungi are pathogenic and their infection is opportunistic. Fungi can occur in the form of yeast, mould, and dimorph. In children fungi can cause superficial infection, subcutaneous fungal infection and lastly it causes systemic infection.[2]

Currently, use of standard antifungal therapies can be limited because of toxicity, low efficacy rates, and drug resistance.[3] Traditional herbal medicine, in form of plant extracts, may be alternative antifungal drugs because of for treatment of fungal infections and some of these have been tested for in vitro antifungal effect with minimal or no side effect. A systematic review evaluates antifungal herbal preparations that have been tested in controlled clinical trials. [4] Herbal medicine is the oldest form of health care known to humanity and has been used in all cultures throughout history. India is the largest producer of medicinal herbs and is called as botanical garden of the world[5]. Herbs can be viewed as biosynthetic chemical laboratories, producing a number of chemical compounds. Herbal drugs ranged from parts of plants to isolated, purified active constituents and come from any part of the plant but most commonly derived from leaves, roots, bark seeds, and flowers. They are eaten, swallowed, drunk, inhaled, or applied to the skin[6]. All three plants, Haridra (*Curcuma longa*), Apamarga (*Achyranthes aspera*) and Kadali (*Musa paradisiaca*) are used in different therapeutic purpose in Ayurveda and also have a potent antifungal activity in vitro as well as clinically.[7, 8]

MATERIALS AND METHODS

Collection of plant materials

All three plants, Haridra (*Curcuma longa*), Apamarga (*Achyranthes aspera*) and Kadali (*Musa paradisiaca*) were collected from Banaras Hindu University Campus. The plant materials were taxonomically identified and authenticated by Prof. N K Dubey Department of Botany Banaras Hindu University. The plant materials were shade dried until all the water molecules evaporated and plants became well dried for grinding. After drying, the plant materials were grind well using mechanical blender into fine powder and transferred into airtight containers with proper labeling for future use. Drugs extraction was carried out in Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University.

Method

1. Solvent extraction

The shade dried Drugs were reduced to fine powder (# 40 size mesh) and around 450 gm of powder was subjected to successive hot continuous extraction (Soxhlet) with petroleum ether (60-80^{0}C), ethanol and aqueous. Each time before extracting with the next solvent the powdered material was air dried in hot air oven below 50^{0}C. After the effective extraction, the solvent was distilled off, and made it concentrated on water bath. The same method was adopted for each drug extracted from different solvent and finally weighed after air drying. Its percentage was be calculated in terms of air-dried weight of plant material color and consistency of the extracts were also noted[9, 10, 11].

2. *In vitro* antifungal susceptibility testing

2.1 Media and test microorganisms: Sabouraud dextrose agar (Hi-media) was used for growth of fungal isolates and Muller-Hinton agar was purchased from Hi-media for antifungal susceptibility testing. The extract activity was observed on three different fungal isolates namely, *Candida albicans* (yeast), *Cryptococcus neoformans* (yeast) and *Trichophyton mentagrophytes* (dermatophyte).

2.2 Extract preparation for antifungal assay: The dried extract was weighed and stock suspension of 100mg/mL was prepared. For working concentration, the stock was diluted to obtain 100µg/ml of which 5µL was dispensed on a sterile disc of Whatman's filter paper for antifungal activity.

2.3 Disc diffusion method: For yeasts, *C. albicans* and *C. neoformans*, antifungal activity was done according to CLSI guidelines M44 A2. Briefly, Muller Hinton agar (MHA) plates were prepared by pouring 15 mL of molten media into sterile petri plates. The freshly grown yeasts were suspended in sterile saline to achieve concentration of 10^6 cfu/mL. Likewise, *T. mentagrophytes* suspension was prepared and left for 5 min to settle down the heavy particles. In continuation, sterile cotton swabs were dipped into the suspension and lawn culture of each microorganisms was done. The plates were dried for 5 min and then different extract were put on 6 mm sterile disc of Whatman filter paper No.1. The disc was then placed on the surface of medium and the extract was allowed to diffuse for 5 minutes. Plates containing yeasts then were kept for incubation at 37 $^{\circ}$C for 24 h while dermatophyte containing plates kept at 28 $^{\circ}$C for 7-14 days. At the end of incubation, inhibition zones were examined around the disc which if present were measured with transparent ruler in millimeters.

Results:

Table 1 shows inhibition Zone for C. *albicans* at 100µg/ml concentration of different drugs extracts.

Table: 1	
Extracts type of drug	Inhibition Zone (mm)
Achyranthes aspera (petroleum ether)	16
Achyranthes aspera (Ethanolic)	12
Achyranthes aspera (Aqueous)	10
Curcuma longa (petroleum ether)	18
Curcuma longa (Ethanolic)	9
Curcuma longa(Aqueous)	12
Musa paradisiaca (petroleum ether)	Resistant
Musa paradisiaca (Ethanolic)	9
Musa paradisiaca (Aqueous)	12

Curcuma longa (petroleum ether) and *Achyranthes aspera* (petroleum ether) extract shows, maximum Inhibitory Zone while *Musa paradisiaca* (petroleum ether) was resistant against *C. albicans* at 100µg/ml.

Table 2 shows inhibition Zone for ***Trichophyton mentagrophytes*** at 100µg/ml concentration of different drugs extracts.

Table: 2	
Extracts of drug	Inhibition Zone (mm)
Achyranthes aspera (petroleum ether)	14
Achyranthes aspera (Ethanolic)	13
Achyranthes aspera (Aqueous)	10
Curcuma longa (petroleum ether)	12

Curcuma longa (Ethanolic)	9
Curcuma longa(Aqueous)	10
Musa paradisiaca (petroleum ether)	Resistant
Musa paradisiaca (Ethanolic)	10
Musa paradisiaca (Aqueous)	8

Achyranthes aspera (petroleum ether) and *Curcuma longa* (petroleum ether) extract shows, maximum MIC while *Musa paradisiaca* (petroleum ether) was resistant against ***Trichophyton mentagrophytes*** at 100µg/ml.

Table 3 shows inhibition zone for *Cryptococcus neoformans* at 100µg/ml concentration of different drugs extracts.

Table: 3	
Extracts of drug	Inhibition Zone (mm)
Achyranthes aspera (petroleum ether)	12
Achyranthes aspera (Ethanolic)	08
Achyranthes aspera (Aqueous)	10
Curcuma longa (petroleum ether)	14
Curcuma longa (Ethanolic)	12
Curcuma longa(Aqueous)	10
Musa paradisiaca (petroleum ether)	Resistant
Musa paradisiaca (Ethanolic)	Resistant
Musa paradisiaca (Aqueous)	Resistant

Curcuma longa (petroleum ether) and *Achyranthes aspera* (petroleum ether) extract shows, maximum MIC while *Musa paradisiaca* (petroleum ether, Ethanolic and Aqueous) was resistant against *Cryptococcus neoformans at* 100µg/ml.

Table 4 shows inhibition Zone (mm) for mixed drug extract of all three drugs at 100µg/ml.

Table: 4

Extracts of drug	*C. albicans*	*Trichophyton mentagrophytes*	*Cryptococcus neoformans*
Achyranthes aspera + *Curcuma longa* + *Musa paradisiaca* (petroleum ether)	16	14	10
Achyranthes aspera + *Curcuma longa* + *Musa paradisiaca* (Ethanolic)	18	14	10
Achyranthes aspera + *Curcuma longa* + *Musa paradisiaca* (Aqueous)	16	12	08

A combination of extract of *Achyranthes aspera* + *Curcuma longa* + *Musa paradisiaca* (Ethanolic) extract show maximum inhibition zone against the *C. albicans, Achyranthes aspera* + *Curcuma longa* + *Musa paradisiaca* (petroleum ether and Ethanolic) extract show maximum MIC against *Trichophyton mentagrophytes* and *Achyranthes aspera* + *Curcuma longa* + *Musa paradisiaca* (petroleum ether and Ethanolic) also show maximum MIC against *Cryptococcus neoformans* at 100µg/ml.

Discussion: The use of medicinal plants plays a vital role in the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms. [12,13] Different plant extracts were evaluated for antifungal activity in this study. The results of the present study justified that the petroleum ether, ethanolic and aqueous extracts of *Apamarg, Kadali* and *Haridra* exhibited good inhibitory activity against *Candida albicans*, *T. mentagrophytes* and *Cryptococcus neoformans* while Kadali displayed Resistant against *Cryptococcus neoformans*. As the work for the development of herbal medicine in progressing worldwide, the present work will help in isolation of new products. Kadli, Apamarga and Haridra may be potential source of Antifungal drugs that can be used for the treatment of refractory fungal infection. This study provokes to explore further the antimicrobial effect of each and every component on the spectrum of the bacteria, parasites, and viruses, only then exact resolution can be drawn to label them as specific antimicrobial agents.

Other in vitro studies also support the antifungal efficacy of Haridra (*Curcuma longa*), Apamarga (*Achyranthes aspera*) and Kadali (*Musa paradisiaca*) in vitro. [14, 15, 16]

CONCLUSION

The inhibitory effect of the extract of *Haridra (Curcuma longa), Apamarga (*Achyranthes aspera*)* and *Kadali (*Musa paradisiaca*)* against pathogenic fungal strains can introduce the plants as a potential candidate for drug development for the treatment of ailments caused by human pathogens. The ability of the extracts to inhibit the growth of several fungal species is an indication of the broad spectrum antimicrobial potential of various parts of drugs, which makes the complete plant a candidate for bioprospecting for an antifungal drugs. Isolation of the antifungal phytoconstituents of individual components are highly needed for further clinical uses.

ACKNOWLEDGEMENT

Authors are thankful to the Department of Dravyaguna and Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India for providing the necessary laboratory facilities for the work.

REFERENCES

1. Havlickova B, Czaika VA, Friedrich M. Epidemiological trends in skin mycoses worldwide. Mycoses 2008;51 Suppl 4:2-15.
2. Walsh, Fungal Infections of the Central Nervous System in Children, Journal of the Pediatric Infectious Diseases Society, Volume 6, Issue 3, September 2017, Pages e123–e133, https://doi.org/10.1093/jpids/pix059
3. Scorzoni L, de Paula e Silva ACA, Marcos CM, Assato PA, de Melo WCMA, de Oliveira HC, Costa-Orlandi CB, Mendes-Giannini MJS and Fusco-Almeida AM (2017) Antifungal Therapy: New Advances in the Understanding and Treatment of Mycosis. *Front. Microbiol.* 8:36. doi: 10.3389/fmicb.2017.00036
4. Karen W Martin et al Herbal medicines for treatment of fungal infections: a systematic review of controlled clinical trials, Mycoses 2004 Apr; 47(3-4):87-92.
5. Seth S.D., Sharma B. Medicinal plants of India. Indian J. Med. Res. 2004;120:9–11
6. Akerele, O. (1993) Summary of WHO Guidelines for the Assessment of Herbal Medicines. HerbalGram, 28, 13-19.
7. In-vitro Evaluation of Antifungal Effect (Specific to Dermatophytes) of Nisha-Durva, Nisha-Arkpatra, Nisha-Kadali Kshar and Apamarga-Mulaka Seeds, published in The Indian journal of research Anvikshiki ISSN 09739777, volume-5 number -3 may june 2011.
8. Antidermatophytic activity of Apamarga – Mulaka seeds specific to Sidhma (Pityriasis versicolor) in children- A clinical study , published in The Pharma Innovation a Peer Reviewed & indexed Journal ISSN: 2277- 7695, Vol. 2 No. 9 2013.
9. Kokate CK. Practical Pharmacognosy, Vallabh Prakashan, New Delhi, 1994; 107-111.
10. Sukhdev Swami Handa, Suman Preet Singh Khanuja, Gennaro Longo, Dev Dutt Rakesh. 2008. Extraction technologies for medicinal and aromatic plants, International centre for science and high technology.

11. Amita Pandey, Shalini Tripathi; Concept of standardization, extraction and pre Phytochemical screening strategies for herbal drug; Journal of Pharmacognosy and Phytochemistry 2014; 2 (5): 115-119.Iqbal Ahmad.
12. Castronovo LM, Vassallo A, Mengoni A, Miceli E, Bogani P, Firenzuoli F, Fani R, Maggini V. Medicinal plants and their bacterial microbiota: a review on antimicrobial compounds production for plant and human health. Pathogens. 2021 Feb;10(2):106.
13. Joshi B, Panda SK, Jouneghani RS, Liu M, Parajuli N, Leyssen P, Neyts J, Luyten W. Antibacterial, antifungal, antiviral, and anthelmintic activities of medicinal plants of Nepal selected based on ethnobotanical evidence. Evidence-Based Complementary and Alternative Medicine. 2020 Apr 22;2020.
14. Varun S. Mali et al Preparation and evaluation of antifungal property of a polyherbal formulation containing Achyranthes Aspera and Plectranthus Amboenicus IJPSR (2013), Vol. 4, Issue 10 E-ISSN: 0975-8232; P-ISSN: 2320-5148.
15. Jeevitha Murugesh et al Evaluation of the antifungal efficacy of different concentrations of *Curcuma longa* on *Candida albicans*: An *in vitro* study JOMFP 2019 Vol.23 Issue 2 Page 305.
16. S. I. Okorondu et al Antifungal properties of Musa paradisiaca (Plantain) peel and stalk extracts Int. J. Biol. Chem. Sci. 6(4): 1527-1534, August 2012.