Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Online International Journal Available at http://www.cibtech.org/jls.htm
2012 Vol. 2 (2) April-June, pp.1 -6 /Pathak
Research Article

ANTI-CANDIDA ACTIVITY OF AQUEOUS EXTRACTS OF SOME HERBALS

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ABSTRACT
Various medicinally important herbs have been used for centuries in the traditional systems of medicine of thirds world countries. All most every part of plants is used in indigenous system of medicine as a health promoter and restorers. The juice of the leaves of various plants has proven benefits. Aqueous extracts of five plant species traditionally used in medicine were investigated for in vitro antimicrobial activity against five different Candida species isolated from human oral cavity, by disc diffusion method. Aqueous extracts of Allium sativum bulb showed the highest toxicity against all the Candida species tested. Aqueous extracts of Azadirachta indica showed week anti Candida activity whereas Ocimum sanctum, Murraya koenigii and Withania somnifera did not displayed any anti-Candida activities. Based on paired t-test, there is no significant difference between the mean values of the size of zone of inhibition of Amphotericin B and herbal extracts were reported.

Key Words: *Aqueous Extracts, Candida, Disc Diffusion, Herbals*

INTRODUCTION
Herbal drugs were used in various aboriginal or traditional systems of medicine since the beginning of human civilization. In India along with Ayurveda and Yoga; Unani, Siddha and Homoeopathy, are practised under the system of AYUSH as a well established branch of medicine. Lack of standardisation, scientific information and quality control are some of the area of concerned with naturopathy. According to Joy *et al*., (2001) the major problem in standardising these drugs are that though these drugs are very effective in its crude forms yet after extracting its active principal compounds these drugs were failed to respond. It is estimated that less than 0.6% of herbal drugs were evaluated in the light of modern scientific knowledge, despite the fact that nearly 80% of the population residing in developing countries have their faith in these systems of medicine.
With increasing incidence of drug resistance in prevalent pathogens and associated risk with chemotherapeutic agents makes it essential to find an alternative to existing drugs. The herbals which have known pharmaceuticals properties could be the best source of these alternative drugs, if the dose response relations, interactions and the risk associated with these herbal medicines were fully evaluated. Recent technological advancements have leads to the discovery and development of many therapeutic agents from herbals with minimum side-effects. More emphasis was given to the anti microbial properties of these herbals either in the form of active components derived from plants or in the form of crude extracts. In most of the indigenous system of medicines herbals were used in its crude form; it has also been reported that the active substances of herbals are unstable in nature when fractionated (Elmore, 1994).
It has been reported in a number of in vitro studies that the herbals that were used to cure infectious diseases have wide antimicrobial spectrum. Therefore, in this study, in order to avoid loss of the volatile and thermo labile active ingredients of the herbals, freshly prepared, unfractionated crude extracts of Garlic (*Allium sativum* L.), Curry leaf or Meethi Neem (*Murraya koenigii* (L.) Spreng), Neem (*Azadirachta indica A. Juss.*), *Shyama Tulasi* (*Ocimum sanctum* L. syn. *Ocimum tenuiflorum*) and Ashwagandha (*Withania somnifera (L.) Dunal*) were screened for their antifungal activity against the clinical isolates of some species of *Candida*. In this present study, we compare the antifungal activity of

known antifungal agents and herbal extracts against the clinical isolates of five different *Candida* species, using disk diffusion susceptibility method and by using the paired Student's t-test.

MATERIALS AND METHODS

Preparation of Extracts

The crude extracts of leaves of selected species of plants were prepared as described by Ghasi *et al*., (2000) with some modification. The leaves of authenticated plants were collected from a garden maintained by Modern Dental College and Research Centre, Indore, India, after collecting the twigs the stalk were removed. Mature fresh leaves were washed thoroughly in sterilized distilled water to remove dust and other foreign matter from surface and then with 1% mercuric chloride, followed by washing 2-3 times in sterilized phosphate buffer saline solution to remove all the chlorine contents.

For the preparation crude juice of leaves and garlic; the fresh sterile leaves of the samples and the garlic cloves were crushed in sterile Phosphate buffer Saline (Merk, India) (1:1 w/v) by using sterile grinded vessel tissue homogenizer (HiMedia, India) with pestle. The solution was thereafter filtered by using a sterilised four layer of muslin cloth, served as 100% concentration; fresh extract of plants leaves and cloves of garlic were used separately for each batch.

Disc Diffusion Method

Preparation of Discs

The prepared extracts were loaded on to the filter paper discs placed in Petri plates (Sterilized Whatmann No. 1 filter paper discs of 6 mm diameter) and allowed to soak in extract for 6 hr. for proper absorption, after which they were removed and allowed to dry overnight in hot air oven at 40°C under aseptic condition.

Micro organisms used

The screening of the anti fungal activity of crude extracts was carried out on clinical isolates of the species of *Candida.* The test organisms for the screening of the anti microbial activity of crude extracts were included clinical isolates of five different species of *Candida,* i.e. *C. albicans, C. dubliensis, C. kruzi, C. glabrata and C. tropicalis* isolated from the oral lesions of patients with Oropharyngeal Candidiasis (OPC); the patients from whom the specimens were collected have not been exposed to prior antifungal or antibacterial drugs.

Antimicrobial activity

Standard method of disk diffusion assay was used to test the yeast isolates (Lee *et al*., 2001). Inoculums were prepared by picking five distinct colonies of approximately 1mm from 24 hours each old culture of *Candida* spp. grown on Sabouraud Dextrose Agar (HiMedia, India) and incubated at 35±2°C. Colonies were suspended in 5ml of sterile 0.85% Saline. Vortexes the resulting suspension and adjusted the turbidity to yield $1X10^7$ cells/ml (i.e.0.5 McFarland standard). A sterile cotton swab moistened with the inoculums suspension was used to apply to a 90 mm diameter plate containing Mueller-Hinton agar (HiMedia, India) supplemented with 2% glucose and 0.5 µg/ml methylene blue for *Candida* spp. The plates were allowed to dry for 5-15 minutes before disks were placed in the centre of the agar. The plates were incubated for 18-24 hours at 37±2°C and the slowly growing isolates were again read after 48 hours incubation. Zone sizes were measured in millimetres with Zone Scale (HiMedia, India), the zone diameter to the nearest whole millimetre the point at which there is prominent reduction in growth were taken into consideration. All the disk diffusion experiments were performed in triplicates and mean were taken. For positive control and comparison, a series of antifungals, namely, amphotericin-B (100 Units), clotrimazole (10 µg/ml.), fluconazole (25 µg/ml.), itraconazole (10 µg/ml.) and voriconazole (1 µg/ml.) were used.

Statistical Analysis

Zone sizes obtained by using disk diffusion experiments of antifungal drugs and herbal extracts for *Candida* species, were compared by the paired Student's t-test by using the SPSS Win 12.0 program (SPSS Inc, Chicago, U.S.A.). Differences in the zone size of antifungal drugs and herbal extracts were

considered significant for *P* of 0.05. The null hypothesis (HO) rejected in favour of the alternative hypothesis (H1) at significance level α (0.05) *if*; $T > t_{n-1}, \alpha/2$ (value of the Student table with *n* -1 degrees of freedom). The null hypothesis was *H*0: $\delta = 0$ (there is a difference in zone of inhibition between antifungal disk and herbal extracts) and the alternative hypothesis is (was) *H*1: $\delta = 0$ (there is no difference in zone of inhibition between antifungal disk and herbal extracts).

RESULTS

The result of the anti candidal activity of aqueous extracts of the selected plant species were shown in table 1. The inhibition zone diameters by the test isolates against different plant extract shows that aqueous extracts of *O. sanctum* and *W. somnifera* were not effective against any tested species of *Candida*. However, *A. indica* and *M. koenigii* were showing week and the extract of *A. sativum* bulbs displaying a strong anti candidal activity, against all the species of *Candida* tested.

Table 1: The inhibition zone diameters by the test isolates against different herbal extract

	Allium sativum	*Azadirachta indica*	*Murraya koenigii*	*Ocimum sanctum*	*Withania somnifera*
C. albicans	14	7	7	-	-
C. krusei	16	9	6	-	-
C. tropicalis	13	6	6	-	-
C. glabrata	17	10	7	7	-
C. dublinensis	16	8	7	-	-

Table 2 shows the inhibition zone diameters shown by the anti fungal agents on the Candidal isolates, there were varying degree of inhibition of all the isolates used in the study. The species of C. albicans, C tropicalis were shown resistance towards all the azole compounds tested, whereas, C.krusei and C glabrata were sensitive towards all the azoles except itracnozole and flucanozole respectively. C. dublinensis was found sensitive to all the antifungal agents tested. None of the *Candida* spp. was shown resistance towards amphotericin-B.

Table 2: The inhibition zone diameters by the test isolates against different anti fungal agents

	Fluconazole	itraconazole	Amphotericin B	clotrimazole	voriconazole
C. albicans	-	-	20S	14R	-
C. krusei	15S	12R	17S	22S	14S
C. tropicalis	-	-	20S	-	12R
C. glabrata	12R	16S	22S	16S	23S
C. dublinensis	30S	17S	18S	21S	38S

To test the hypothesis of no difference or no relationship between the size of zone of inhibition of antifungal drugs and herbal extracts paired t-test was performed (Table 3). For Nine pairs, 56% of pairs rejected null hypothesis in favour of alternate hypothesis, it means that there is no significant difference between the mean values of the size of zone of inhibition of Amphotericin B and herbal extracts, Clotrimazole was significantly related with Azadirachta indica and Murraya koenigii, but not with Allium sativum, and Voriconazole shown non-significant relation with all the herbal extracts tested.

Table 3: Paired Samples t-Test of anti fungal agents and Aqueous extracts of plant species

	Paired Samples Test			
		t	Sig. (2-tailed)	**Decision (α = 0.05)**
Pair 1	Amphotericin B - *Allium sativum*	3.628	0.022	Significant
Pair 2	Amphotericin B - *Azadirachta indica*	10.585	0.000	Significant
Pair 3	Amphotericin B - *Murraya koenigii*	11.513	0.000	Significant
Pair 4	Clotrimazole - *Allium sativum*	1.665	0.195	Non-significant
Pair 5	Clotrimazole - *Azadirachta indica*	5.620	0.011	Significant
Pair 6	Clotrimazole - *Murraya koenigii*	7.028	0.006	Significant
Pair 7	Voriconazole - *Allium sativum*	1.127	0.342	Non-significant
Pair 8	Voriconazole - *Azadirachta indica*	2.335	0.102	Non-significant
Pair 9	Voriconazole - *Murraya koenigii*	2.497	0.088	Non-significant
Pair 10	Allium sativum - *Azadirachta indica*	36.000	0.000	Significant
Pair 11	Allium sativum - *Murraya koenigii*	14.063	0.000	Significant
Pair 12	Azadirachta indica - *Murraya koenigii*	1.826	0.142	Non-significant

DISCUSSION

Several studies have previously been done which demonstrate that; Garlic (Shuford *et al.*, 2005; Lee *et al*., 2011), Curry leaf (Vohra and Gupta, 2011 ; Mathur *et al*., 2011), Neem (Biswas *et al*., 2002, Timothy *et al.*, 2011) and Ashwagandha (Singh *et al.*, 2010; Jain and Varshney, 2011,) possesses good antibacterial and antifungal properties. This study has not been reported antifungal activity of the aqueous extract of Ashwagandha and Tulsi against the species of *Candida* tested. Previous scholars while evaluating the aqueous extract of Ashwagandha (Kambizi and Afolayan, 2008) and Curry leaf (Malwal and Sarin, 2011) have not been reported anti-candidal activity. The anti candidal activity of Neem was concentration dependent; at lower concentration neem extract losses its antifungal property reported during this study was similar to previous findings (Grover *et al*., 2011).

Presence of wide range natural sulfur compounds in garlic exhibited both bacteriostatic and fungistatic properties; inhibition of ADP phosphorylation, interfere with membrane associated enzyme proteins, and inhibition of the synthesis of DNA, RNA, proteins and polysaccharides of microorganism, are some of the mode of action were suggested by previous workers (Bagiu, 2012). Previous workers have been reported the most effective concentration of the extract of *Allium sativum* and the other species of *Alllium* against *C. albicans* and Non *C. albicans Candida* species were ranging from 0.5 to 4.0 mg/ml (Bagiu, 2012). The extract of *A. sativum* bulbs (1 mg/ml.) used in this study was reported effective in controlling Candidal growth under in vitro condition.

Bhadauria and Kumar, 2011 have reported that Candida *albicans* was most resistant to water extracts taken from *Allium sativum* Linn., *Cymbopogon martinii* (Roxb) Wats. and *Catharanthus roseus* (Linn.) G. Don., according to them water and methanolic extracts of all plant parts were least effective against dermatophytes, this work is also in agreements to Bhadauria and Kumar, (2011) except for *Allium sativum*. Present study reported a strong anti candidal activity of extract of *A. sativum* bulbs under in vitro condition.

REFERENCES

Bagiu RV, Vlaicu B and Butnariu M (2012). Chemical Composition and *in Vitro* Antifungal Activity Screening of the *Allium ursinum* L. (Liliaceae). *International Journal of Molecular Sciences* **13** 1426-1436.

Bhadauria S and Kumar P (2011). *In Vitro* Antimycotic Activity of Some Medicinal Plants Against Human Pathogenic Dermatophytes. *Indian Journal of Fundamental and Applied Life Sciences*. [Online], **1** (2) 1-10.

Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U (2002). Biological activities and medicinal properties of neem (*Azadirachta indica*). *Current Science* **82**(11) 1336-1345.

Collee JG, Fraser AG, Marmion BP and Simmons A (1999). Practical Medical Microbiology.14th ed. *Charchil Livingstone.* London.

Duraipandiyan V, Ayyanar M and Ignacimuthu S (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complementary and Alternative Medicine* **6** 35.

Elmore, GS and Feldberg, RS (1994). Alliin lyase localization in bundle sheaths of garlic clove (*Allium sativum*). *American Journal of Botany* **81** 89-94.

Geeta, Vasudevan DM, Kedlaya R, Deepa S and Ballal M (2001). Activity of *Ocimum sanctum* (the traditional Indian medicinal plant) against the enteric bacteria. *Indian Journal of Medical Sciences* **55** 534–538.

Ghasi S, Nwobodo E and Ofili JO (2000). Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high-fat diet fed wistar rats. *Journal of Ethnopharmacology* **69** 21–25

Goyal P and Kaushik P (2011). *In vitro* Evaluation of Antibacterial Activity of Various Crude Leaf Extracts of Indian Sacred Plant, *Ocimum sanctum* L. *British Microbiology Research Journal* **1**(3) 70-78.

Grover A, Bhandari BS and Rai N (2011). Antimicrobial Activity of Medicinal plants- Azadirachta indica A. Juss, Allium cepa L. and Aloe vera L. *International Journal of PharmTech Research* **3**(2) 1059-65.

Jain P and Varshney R (2011). Antimicrobial activity of aqueous and methanolic extracts of *Withania somnifera* (Ashwagandha). *Journal of Chemical and Pharmaceutical Research* **3**(3) 260-263.

Janssen AM, Scheffer JJ, Ntezurubanza L and Svendsen AB (1989). Antimicrobial activities of some *Ocimum* species grown in Rwanda. *Journal of Ethnopharmacology* **26** 57-63.

Kambizi L and Afolayan AJ(2008). Extracts from *Aloe ferox* and *Withania somnifera* inhibit *Candida albicans* and *Neisseria gonorrhoea. African Journal of Biotechnology* **7**(1) 12-15.

Lee HJ, Park HS, Kim KH, Kwon TY, Hong SH (2011). Effect of garlic on bacterial biofilm formation on orthodontic wire. *Angle Orthodontist* **81**(5) 895-900.

Lee SC, Fung CP, Lee N, See LC, Huang JS, Tsai CJ, Chen KS, Shieh WB (2001). Fluconazole disk diffusion test with methylene blue and glucose-enriched Mueller-Hinton agar for determining susceptibility of Candida species. *Journal of Clinical Microbiology* **39** 1615–17.

Malwal M and Sarin R (2011). Antimicrobial efficacy of *Murraya koenigii* (Linn.) Spreng. root extracts. Indian journal of natural products and resources **2** (1) 48-51.

Mathur A, Verma SK, Singh SK2, Prasad GBKS, DuaVK (2011). Investigation of the antimicrobial, antioxidant and antiinflammatory activity of compound isolated from *murraya koenigii*. *International Journal of Applied Biology and Pharmaceutical Technology* **1**(2) 470-477.

National Committee for Clinical Laboratory Standards (2002). *Performance Standards for antimicrobial susceptibility testing*. 8th Informational Supplement. M100 S12. *National Committee for Clinical Laboratory Standards*, Villanova, Pa.

Pathak AK (2011) Antibiogram of some selected species of gram negative bacteria isolated from hospital environment. Indian Journal of Fundamental and Applied Life Sciences [Online], **1**(4) 145-150.

Shuford JA, Steckelberg JM, Patel R (2005). Effects of Fresh Garlic Extract on *Candida albicans* Biofilms. *Antimicrobial Agents* and *Chemotherapy* **49**(1) 473.
Singh SP, Tanwer BS, Khan M (2010). Antifungal potential of ashwagandha against some pathogenic fungi. *International Journal of Biopharmaceutics* **1**(2) 72-74.
Timothy SY, Goji SY, Abdussalam B, Mava Y, and Galadima IH (2011) Antibacterial and phytochemical screening of the ethanolic leaf extract of *azadirachta indica* (neem) (meliaceae). *International Journal of Applied Biology and Pharmaceutical Technology* **2**(3) 194-199.
Vohra K, Gupta VK (2011). *Murraya koenigii* (Linn.) Spreng (Rutaceae): A precious Gift from the Nature. *International Journal of Pharma Recent Research* **3**(1) 18-25.