

Full Length Research Paper

Screening of some plant extracts for antifungal activity against *Candida* species

Marwan Khalil Qader*, Nezek Salim Khalid and Asia A.M. Saadullah

Faculty of Science, University of Duhok, Duhok, Iraq.

Accepted 19 September, 2013

Plants are very useful and utilized as medicine due to their medicinal properties. Screening of plants for biologically active compounds against human pathogens is a renewed interesting research field. In this study, seven plants extracts were used namely: *Plantago media subsp. stepposa*, *Quercus infectoria*, *Punic granatum*, *Thymus otschyana*, *Zingier officinalis*, *Rhus angustifolia* and *Cinnamomun* were screened for its potential antibacterial activity against *Candida tropicalis*, *Candida albicanus*, *Candida dublinensis*, *Candida krusei* and *Candida glabrata*. The antimicrobial activity was determined in ethanol extracts and distill water extracts of these plants. The distill water extracts showed minimum antimicrobial activity when compared to ethanolic extracts. The ethanolic extracts of *Q. infectoria* showed the maximum activity against all species of *Candida* fungus.

Key words: *Candida*, plant extracts, antifungal agent.

INTRODUCTION

Medicinal plants are part of human medicine since the dawn of civilization. These plants are making backbone of traditional medicinal systems in India (Nayak et al., 2011). The use of plant extracts to treat microbial infections is also reported in our ancient Ayurvedic compendium 'Charak Samhita' and 'Sushrat Samhita' (Kelmanson and Staden, 2000). Due to increased prevalence of drug resistant microorganisms, there is great need to search for new effective drugs having natural or synthetic origin (Pai et al., 2004).

Generally, phytopathogenic fungi are controlled by synthetic fungicides; however, the use of these is increasingly restricted due to the harmful effects of pesticides on human health and the environment (Harris et al., 2001). The increasing demand of production and regulations on the use of agrochemicals and the emergence of pathogens resistant to the products employed, justifies the search for novel active molecules and new control strategies. Since antiquity, the plant kingdom has provided a variety of compounds of known therapeutic properties, like analgesics, anti-inflammatories, medicines for asthma, and others. In recent years, antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world (Cowan, 1999). Plant extracts

and their products are clinically safer than antibiotics (Srinivasan et al., 2001). The research for new therapeutic treatments for various disease conditions is expanding. In many poor countries, plants have been looked at as a very promising source of new lead compounds for drug discovery and development (Kong et al., 2003). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs (Nascimento et al., 2000). Therefore, such plants should be investigated to better understand their properties, safety and efficacy. The success story of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains of microorganisms. The investigation of certain indigenous plants for their antimicrobial properties may yield useful results. Many studies indicate that some plants have substances such as peptides, unsaturated long chain aldehydes, alkaloids, essential oils, phenolics, as well as different ethanol, chloroform, methanol and butanol soluble compounds. These plants have emerged

*Corresponding author. E-mail: marwan.qader@yahoo.com.

Table 1. Results of antifungal susceptibility test of the plant extracts (diameter of clearing zones produced by the ethanol extracts in mm, 0.2 gm/ml).

Extract	<i>C. tropicalis</i>	<i>C. albicanus</i>	<i>C. dublicans</i>	<i>C. krusei</i>	<i>C. glabrata</i>
<i>Plantago media</i> subsp. <i>stepposa</i>	10	15	10	15	15
<i>Quercus infectoria</i>	50	40	20	15	15
<i>Punic granatum</i>	25	30	20	10	12
<i>Thymus kotschyana</i> ,	20	15	20	20	17
<i>Zingiber officinalis</i> ,	10	10	15	15	10
<i>Rhus angustifolia</i>	15	10	8	20	15
<i>Cinnamomum sp.</i>	30	10	10	14	17
The mixture of all plants	40	35	35	20	20
Fluconazole	Resistant	Resistant	Resistant	Resistant	Resistant

as plants with compounds possessing significant therapeutic potential against human pathogens, including bacteria, fungi or virus (El astal et al., 2005).

MATERIALS AND METHODS

Collection and preparation of extracts

Plant specimens were collected and identified from different locations of Kurdistan region of Iraq. Samples were dried at room temperature for 5 days, after which they were ground dried to powder plants and then dissolved by using solvents (ethanol and distilled water). The dried plant materials of 5 g each were dissolved in 25 ml (0.2 gm/ml). After the plant materials were successively extracted with ethanol and distilled water separately, the extract was filtered through Whatman filter paper and stored at -2°C until it was used.

Culture media

The media used for antifungal test was Sabouraud's dextrose agar/broth of Himedia Pvt. Bombay, India.

Fungal strains used

Fungi were obtained and an examination of its sensitivity was carried out in Mycology Laboratory in the Biology Department, Faculty of Sciences, University of Dohuk, Kurdistan region of Iraq and the species were as follows: *C. tropicalis*, *C. albicanus*, *C. dublicans*, *C. krusei* and *C. glabrata*.

Preparation of inoculums

The suspension of fungus was prepared as per MacFarland Nephelometer Standard. A 24 h old culture was used for the preparation of fungus suspension. A suspension of fungus was made in a sterile isotonic solution of sodium chloride and the turbidity was adjusted such that it contained approximately 1.5×10^6 cells / ml.

It was obtained by adjusting the optical density (650 nm) equal to 1.175% barium chloride in 100 ml of 1% sulphuric acid.

Antifungal susceptibility test

Stock fungi were maintained at room temperature on Potato Dextrose Agar. Active fungi for experiments were prepared by seeding a loopful of fungi into Potato dextrose broth and incubated without agitation for 48 h at 25°C. The broth was diluted with Potato dextrose broth to achieve optical densities corresponding to 2.0×10^5 spore/ml for the fungal strains. The disc diffusion method was also used to screen for antifungal properties. *In vitro* antifungal activity was screened by using Potato Dextrose Agar (PDA). The PDA plates were prepared by pouring 15 ml of molten media into sterile Petri plates. The plates were allowed to solidify for 10 min and 1 ml of the test culture was introduced into agar and allowed to spread while the excess was drained off. The plate was incubated at room temperature for 10 min. A sterile cork borer of 5 mm diameter was used to make two ditches (wells) on each plate and filled with 1 ml (200 mg) of the plants extract. The same was repeated for each fungus strain using the extract. These were carried out in triplicate for each fungus. The plates were incubated at 25°C for 96 h and the resulting zone of inhibition around the ditches were measured to the nearest millimeter along two axes and the mean of the two measurements was calculated. Each set of seeded plates were compared for confirmation. Control test was carried out using 10 mg/ml of fluconazole.

RESULTS AND DISCUSSION

Antifungal susceptibility test showed that all the fungi were effective against plant extracts with different zones of inhibition (Tables 1 and 2). The results obtained from this work showed that plant extracts exhibit antifungal effects against *Candida* spp. In particular, ethanolic extracts offer effective bioactive compounds for growth

Table 2. Results of antifungal susceptibility test of the plant extracts (diameter of clearing zones produced by the water plant extracts in mm, 0.2 gm/ml).

Extract	<i>C. tropicalis</i>	<i>C. albicanus</i>	<i>C. dublicans</i>	<i>C. krusei</i>	<i>C. glabrata</i>
<i>Plantago media</i> subsp <i>stepposa</i>	8	5	10	10	15
<i>Quercus infectoria</i>	20	22	20	11	11
<i>Punic granatum</i>	Resistant	15	10	9	7
<i>Thymus kotschyana</i> ,	18	16	Resistant	18	16
<i>Zingiber officinalis</i> ,	17	20	14	12	11
<i>Rhus angustifolia</i>	8	5	6	12	14
<i>Cinnamomum sp.</i>	15	15	10	11	10
The mixture of all plants	10	14	12	9	12
Fluconazole	Resistant	Resistant	Resistant	Resistant	Resistant

inhibition of the fungi. Even at low concentrations, these species showed antifungal activity nearly equal to that of the commercial fungicide used as a positive control, suggestive of antimicrobial activity, or previous studies that have demonstrated antifungal properties using different kinds of extracts (Wilson et al., 1997; Zhu et al., 2005).

The presence of antimicrobial substances in the higher plants is well established. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health (Rana and Jain, 2011).

The higher antifungal activity of most of the ethanolic extracts as compared to water extracts might be due to the lack of solubility of active constituents in water. Out of the 7 plant extracts tested for their antifungal activity, most of them showed antifungal activity against the species, indicating that its ethanolic extract was more effective as compared to the water extract. As observed in Table 1, *Q. infectoria* (50 mm) showed the maximum antifungal activity against *C. tropicalis* whereas *R. angustifolia* (8 mm) showed the minimum antifungal activity against *C. dublicans*. The best results were obtained when all the plant extracts were mixed against all kinds of fungi used.

REFERENCES

- Cowan MM (1999). Plant products as antimicrobial agents. *Clin. Microbiol. Rev.*, 10: 564-582.
- El astal ZY, Aera A, Aam A (2005). Antimicrobial activity of some medicinal plant extracts in Palestine. *Pak. J. Med. Sci.*, 21(2): 187. www.pjms.com.pk.
- Harris CA, Renfrew MJ, Woolridge MW (2001). Assessing the risk of pesticide residues to consumers: recent and future developments. *Food Addit. Contamin.*, 18: 1124-1129.
- Kelmanson JE, Staden JZ (2000). Medicinal plants with antimicrobial activity. *J. Ethnopharmacol.*, 69: 241-246.
- Kong JM, Goh NK, Chia LS, Chia TF (2003). Recent Advances in traditional plant drugs and orchids. *Acta Pharmacol. Sin.*, 24: 7-21.
- Nascimento GGF, Lacatelli J, Freitas PC, Silva GL (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Braz. J. Microbiol.*, 31(4): 886-891.
- Nayak A, Nayak RN, Soumya B, Bhat K, Kudalkar M (2011). Evaluation of antibacterial and anticandidal efficacy of aqueous and alcoholic extracts of Neem (*Azadirachta Indica*) and *in vitro* study. *IJPAP*, 1: 230-235.
- Pai MR, Acharya LD, Varun N (2004). Evaluation of antiplaque activity of *Azadiachta Indica* leaf extract get a 6 week clinical study. *J. Ethnopharmacol.*, 90: 99-103.
- Rana PS, Jain DA (2011). Screening For Anti-Fungal Activity of Some Medicinal Plant Species from North India. *Asian J. Biochem. Pharm. Res.*, 1(2): 2231-2560.
- Srinivasan D, Thirumurugan N, Hansi T, Persumalasy PL (2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *J. Ethnopharmacol.*, 74: 217-220.
- Wilson CL, Solar JM, El Ghaouth A, Wisniewski ME (1997). Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. *Plant Dis.*, 81:204-210.
- Zhu XF, Zhang HX, Lo R (2005). Antifungal activity of *Cynara scolymus* L. extracts. *Fitoterapia*, 76: 108-111.