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Evaluation of phenolic compounds of methanolic and aqueous extracts of water hyacinth leaves And its antifungal effect on Candida Albicans

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Abstract

Water hyacinth (Echhornia crassipes) has become one of the world's worst weeds due to rapid proliferation rate and ability to quickly spread over the surface of fresh water bodies despite abundant phytochemical content. Candida albicans is an opportunistic fungal pathogen causing candidiasis fungal infection in humans in the presence of predisposing factors. The present study aimed to evaluate phenolic composition of aquatic and hydromethanolic extracts of water hyacinth, and its antifungal effect on standard strain of Candida albicans. Dry leaf powder of water hyacinth was soaked in 80% methanol and water to prepare extracts under in vitro. Spectrophotometric assays were used to determine total phenolic and flavonoid contents of the extracts. Antifungal activity of the extracts against Candida albicans was evaluated.

The growth inhibition zone diameter was assessed using agar diffusion assays at 6.3, 12.5, 25, 50 and 100 mg/ml concentrations. Total phenolic contents of aqueous and hydromethanolic extracts were 491.1 ± 31.9 and 76.8 ± 7.8 mg gallic acid/gr and total flavonoid contents were 258.3 ± 0.8 and 46.1 ± 6 mg quiricetin/g. Both extracts showed inhibitory effect on Candida albicans at 50 and 100 mg/ml concentrations. Inhibitory effect of the hydromethanolic extract of water hyacinth on Candida albicans strains was significantly higher than aqueous extract of the plant ($P \leq 0.05$). Phenolic compounds and other secondary metabolites of the extracts play an important role in antifungal activity of the plant.

Key words: Hydromethanolic extract, Water hyacinth, aquatic extract, Candida albicans

Introduction

Water hyacinth is native to Brazil. It was first found in Aynak Lagoon in Gilan province in 2012 (Mozaffarian and Yaghoubi, 2015). Rapid growth rate of the plant guarantees that water hyacinth can be doubled within a week to ten days under appropriate ecological condition (Khatun et al., 2014). Water hyacinth has been identified by the International Union for Conservation of Nature (IUCN) as one of the 100 most aggressive invasive species and recognized as one of the top 10 worst weeds in the world (Téllez et al., 2008). Stems and leaves of water hyacinth were used to treat swelling and wounds since ancient times (Rorong et al., 2012). Studies have shown that the plant can be used as an effective drug to treat cancer and combat clinical and aqueous microbial infections in vitro due to its active compounds and secondary metabolites (Jayanthi and Lalita, 2013; Thamaraiselvi and jayanthi, 2012; Rufchaie et al., 2018).

Fungi are known as one of the most common pathogens worldwide and are still the leading cause of morbidity and mortality despite advancement of scientific knowledge. Candida albicans in people with compromised immune systems due to AIDS, cancer, bone marrow transplantation, solid organ transplantation, etc. causes infections that later lead to death (Menon et al., 2001). Chemical antifungal drugs might not only act on infected cells but also interact with host tissue cells since fungi like humans consist of eukaryote cells (Falahati et al., 2012). Therefore, natural agents such as medicinal plants are recently used to treat fungal diseases since the herbs have fewer side effects (Rafieian-Kopaei et al., 2016). The habitat and ecological conditions of the plants determine the amount and composition of active compounds of the plant (Jafari Nodoushan et al., 2007). The present study sought to take advantage of active compounds of this invasive plant and turn the challenge into opportunity by accelerated proliferation of therapeutic effects. Efficient use of this weed not only reduces its detrimental effect on the environment, but also offers a valuable oral supplement.

Material and Method

Sample Collection and Extraction

Water hyacinth was transferred from Anzali Lagoon to food and live feeding station in Anzali Port. Scientific name of the plant was confirmed in systematic classification system of weeds (Holm et al., 1977). Leaves were isolated from the plant, washed with water, dried at 30 ° C and then grinded in a mill. The leaf powder was soaked in two solvents of water and 80% hydromethanol for 48 hours to prepare extracts. It was repeated three times. The

extracts were filtered through a filter paper and concentrated using vacuum distillation process at 40°C (Arabshahi-Delouee and Urooj, 2007). The extracts were kept in the freezer at 20°C until the test.

Total phenolic content measurement

Total phenolic content of the extract was measured using the Folin and Ciocalteu reagent with slight modification (Chaouche et al., 2013). Ten milligrams of water hyacinth powder was dissolved in one milliliter of water. Then, 100 µl (1 mg) of the suspension was mixed with 2.8 ml of water, 2 ml of 2% sodium carbonate and 0.1 ml 50% Folin for 30 minutes and left at room temperature. The absorbance was measured by the spectrophotometer and read at 750 nm. Total phenolic content was calculated as mg gallic acid equivalent per g using the equation from the calibration curve.

Total flavonoid content measurement

Ten milligram of extract powder was dissolved separately in 5 ml of water. Then, 0.5 ml (1 mg) of the suspension was added to 1.5 ml of 95% ethanol, 100 µl of 10% $AlCl_3$, 100 µl of 1M potassium acetate, and 2.8 ml of water. The mixture was incubated at 20°C for 40 minutes. Its absorbance was measured by the spectrophotometer and read at 415 nm. Quercetin was prepared through serial dilution and its concentration was determined according to the standard curve (Meda et al., 2005).

Biometric Test (Antifungal Activity Test)

Antifungal activity of aqueous and hydromethanolic extracts of water hyacinth was evaluated against Candida albicans (standard number = ATCC10231) in this test

Evaluation of antifungal effect of extracts - well diffusion method

Antifungal activity of different extracts of water hyacinth against various pathogens was investigated by measuring diameter of growth inhibition zone using well diffusion method. To prepare the inocula, yeast cell suspensions were adjusted to match the turbidity of a 0.5 McFarland standard (1.5 x 108 10^8 cfu / mL). The fungal specimens were streaked into the Sabouraud Dextrose Agar (SDA) using sterile swabs to obtain uniform fungal culture. Then, wells in 6 mm diameter were drilled in the medium using sterile Pasteur pipettes. The wells were coated with 20 µL of fused media. The plates were refrigerated for 15 minutes. Then, 50 µl of each extract was prepared at concentrations of 6.3, 12.5, 25, 50 and 100 mg/ml. Prepared extracts were poured into the well. The plates were incubated at 37°C for 24 hours. Bacterial susceptibility or resistance to different extracts was assessed by measuring diameter of growth inhibition. Extraction solvents were used as negative control and nystatin antifungal disk (100 I.U) as positive control (CLSI, 2012).

Determination of minimum inhibitory concentration (MIC)

MIC was determined based on clinical and laboratory standard institute (CLSI) guidelines. A stock solution of extracts was prepared using DMSO solvent. A serial dilution of the stock (from 128 to 0.06 mg/ml) was also prepared in 96-well plates using SDA. A suspension of the fresh culture (18–24 h) of 0.5 McFarland standard media was also prepared using saline solution. A suspension of the latter + SDA at a ratio of 1: 100 (suspension to SDA) was prepared and added to each well (1.5×10^6 CFU/ml) to reduce final concentration of the well to half of the origin. The plates containing serial dilution of the sample and fungi were incubated at 37°C. Turbidity of the wells was assessed and MIC was determined as mg/ml. Resazurin reagent was used in case that the extract dissolved in the culture media and made the culture turbid. Resazurin was used to distinguish inhibited wells from non-inhibited wells. All MIC determination tests were repeated three times. Some wells were used as negative control for quality control (wells containing only 200 µL of liquid culture medium).

Determination of minimum fungicidal concentration (MFC)

MFC shows the concentration of the extract that kills 99.9% of fungi. After MIC determination, 100 microliters of each inhibited well was shaken and grown on SDA in form of lawns. The wells were heated at a suitable temperature for 24 hours. MFC was determined in the wells with inhibited fungal growth.

Data analysis method

The tests were repeated three times to compare antifungal activity of the extracts. SPSS v.18, one-way ANOVA, Duncan test, and Tukey test were used to analyze the difference between the means at 0.05 significance level. The independent t-test was used to evaluate antifungal activity of the extract after ensuring normal data distribution.

Result

Table1: Comparison of total phenol and flavonoid values of aqueous and hydromethanol E. crassipes extract

Solvent	) mg Galic acid/g DW(Total phenol	)mg Quercetin /g DW(Total flavonoid
Aqueous	491/2± 31/9 [a]	76/8±7/8 [a]
Hydro-methanol	258/3±10/8 [b]	46/1±6/0 [b]

Data in the same colume with different letters are significantly different ($P < 0.05$). Values are mean ± SE

Table 1 shows that total phenolic content of hydromethanolic extract was higher than aqueous extract of water hyacinth (491.2±31.9 vs. 258.3±10.8 mg gallic acid per gram of dry sample) ($P \leq 0.05$). Total flavonoid content of hydromethanolic extract was also significantly higher than

aqueous extract of water hyacinth (76.8±7.8 vs. 46.1±6 mg quercetin per gram of dry plant) (P≤0.05). Table 2 shows greater inhibitory effect of hydromethanolic extract (28.3±0.3 mm) against Candida albicans compared to aqueous extract at 100 mg/ml concentration. The results also showed significantly greater antifungal activity as concentration of the extract increased (p≤0.05). Independent t-test results showed that hydromethanolic extract had significantly richer phenolic content and greater antifungal effect than aqueous extract at most studied concentrations (p≤0.05).

Table 3 shows MIC was determined in the hydromethanolic extract and MFC was estimated as 32 mg/ml in this extract.

Table2: Antifungal activity of E. crassipes hydro-methanol and aqueous extracts against different candida sp. based on well diffusion method.

Extract (mg/ml)	Inhibition zone diameter around test disc					Control	
	6.3	12.5	25	50	100	** Negative	* positive
Aqueous	7±0.0^{e}	9±0.3^{e}	11±0.6^{d}	18±0.5^{c}	28.3±0.3^{b}	6	32^{a}
Hydro-methanol	6	6	9±0.5^{d}	13.3±0.6^{c}	18±1.0^{b}	6	32^{a}

*Standard nistatin discs (100 I.U) **DMSO

Data in the same row with different letters are significantly different (P < 0.05). Values are mean ± SE.

Table 3: The MBC and MIC values of E. crassipes hydro-methanol and aqueous extracts against the microorganisms tested

Antifungal solution	MIC(mg/ml)	MFC(mg/ml)
Aqueous	64>	64>
Hydro-Methanol	16	32
Nistatin	0.005	0.005

Discussion

Water hyacinth has spread widely in the world and extend from tropical and subtropical rainforests (Joshi and kaur, 2013) to arctic areas (e.g. Peruvian Mountains and Clair Lake in Canada).

Novel research approach aims to take advantage of therapeutic effects of weeds (turn challenge to opportunity) (Haggag et al., 2017). Secondary metabolites and active compounds guarantee medicinal properties of the plants (Kumar et al., 2014). Quality and quantity of these active compounds are determined by the solvent types, the extraction method, selective

tissue, as well as season and geographical location of sample collection. It is essential to search for effective methods to extract maximum active compounds of the plant since composition of the extract also determine synergistic or inhibitory effect of the extract (Derita et al., 2009). Phytochemical analysis of the extract revealed presence of bioactive compounds. Tables 3 and 4 show that the plant contains considerable phenolic compounds. These compounds were richer in the hydromethanolic extract than the aqueous extract. These results are consistent with the results of the study by Ho and Rorong (Ho et al., 2012; Rorong et al., 2012; Shanab et al., 2010). Antioxidant activity and redox capacity are directly related to the phenolic content (Aboul-Enein et al., 2014; Surendraraj et al., 2013). Certain studies on herbaceous and medicinal plants have shown considerable effect of phenolic compounds on antioxidant activity of these plants (Nakatani, 2000). The results showed that the growth inhibition zone diameter against Candida albicans was significantly higher in hydromethanolic extract of water hyacinth (MIC = 16 mg/ml). Studies have shown that alcoholic extract of the plant can control leaf spot disease caused by Fusarium in wheat in vivo (Haggag et al., 2017). It should be noted that the results under in vivo cannot be generalizable to in vitro and depends on the invading strain, the host and its immunological conditions as well as pharmacology of the plant extract (Arbabi Kalati et al., 2013).

Scavenging and antioxidant activity of free radicals enhance as concentration of phenolic compounds increases since the number of hydroxyl groups bonded to the aromatic ring and the likelihood of hydrogen donation increase (Aboul-Enein et al., 2014). Antimicrobial effects of hydromethanolic extract of invasive water hyacinth was also assessed in Anzali Lagoon in vitro. The results indicated the inhibitory effects of this extract on clinical and aquatic pathogenic strains (Rofchaei et al., 2018). Flavonoids consist of a large group of polyphenolic compounds that exhibit antimicrobial activity since they scavenge free radicals and protect cell membranes (Kumar et al., 2014). Studies have also shown that flavonoids inhibit the growth of opportunistic Candida strains in an effective manner, especially C. albicans, Aspergillus flavus. High potential of flavonoids to inhibit spore germination announced this secondary metabolite as a suitable candidate for treatment of fungal diseases (Cushnie and Lamb, 2005).

Flavonoids content of hydromethanolic extract was measured and showed rich phenolic content of the extract (Table 2). Phytochemical analysis of different fractions of different percentages (hexene-methyl acetate) of hydromethanolic extract of water hyacinth indicated the presence of

Yohimbine alkaloids and phthalate compounds such as 2-ethylhexyl. Antifungal properties of this secondary metabolites was confirmed in many studies (Shanab et al., 2010). Assessment of mechanism of action of these compounds on 6 types of human and plant fungi showed that 2-methylhexyl inhibited spore proliferation and cell wall growth (El-Mehalawy et al., 2008). Table 5 shows presence of other active compounds such as saponin, tannin, terpenoids in the water hyacinth extract. Assessment of these compounds have shown that saponin is a toxin in various plant organs and has antibiotic and fungicidal properties (Ncube et al., 2011). Various studies have shown that antifungal activity of hydromethanolic extract of sumac (Behzadi Rad et al., 2015), cinnamon and clove (Arbabi Kalati et al., 2011) garlic (Ayatollahi Mousavi et al., 2008), Ferulago angulate (Khodavandi et al., 2017), and Origanum vulgare and O. majorana (Movaghari Pour et al., 2018) were significantly greater than other solvents against Candida albicans. This indicated the effect of solvent type on extraction of active compounds that lead to greater efficacy of the extract in controlling fungal agents. These results were consistent with the results of this study. Anti-Candida activity of water hyacinth extract and other medicinal plants were compared, which showed lower anti-Candida activity of Scrophularia striata (Havasian et al., 2012) and Glycyrrhiza glabra (Arbabi Kalati et al., 2013). Such medicinal herbs as Myrtus communis (Bidrighe et al., 2008) and garlic (Ayatollahi Mousavi et al., 2008) had a significant antifungal activity against candidiasis. The efficacy of this invasive plant is comparable to jensing (Tajik Ijdan et al., 2018) and sumac (Behzadi Rad et al., 2015).

Synergistic activity of secondary metabolites on fungal agents can also be explored. Studies have shown suitable efficacy of a medicinal plant does not depend on a sole compound, rather on a combination of various active compounds. Age of the medicinal plant was also assessed in some studies. The results showed greater antifungal effect as age of the plant increased

(Lata and Dubey, 2010). The results of this study and other studies show that polarity of the selective solvent affect the quantity and quality of secondary metabolites and the target drug. The results of this study showed that anti-Candida activity of invasive water hyacinth is comparable to many medicinal plants due to its suitable phytochemical potential and composition. Identification of active compounds of the invasive and resistant water hyacinth and use of effective isolation methods for identification of active compound or compounds

Table 5: Review of Phytochemical Screening Investigations of E. crassipes with Methanol and Aquatic Solvent in Different Biogeography.

Type of extract	Parts	Geographic	Phytochemicals	Refrence
Aqua.metanolic	leaf	Egipt	Flav, Ste,Terp, Alka	Fareed et al., 2008
Metanolic	leaf	Indonesia	Flav, phe, Tan	Rorong et al., 2012
Metanolic	leaf	India	Alk,phen,streroied,Tan	Joshi and kaur, 2013
Aquatic	leaf	India	Phe, Alka, Ter,sap	Kumar et al 2014
Metanolic	leaf	Egipt	Alk,Terp,Flav,Tan	Ho et al. 2012
Metanolic	leaf	Egipt	Flav,Alk, Terp,Tan	Haggag et al., 2017

Abbreviations: Phe,Phenolic; Flav, Flavonoid ; Alk,Alkaloid; Qui,quinone ;Antra,Anthraquinone ; Sap, Saponin ; Ste,steroid ; Tan,Tanine ;Terp,Terpenoids

Refrence:

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