Research Article

Efficiency of common water hyacinth (*Eichhornia crassipes*) in controlling growth of fungal and bacterial clinical strains

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Abstract

In this paper we have studied the anti-bacterial and anti-fungal potential of *Eichhornia crassipes* (Mart.) Solms (the common water hyacinth). The main aim was to find a new source against selected bacterial and fungal species was fractionated. Five fractions i.e. n-hexane, aqueous, chloroform, methanolic extracts and ethyl acetate were prepared and tested against four bacterial strains including MRSA (Methicilline resistance *staphylococcus aureus*), *Streptococcus mutans* and, *Staphylococcus aureus* and *Serratia marcescense* and four fungal strains i.e *Alternaria alternate*, *polyspondylium pallidum*, *Fusarium oxysporum*, *Aspergillus flavous*. The highest anti-bacterial activity was displayed by n-hexane fraction (46-48%) and the lowest activity was shown by crude methanolic extracts (38-44%) against all the selected bacterial strains. However in anti-fungal activities the highest activity was shown by crude methanolic extracts (35-65%) against the four selected fungal species and lowest activity was displayed by the aqueous fractions. The other fractions have also shown significant activities.

Keywords: Antibacterial; Antifungal; *Eichhornia crassipes*; Inhibition; Water Hyacinth

Introduction

At the present time most of the research work is performed to find out the treatment of microbial (fungal and bacterial) diseases from medicinal plants. Such drugs are called traditional drugs with no or very little side effect. Plants contain different chemical compound such as phenols, alkaloids and flavonoids and tannins which play an essential role in the development of medicine

and human health. Different extracts obtained from plants such as methanol, ethanol and chloroform have significant effect against antibacterial, antiviral and antitumor diseases [1]. Other natural products of plants are primary and secondary metabolites. The primary metabolites required in sufficient amount for plants and cannot survive without it while the secondary metabolites derived from the primary

metabolites by different pathways and required in small amount and possess medicinal properties and also have ecological functions [2]. The usage of waste and treatment of high rate algal pond (HRAP) has been researched and developed in 1950s [3]. The comprehensive study of this work was done by [4]. The main aim of this high rate of algal pond (HRAP) is to assimilate nutrient with the help of algae growth which leads to the formation and development of soil. Water hyacinth (*Eichhornia crassipes*) belongs to monocotyledonous family Pontederiaceae and is a free floating aquatic plant (hydrophyte). Initially it was invaded in aquatic areas of eastern and southern America where it possesses ovate thick and broad leaves and grows up to 1m in height [5]. With the help of its complex root system and rapid rate of reproduction it forms dense and interlocking mats which cause ecological and socioeconomic changes [6, 7]. It contains calcium, magnesium, boron, copper, zinc, aluminum, molybdenum, ferric and potassium which make its growth best over other aquatic species [8, 9]. After dying and decomposition of *Eichhornia crassipes* the water become eutrophic due to addition of such elements [10, 11]. Although many studies were carried out over the selected plant species such as its role in ecological ecosystem [12, 13]. Antialgal activity was also studies in which different allelochemicals were extracted by fractionation which shown effect over other algal species and also cause inhibition of seeds in many crop plants [14]. The marine and freshwater hydrophytes at one side run the ecosystem and on the other side accumulate different nutrients which are important for human health and other organism [15]. About 25% medication and 60% anti-inflammatory and anti-cancer drugs in the markets were prepared in the laboratories from the plant constituents [16]. Initially the antibiotics were very active

against different microbes but with the passage of time they loss their activity because of modification in microbes against these antibiotics [17]. These microbes cause diseases and about 50% of the death globally occurred due to these microbial diseases. In this regard the present study was carried out with the aim to find the anti-bacterial and anti-fungal activity of *Eichhornia crassipes* against four bacteria and four fungal species. The bacterial and fungal species were selected due to their frequent occurrence in local hospitals of Khyber Pakhtunkhwa (KPK) and also showing resistance to different drugs.

Material and methods

Antibacterial activity

Collection and processing of plant specimen

Different areas of the Khyber Pakhtunkhwa were visited for the collection of *E. crassipes* plant (Voucher No. H.ICP-1357. The collected plants were washed and dried in shadow followed by oven drying in the oven. The completely drying plants were powdered with the help of grinder. Then the powder stored at cool place before extraction.

Preparation of extraction and fractions

Cold maceration method was used for the extraction of the active metabolites. One and half of the powder was dipped in two later of ethanol and incubated for 5 days at 40°C. The material filtered thrice and a clear filtrate was obtained. The filtrate was subjected for evaporation via rotary evaporator at 40°C. The obtained extract was dried and then dissolved in 100ml distilled water. The solution was then fractionated by using different organic solvents including ethanol, n-hexane, chloroform, methanol and ethyl acetate by using separating funnel. All the fractions thus obtained were concentrated by rotary evaporator and designate for that solvent fraction.

Media preparation

Agar well diffusion method was used during antibacterial activity. A liter of distilled water was used to liquefy 25 g of Luria Broth; neutral pH (7.0) was maintained for Miller powder. The media put in autoclave in 250ml flask. The selected four bacterial stains were introduced into the flask and kept overnight at 150RPM at 37°C. After that agar converted into solid form and with the help of sterilized borer five holes were tunneled. The inoculum was introduced into the tunneled. The methods of [18, 19] were followed.

Test for bacterial strains

Among the four selected bacterial strains three were gram positive and one strain was gram negative i.e. MRSA (*Methicillin resistance staphylococcus aureus*), *streptococcus mutans* and *staphylococcus aureus*; while the gram negative bacterium was *Serratia marcescens*.

Measurement of zones of inhibition

Dimethyl sulfoxide 20mg/ml was used as a negative control in which the extracts were dissolved. While Cefotaxime (standard antibiotics) was used as a positive control. The plant fractions about 75µl were introduced into the wells of petri dish and that petri dishes were placed in the incubator for 24 hours at 37°C (Figure 1). When the incubation period was completed then the diameter of each transparent zone was measured. The experiment was repeated again and again to calculate the standard data.

Test for fungal strains

During antifungal activity the four fungal strains were selected i.e. *Fusarium oxysporum*, *Aspergillus flavus*, *polyspondylium pallidum*, and *Alternaria alternata*.

Results and discussion

The current research work was carried out to evaluate the anti-fungal and anti-bacterial potential of the *Eichhornia crassipes* (Mart.) Solms (the common water hyacinth). The results revealed that this plant is an effective

source for the inhibition of the growth of tested species of both bacteria and fungi. The plant can be investigated for active metabolites to evolve the anti-bacterial and anti-fungal drugs through biochemical and biophysical essays.

Antibacterial activities of *Eichhornia crassipes*

In the current research work five fractions of *Eichhornia crassipes* extracts were used to know their potential use against the selected bacterial strains that are *Staphylococcus aureus*, *Streptococcus mutans* and Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Serratia marcescens*. Results of the anti-fungal activity as shown in (Table 1) showed that all the fractions were active against the tested bacterial strains. The growth of *Staphylococcus aureus*, *Streptococcus mutans*, *Serratia marcescens* and Methicillin-resistant *Staphylococcus aureus* (MRSA) was inhibited by the crude methanolic extract with 10.0mm, 12.0mm, 09.0mm and 11.0mm zones of inhibition respectively. The *n*. hexane extracted fractions inhibited the growth of *Staphylococcus aureus*, *Streptococcus mutans*, *Serratia marcescens* and Methicillin-resistant *Staphylococcus aureus* (MRSA) with 12.0mm, 13.0mm, 14.0mm and 10.0mm zones of inhibition respectively. The chloroform fractions were produced 11.0mm, 14.0mm, 07.0mm and 14.0mm zones of inhibition against *Staphylococcus aureus*, *Streptococcus mutans*, *Serratia marcescens* and Methicillin-resistant *Staphylococcus aureus* (MRSA) respectively. The ethyl acetate fraction was most active against *Streptococcus mutans* (ZOI=10.0mm) followed by Methicillin-resistant *Staphylococcus aureus* (ZOI=09.0mm), *Staphylococcus aureus* (ZOI=08.0mm) and *Serratia marcescens* (ZOI=08.0mm). The aqueous extracted fraction inhibited the growth of tested bacterial strains in the order of Methicillin-

resistant *Staphylococcus aureus* (ZOI=08.0mm), *Staphylococcus aureus* (ZOI=05.0mm), *Serratia marcescens* (ZOI=05.0mm) and *Streptococcus mutans* (ZOI=04.0mm) respectively (Figure 2). According to [20] in the previous century most of the faculties in the medicine sector switched their concern from natural to synthetic drugs. But for the last few decades this trend is shifting in the reverse direction [21]. In a survey 25% of the drug prescriptions in 35 countries are plants derived in non-modified or little modified [22, 23].

Antifungal activities of *Eichhornia crassipes*

Results of the anti-fungal activity as shown in (Table 2) revealed that the n-hexane extracted sample inhibited the growth of *Fusarium oxysporum* completely. Chloroform and Ethyl acetate inhibited the growth of *Polysphondylium pallidum*. While the aqueous extract showed significant activity against *Aspergillus flavus* and *Alternaria alternate*. Crude methanolic extract showed good results against *Polysphondylium pallidum*. Trend in results revealed that *Fusarium oxysporum* is comparatively more sensitive to the n-hexane extracts, *Polysphondylium pallidum* to chloroform and Ethyl acetate extracts while *Aspergillus flavus* and *Alternaria alternate* to the aqueous extracts of plants (Figure 3). The usage of medicinal plants as anti-fungals is

quite old practice performed by human beings. These result in the production of important compounds that are used to develop drugs against fungal pathogens. One of the positive aspects of these drugs is that these drugs have very little adverse/side effects on the human health [24]. The demand for the development anti-fungal drugs has enormously increased. It is established that among others fungal pathogens are causing serious losses in the quantity, quality, shelf life and mortality of the crop plants [25]. In regard to humans, fungi as the cause of diseases offer a new aspect of human pathology [26]. In this regard medicinal plants are proved to be the sources for the discovery of new drugs [27]. In the developing countries, which face the problems of the quantity and quality of modern drugs, medicinal plants derived compounds have recently gained much importance and their old traditional uses are valued more nowadays [28]. The current study revealed that antifungal activity of the wild plants proved to be more significant and can be a very suitable substitute of the modern medicines. Similar findings have been reported by [29] for the fungus *Alternaria alternate*. Similarly [30] revealed that the crude methanolic extract of the medicinal plants showed maximum anti-fungal properties which is in agreement with our work. Similar results with other medicinal plants were reported by [31].

Figure 1. Plates showing results of antibacterial and antifungal activities

Figure 2. Antibacterial efficacy of *Eichhornia crassipes* (Mart.) Solms in different solvents

Table 1. Antibacterial activities of *Eichhornia crassipes* (Mart.) Solms against selected strains

Bacterial Species	Standard	Cr. Met. Ext		n- hexane		CHCl ₃		EtOAc		Aqueous	
		ZOI	%	ZOI	%	ZOI	%	ZOI	%	ZOI	%
MRSA	25.0±0.67	16±0.56	44.0	13±0.90	48	12.0±0.57	40	15.0±0.87	36	09.0±0.23	32
<i>S. marcescens</i>	21.0±0.87	13.0±0.56	42.8	12±0.88	47.6	10±0.45	33.3	14.0±0.44	38.1	06.0±0.22	23.8
<i>S. mutans</i>	28.0±0.60	15.0±0.67	42.8	11±0.66	46.4	11.0±0.56	50	17.0±0.34	35.7	05.0±0.34	14.2
<i>S. aureus</i>	26.0±0.56	14.0±0.87	38.4	14±0.93	46.1	11.0±0.21	42.3	16.0±0.33	30.7	05.0±0.50	19.2

Figure 3. Antifungal efficacy of *Eichhornia crassipes* (Mart.) Solms in different solvents

Table 2. Antifungal activities of *Eichhornia crassipes* (Mart.) Solms against selected strains

Fungal Species	Standard	Cr. Met. Ext		<i>n</i> - hexane		CHCl ₃		EtOAc		Aqueous	
		ZOI	%	ZOI	%	ZOI	%	ZOI	%	ZOI	%
<i>A.flavus</i>	100.0±0.00	43±0.52	43	47.0±0.78	47	34.0±0.53	34	52.0±0.44	52	17.0±0.11	17
<i>A. alternate</i>	100.0±0.00	68.0±0.53	68	39.0±0.88	39	45.0±0.61	45	48.0±0.14	48	15.0±0.12	15
<i>F. oxysporum</i>	100.0±0.00	59.0±0.21	59	21.0±0.00	21	37.0±0.45	37	59.0±0.19	59	23.0±0.34	23
<i>P. pallidum</i>	100.0±0.00	39.0±0.14	39	32.0±0.67	32	20.0±0.34	20	36.0±0.23	36	25.0±0.23	25

Conclusion

It is evident from the results of anti-bacterial and anti-fungal activities that the selected plant i.e. *Eichhornia crassipes* possessed very significant properties. The crude methanolic and ethanolic extracts were comparatively more active which clarified that polar solvents dissolved more properly in the polar solvents.

Authors' contributions

Conceived and designed the experiments: S Wali, K Rehman & B Ullah, Performed the experiments: K Rehman, T Yaseen & G Ahmad, Analyzed the data: S Wali, K Rehman & G Ahmad, Contributed materials/ analysis/ tools: K Rehman, S Wali & G Ahmad, Wrote the paper: S Wali, K Rehman, B Ullah, T Yaseen & G Ahmad.

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