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Research

Bioactivity and biochemical analysis of water hyacinth (*Eichhornia crassipes*)

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Abstract

Water hyacinth (*Eichhornia crassipes*) is an invasive aquatic weed causing serious threats to water ecosystems throughout the world. Recently, considerable attention has been given at harvesting the plant for practical uses. An experiment on the bioactivity of water hyacinth was conducted using the soxhlet extraction (hot method) and cold percolation method in chloroform and ethanol in order to evaluate the antimicrobial properties of the plant. Plant samples were also analyzed for the presence of major pharmacologically active compounds. The antimicrobial assay was performed using well diffusion method against nine different clinical bacterial strains and six phytopathogenic fungal strains. The chloroform hot extract showed activity against 22.22% (Zone of Inhibition, ZOI ≤ 13mm) bacteria and 66.66% (ZOI ≤ 12mm) fungi; while the cold extract showed activity against 50% (ZOI ≤ 13mm) fungi, but no activity against bacteria. Similarly, the ethanol hot extract showed activity against 77.77% (ZOI ≤ 19mm) bacteria and 66.66% (ZOI ≤ 20mm) fungi, while the cold extract showed activity against 77.77% (ZOI ≤ 10mm) bacteria and 50% (ZOI ≤ 14mm) fungi. The ethanolic hot and cold extract proved to be far better than the chloroform fraction showing more antibacterial activity, while they share the same value and possess same effectiveness against the different fungi. Chemical analysis indicated that the major components in these extracts were saponins, polyoses, alkaloid salts, and reducing compounds. The present study showed that the devastating aquatic weed, with strong antimicrobial potentials and presence of biologically active phytochemicals, may be useful for developing alternative compounds to treat infectious diseases caused by bacterial and fungal pathogens.

Key-words: biological screening, extracts, phytochemical assay, zone of inhibition.

Introduction

For centuries, plants have been used as a source of medicines. Traditional medicine using plant extracts still continues to provide health services for over 80% of the world's population, especially in the developing countries (WHO 2003). Plant extracts have great potential as biologically active compounds against pathogens, including microorganisms (Fischer *et al*. 2004). Also, the synergistic effect from the association of antibiotics in plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases which enables the plant as a potential candidate for drug development. In recent years, novel active compounds have been discovered from variety of plant species based on the study of traditional medicines (Fabricant and Farnsworth 2001; Tomoko *et al*. 2002).

In contrary to the synthetic drugs, antimicrobials of plant origin do not have side effects and possess massive therapeutic potential to heal many infectious diseases. Development of the antimicrobials from higher plants appears rewarding, leading to the development of phytochemicals to act against microbes (Iwu *et al*. 1999). Antimicrobial activities reported in several plant taxa are due to the presence of such

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active constituent as phenols, quinones, flavones, flavonoids, flavonols, tannins, terpenoids, essential oils, alkaloids and so on (Cowan 1999; Harborne and Williams 2000).

Water hyacinth [*Eichhornia crassipes* (Mart.) Solms] is one of the world's most obnoxious water weeds when not controlled (Reddy and Sutton 1984). The plant is known for its well ability to grow in severe polluted water (So *et al*. 2003). A large number of biologically active compounds have been extracted from this weed (Greca *et al*. 1991, 1992; Wang *et al*. 2011). Recently, considerable attention has been given at harvesting the aquatic plant for practical uses to partially defray the cost of removing plants from water ways and use it as economical sources in many parts of the world (Widjajanto *et al*. 2003; Sharshar and Haroon 2009). This study was designed with an aim to evaluate the antimicrobial properties of the extracts of *Eichhornia crassipes* and assess the major pharmacologically active substances.

Materials and Methods

SAMPLING

The samples of *Eichhornia crassipes* for the laboratory research were collected from Fewa Lake, Pokhara, central Nepal. The whole plants were shade dried, ground to a fine powder and used for further analysis.

PREPARATION OF PLANT EXTRACTS

The extraction of the powdered plant materials was carried out by soxhlet extraction method and cold percolation method using chloroform and ethanol as extracting solvent following the protocol of Rosenthaler (1930). Removal of the solvent from the extracts was done using rotary vacuum evaporator and the concentrated extracts were stored at 4°C. Different working solutions of the extracts (10%, 20%, 30% and 40%) were prepared in dimethyl sulfooxide (DMSO). The yield of respective extract was calculated as: percentage yield (%) = (dry weight of extract/dry weight of samples) × 100.

PREPARATION OF STANDARD CULTURE INOCULUM

Nine different clinical bacterial strains and six phytopathogenic fungal strains were employed to detect the antimicrobial potentials of the plant. The bacterial strains used were *Acinetobacter* sp., *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae, Proteus mirabilis*, *Salmonella* Paratyphi, *S.* Typhi, *Schigella dysenteriae* and *Staphylococcus aureus*. Similarly, the fungal strains employed for the experiment were *Curvularia* sp., *Fusarium erundiforme, F. moniliforme, F. oxysporum, F. proliferatum* and *Sclerotium rolfsii*. For antibacterial assay, colonies were selected from 18–20 h old cultures for preparing bacterial inoculums. Turbidity was adjusted to 0.5 McFarland standards. Similarly, the standard culture inoculums of each fungal strain was prepared in potato dextrose broth (PDB) and adjusted to a range of 1×10^6 to 5×10^6 spores ml^{-1}. The standard bacterial cultures were obtained from Central Laboratory, Teku, Kathmandu, Nepal and the fungal cultures from Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur, Nepal.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Well diffusion method using Muller-Hinton agar plates and PDA were used to demonstrate the antibacterial and antifungal properties of the crude extracts. The prepared inoculums for each microbial strain were spread onto agar plates. Wells of 6mm were bored in the culture media using sterile cork borers. In each well, 50µl of each plant extract of different concentrations (10%, 20%, 30% and 40%) was dispensed with the help of micropipette. The plates were incubated at 37°C for 24 h for bacterial culture and at 27°C till 5 days for fungal culture. Zone of Inhibition (ZOI) as indicated by the clear zone i.e. without growth of organism around the well was measured. The solvents used as the negative control was dispensed at the centre. The tests were done in triplicates.

The antimicrobial tests of extracts showing ZOI were further done by two fold broth dilution method to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)/minimum fungicidal concentration (MFC). The MIC and MBC for bacteria were evaluated using Nutrient Broth (NB), while Potato Dextrose Broth (PDB) was used to evaluate MIC and MFC for fungi.

PHYTOCHEMICAL ANALYSIS

Plant samples were analyzed, following standard methods (Harborne 1973, 1998; Sofowora 1993; Mukherjee 2002; Kokate 2003), to demonstrate the presence of the different pharmacologically active compounds.

Test for volatile oils: The obtained hexane extract was concentrated to yield residue, in which methanol was added and shaken vigorously followed by filtration and then spotted

on the filter paper. The presence/absence of the yellow color was noted, confirming the volatile oils in the presence of color.

Tests for alkaloids: The hexane extract was dissolved in 2% v/v HCl (3 ml) and divided equally for Maeyer's test and Dragendorff's tests. The half solution was treated with 3 drops of Maeyer's reagent, and the presence of white precipitate indicated the presence of alkaloids. The next half solution was treated similarly with Dragendroff's reagent (3 drops), and the absence of the white precipitate indicated the absence of alkaloids.

Tests for sterols and triterpenes: The residue of hexane extract was dissolved in acetic anhydride (1 ml) and chloroform (1 ml), followed by the addition of conc. H_2SO_4 from the side of the tube. Presence of the violet ring at the junction of two liquid and the appearance of green color in the upper level indicated the presence of sterols and triterpenes.

Test for carotenoids: The residue of hexane extract was treated with 1 ml of conc. H_2SO_4. Presence of the orange yellow color initially similar to the extract and turning to red indicated the presence of carotenoids.

Test for coumarins: The ether extract was concentrated to yield the residue, dissolved in hot water (4 ml) and was left to cool. The tube was then treated with 10% (v/v) ammonium hydroxide solution drop by drop until pH 8 and was observed under UV light. Observation of greenish yellow fluorescence indicated the presence of coumarins.

Test for emodins (Borntiager's test): 25% (v/v) ammonium hydroxide solution (1 ml) was treated with the ether extract (2 ml) and was shaken vigorously, followed by standing for few minutes. The decolorization of the upper etheric layer and the gaining of the red color by the lower alkaline layer were noted, indicating the absence of the emodins.

Test for quinones: One ml of freshly prepared ferrous sulphate solution was added to etheric solution followed by few crystals of ammonium thiocyanate and conc. H_2SO_4 drop by drop. Presence of deep red color indicated the presence of quinones.

Test for glycosides: The methanolic extract residue was treated firstly with 25% (v/v) ammonium hydroxide solution (2 ml) and was shaken vigorously. Secondly, the test solution was treated with Molisch's reagent (5 drops) and H_2SO_4 was added drop wise from the side of the tube without disturbing the solution. The presence of the cherry-red color in the first tube and a violet ring at the junction of the two liquid on the second test solution indicated the presence of glycosides.

Test for reducing compound: One ml of the methanolic extract was mixed with water (1 ml) followed by Fehling's reagent and warmed over a water bath for 30 min. Presence of a brick red precipitate indicated the presence of reducing compounds.

Test for alkaloid salts: The methanolic extract residue was treated with 2% HCl (4 ml) and was shaken vigorously and filtered. The obtained filtrate was treated with 10% (v/v) NH_4OH solution until pH 8 and was extracted with $CHCl_3$ (15 ml). The lower combined layer was concentrated over a water bath in fume hood discarding the upper layer and 2% HCl (5 ml) was added and was taken for the different tests. Firstly, the test solution was treated with Maeyer's reagent (3 drops) and then with Dragendorff's reagent (3 drops). In both cases, presence of white precipitate indicated the presence of alkaloid salts.

Test for polyphenols: One ml of the methanolic extract was mixed with water (1 ml) followed by 1% (w/v) ferric chloride solution (3 drops). Presence of the greenish blue color indicated the presence of polyphenols.

Results

The yield of the extract was found to be 26.23 % and 17.65% in ethanol by hot and cold extraction, while the yield was 12.28 % and 6.61 % in chloroform by hot and cold extraction respectively.

The ethanolic extract was found far effective against the bacterial pathogens, while varied results were obtained on fungal strains. The soxhlet extract was found to be effective against the bacterial and fungal pathogens than the cold extract except for *P. mirabilis*, *S.* Paratyphi, *F. oxysporum* and *Curvularia* sp. Among the bacterial strains (Table 1), *S.* Typhi was found to be more susceptible against the ethanol (soxhlet and cold) extracts. In the fungal strains, ethanol (soxhlet) extract was found to be more effective against *Sclerotium rolfsii* (Table 2). The cold chloroform extract was found to be ineffective against tested bacterial pathogens.

The extracts showed very little activity against *Klebsiella pneumoniae* and *Salmonella* Paratyphi among bacteria and *Fusarium oxysporum* and *Curvularia* sp. among fungi. The MIC values of bacteria and fungi could not be obtained due to the colored extract. The MBC value of the ethanolic hot and

Table 1. Antibacterial properties (ZOI in mm). The values shown are mean ± SD (n = 3).

Extraction methods	Concentrations (%)	Test organisms*								
		KP	BS	AC	ST	PM	SA	SD	SP	EC
Soxhlet extraction (ethanol)	10	-	9.0 ± 0.4	6.0 ± 0.3	11.0 ± 0.8	-	7.0 ± 0.9	9.0 ± 1.0	-	7.0 ± 0.9
	20	-	10.0 ± 0.6	7.0 ± 0.7	14.0 ± 0.5	-	9.0 ± 0.5	13.0 ± 0.9	-	9.0 ± 0.8
	30	7.0 ± 0.1	10.0 ± 0.7	8.0 ± 0.4	15.0 ± 0.5	-	9.0 ± 0.0	14.0 ± 0.6	-	12.0 ± 0.7
	40	8.0 ± 0.4	12.0 ± 0.6	9.0 ± 0.3	19.0 ± 0.8	-	11.0 ± 0.6	16.0 ± 0.7	-	14.0 ± 0.1
Cold percolation (ethanol)	10	-	-	-	8.0 ± 0.6	7.0 ± 0.3	-	-	-	-
	20	-	7.0 ± 0.7	-	8.0 ± 0.4	8.0 ± 0.8	-	-	-	-
	30	7.0 ± 0.6	7.0 ± 0.8	8.0 ± 0.9	9.0 ± 0.8	9.0 ± 0.7	7.0 ± 0.7	-	-	7.0 ± 0.3
	40	8.0 ± 0.2	9.0 ± 0.4	10.0 ± 0.7	10.0 ± 0.9	10.0 ± 0.4	9.0 ± 0.8	-	-	8.0 ± 0.4
Soxhlet extraction (chloroform)	10	-	-	9.0 ± 0.9	-	-	-	-	-	-
	20	-	-	11.0 ± 0.9	-	-	-	-	7.0 ± 0.8	-
	30	-	-	12.0 ± 0.7	-	-	-	-	9.0 ± 0.3	-
	40	-	-	13.0 ± 1.0	-	-	-	-	12.0 ± 0.6	-
Cold percolation (chloroform)	10-40	-	-	-	-	-	-	-	-	-

*KP: *Klebsiella pneumoniae*, BS: *Bacillus subtilis*, AC: *Acinetobacter* sp., ST: *Salmonella* Typhi, PM: *Proteus mirabilis*, SA: *Staphylococcus aureus*, SD: *Schigella dysentriae*, SP: *Salmonella* Paratyphi, EC: *Escherichia coli*. — No inhibition of growth.

cold extracts against *S.* Typhi was 8.32 mg ml^{-1} and 7.96 mg ml^{-1} respectively. Similarly, the MFC values of the chloroform hot and cold extracts against *Sclerotium rolfsii* were 3.13 mg ml^{-1} and 3.63 mg ml^{-1} respectively. *Salmonella* Typhi and *Schigella dysentriae* were the most susceptible bacterium of all the tested bacteria, while *Sclerotium rolfsii* was found to be the most susceptible fungus.

The phytochemical screening of various solvent extracts revealed the presence of alkaloid salts, reducing compounds, polyoses and saponins (Table 3).

Discussion

The plant extract was found to have a good potential against *Sclerotium rolfsii* followed by *Fusarium erundiforme*, leading to best controlling and regulating mechanism against these phytopathogens. This may be due to the presence of saponins because saponins are active antifungal agents (Sodipo *et al*. 1991) having expectorant action, which is very useful in management of upper respiratory tract inflammation (Finar 1989; Trease and Evans 1989). *Sclerotium rolfsii*, a phytopathogen that mainly attacks stems, roots, leaves and fruits and usually restricted to plant parts in contacts to the soil can widely be controlled by the application of water hyacinth.

The tested plant extract showed less activity against *Curvularia* sp., *Fusarium oxysporum*, *Klebsiella pneumoniae* and *Salmonella* Paratyphi. In comparison to other phytopathogens, *Sclerotium rolfsii* and some *Fusarium* sp. are found to be more susceptible against the extract of water hyacinth. The presence of more pharmacologically active compounds in ethanol extract is presumed to be responsible for the high potency recorded than chloroform extracts. Phytochemicals in plants have been proved to be responsible for their therapeutic effect and by extension, antimicrobial activity.

The variation in the effectiveness of the extract against different microorganisms depends upon the chemical composition of the extracts and membrane permeability of the microbes for the chemicals and their metabolism. It has been suggested that the antimicrobial activity is mainly due to the presence of essential oils, flavonoids and triterpenoids and other natural polyphenolic compounds or free hydroxyl groups (Rojas *et al*. 1992). Plants have an almost limitless

Table 2. Antifungal properties (ZOI in mm). The values shown are mean ± SD (n = 3).

Extraction methods	Concentrations (%)	Test organisms*					
		SR	CU	FE	FM	FP	FO
Soxhlet extraction (ethanol)	10	13.0 ± 0.1	-	9.0 ± 0.9	7.0 ± 0.7	-	-
	20	16.0 ± 0.6	-	11.0 ± 0.9	7.0 ± 0.8	-	7.0 ± 0.8
	30	17.0 ± 0.8	-	11.0 ± 0.4	11.0 ± 0.4	-	9.0 ± 0.6
	40	20.0 ± 1.0	-	12.0 ± 0.4	12.0 ± 0.8	-	13.0 ± 0.2
Cold percolation (ethanol)	10	12.0 ± 0.5	7.0 ± 0.6	9.0 ± 0.8	-	-	-
	20	13.0 ± 0.3	9.0 ± 0.6	10.0 ± 0.8	-	-	-
	30	14.0 ± 0.5	12.0 ± 0.8	10.0 ± 0.9	-	-	-
	40	14.0 ± 0.3	14.0 ± 0.9	10.0 ± 0.8	-	-	-
Soxhlet extraction (chloroform)	10	-	-	7.0 ± 0.8	7.0 ± 0.7	7.0 ± 0.8	-
	20	7.0 ± 0.6	-	7.0 ± 0.3	9.0 ± 1.0	8.0 ± 0.9	-
	30	8.0 ± 0.5	-	7.0 ± 0.9	11.0 ± 0.8	10.0 ± 0.1	-
	40	12.0 ± 0.8	-	8.0 ± 0.5	12.0 ± 0.8	11.0 ± 0.2	-
Cold percolation (chloroform)	10	7.0 ± 0.2	-	7.0 ± 0.7	-	7.0 ± 0.5	-
	20	8.0 ± 0.5	-	8.0 ± 0.4	-	8.0 ± 0.6	-
	30	11.0 ± 0.6	-	8.0 ± 0.5	-	12.0 ± 0.8	-
	40	12.0 ± 0.6	-	9.0 ± 0.7	-	12.0 ± 0.6	-

*SR; *Sclerotium rolfsiii, CU: Curvularia sp.,* FE: *F.erundiforme,* FM: *F. moniliforme,* FP: *F. proliferatum,* FO: *F. oxysporum.*

— No inhibition of growth.

ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Geissman 1963). Alcohol extracts provide a more complete extraction, including less polar compounds, and many of these extracts have been found to possess antimicrobial properties (Ali-Shtayeh and Abu-Ghdeib 1999). Also the chloroform extract was less potent than ethanol extracts. This may probably be due to the solvent and/or method of extraction (Rios and Recio 2005). In addition, Nostro *et al*. (2000) demonstrated the effect of different extractives on the activity of the bioactive molecules on the test organisms, thus supporting the reason for greater potency in ethanol extract than chloroform extract. Ethanol solvent is known with its ability to isolate more antimicrobials from plants including tannins, polyphenols, terpenoids, saponins, xanthoxyllines, totarol, quassinoids, lactones, flavones and phenones, while the chloroform extracts could contain only anthocyanins, starches, tannins, saponins, polypeptides and lectins (Cowan 1999). Many alkaloids contain at least one nitrogen atom in an amine type structure that makes them pharmacologically active. The antimicrobial effect of the extracts could be explained by disturbance of the permeability barrier of the bacterial membrane structure (Cowan 1999).

In summary, our data indicate that the extracts from water hyacinth efficaciously inhibit *Salmonella* Typhi, *Acinetobacter* sp., *Schigella* sp., *Escherichia coli, Sclerotium rolfsii* and *Fusarium proliferatum* which make serious problems worldwide. This indicates that this plant may be useful for developing alternative compounds to treat infections caused by these antibiotic-resistant pathogens. As suggested in a recent report (Betoni *et al*. 2006), these biologically active compounds of plant origin may be used together with known drugs in the development of pharmacological agents against different hazardous pathogens.

The yields of chloroform and ethanol extracts of water hyacinth were quite adequate inhibiting the growth of the test organisms. The degree of inhibition was found to be both organism(s) and concentration dependent. These findings tend to lend credence to the traditional use of *Eichhornia crassipes* for the treatment of microbial infections (Rastogi and Mehrotra 2002). By the use of this plant extract, the different sorts of infections to the humans body can be reduced to a considerable

Table 3. Screening of the chemical constituents.

Phytochemicals	Hexane extract	Chloroform extract	Ethanol extract
Alkaloids	+	+	+
Reducing compound	-	+	+
Polyoses	+	+	+
Saponins	-	+	+

+ presence, - absence.

level. The broad activity of the extracts explains the widespread use of this plant for wound healing and other applications as well.

Scientists from divergent fields are investigating plants anew with an eye to their antimicrobial usefulness. A sense of urgency accompanies the search as the pace of species extinction continues. It would be advantageous to standardize methods of extraction and *in- vitro* testing. Also, alternative mechanisms of infection prevention and treatment should be included in initial activity screenings. Hence, the devastating aquatic weed can have the good antimicrobial properties because of some compounds, which we can use in preparing our pharmaceuticals products. By exploiting its low range medicinal properties, the weed can also be controlled to a certain extent. Attention to these issues could usher in a badly needed new era of chemotherapeutic treatment of infection by using plant and plant-derived products.

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