

PHYTOCHEMICAL SCREENING AND ANTI-MICROBIAL SCREENING OF POTENTIAL INVASIVE SPECIES FOUND AT AROUND PAKLIHAWA, NEPAL

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ABSTRACT

Weed management is one of the great issues in the agroecosystem of this contemporary world. The invasiveness is negatively impacting human and ecosystem as a whole. In this regard, a comparative study was done to investigate the potential phytochemicals and anti-microbial properties present in the problematic invasive species distributed at around Paklihawa Campus, Siddharthanagar, Nepal. Four species viz. *Eichornia*, *Parthenium*, *Pistia* and *Ipomoea* were selected and the chemical was screened using aqueous methanol as selective menstruum through cold percolation. The fractionated extracts showed the presence of some phytochemicals including alkaloids, saponins, carbohydrates and steroids. *Pistia* was screening positively for alkaloids, carbohydrates, flavonoids, saponin and steroid; *Parthenium* for alkaloids, flavonoids and steroids; *Eichornia* for alkaloids and carbohydrates whereas *Ipomoea* showed the presence of alkaloids only. The extracts were also screened for antibacterial and antifungal activities. The leaf extracts were used to assess antimicrobial activities against bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Salmonella typhimurium*, and fungi: *Aspergillus niger*, *Candida albicans*, *Alternaria solani* and *Fusarium solani* using the well diffusion method. The results indicated that the leaf extracts of *Parthenium* exhibited more zone of inhibition against all bacteria and has potential to be used as anti-bacterial agent. In majority of fungus also, *Parthenium* was superior to others. The Minimum inhibitory concentration (MIC) values against the tested bacteria ranged from 11 to 19 $\mu\text{g mL}^{-1}$ and for the tested pathogenic fungi, from 10 to 20 $\mu\text{g mL}^{-1}$. This result showed that these invasive species could be promising anti-bacterial and anti-fungal agents.

Key words: Anti-microbial, invasive, phytochemicals, screening, weeds

INTRODUCTION

A weed is defined as a plant that grows out of a place and is competitive, persistent and pernicious (James *et al.* 1991). The resistance of weeds to microbes is more when compared to cultivated crops. The resistance nature and their sustenance towards the microbial disease made us have the interest to know the potency behind. Invasive alien species are animals, plants or other organisms that are introduced into

places outside their natural range, negatively impacting native biodiversity, ecosystem services or human wellbeing (IUCN, 2017). Hence, the present study is conducted to screen the phytochemicals and antimicrobial potency of weeds that are invasive in nature. The logic behind the selection of species was to see their potential use as botanicals.

Many plants and weeds, in particular, have biologically active chemical components that may be used in the treatment of various

ailments and plant protection measures as well, but, so far, very few have been identified and used accordingly. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments (Srivastava *et al.* 1996; Dahiya and Purkayastha 2012).

Phytochemicals are bioactive chemicals of plant origin and are regarded as secondary metabolites as the plants use them for protective and attractive purpose. They are naturally synthesized in all parts of the plant body, such as bark, leaves, stem, root, flower, fruits, seeds etc. i.e. any part of the plant body may contain these active compounds (Sushma Raj, 2017). Phytochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants using standardized procedure. Some of the bioactive substances that can be identified and derived from plants are flavonoids, alkaloids, carotenoids, tannins, antioxidants and phenolic compounds (Senguttuvan *et al.* 2014). In the last few years, a number of studies have been conducted in different countries to test the efficiency of various plants. It is due to the secondary metabolites which are equipped with several antimicrobial traits. Such metabolites can be of great use to society. Invasive species are problematic in various ecosystems throughout the world. The management of invasive species is a cumbersome task. In this regard, this study focused to analyze the phytochemical profile of various invasive species and to evaluate its antimicrobial activity against various selected pathogens.

MATERIALS AND METHODS

Site selection: For the collection of plant samples to be used in phytochemical screening, wetland area adjacent to Dande River and Paklihawa Campus was chosen. The area is wasteland

where a lot of invasive species are found among them, four of the most problematic ones were selected. Two species were from terrestrial habitat and two were selected from aquatic habitat. The of the selected site is 27.481°N and 83.444°E.

Collection of plant samples

The collection of plant samples was done at around Paklihawa Campus. The leaves were picked and washed with water thoroughly to remove all unwanted plant materials and sand, air dried under diffused light exposure (27°C-30°C for 14 days), pulverized in a mill and stored in an airtight container for further use.

Preparation of extract

Ten gram of plant powder was added to 100 ml of methanol in a conical flask and plugged with cotton wool. After 24 hours the supernatant was collected and the solvent was evaporated to make the crude extract and stored at 4°C (Harbone, 1973; Tiwari *et al.* 2011). The extraction was carried out by cold percolation method and was filtered using Whatman's filter paper no.42. The supernatant was evaporated at 45°C to make the final volume 1/5th of the original volume.

Phytochemical Screening

For the phytochemical screening of selected invasive weed species, standard qualitative methods previously described by (Sasidharan *et al.* 2011) was followed. Following tests were performed for detecting major phyto-constituents.

Detection of flavonoids

Shinoda test: A piece of magnesium ribbon and concentrated hydrochloric acid was added to 2-3 ml of methanolic extracts. Pink red or red coloration of the solution indicated the presence of flavonoids.

Alkaline reagent test: To the 5 ml of the test solution, 2 ml of 2% of NaOH was added.

Formation of an intense yellow color, which turns to colorless on the addition of a few drops of diluted acids, indicated the presence of flavonoids.

Detection of tannins

Braemer's test: 10% of alcoholic ferric chloride was added to 2-3 ml of methanolic extracts (1:1). Dark blue or greenish grey coloration of the solution indicated the presence of tannins.

Detection of terpenoids

Salkowski test: 2 ml of chloroform and 3 ml of concentrated sulphuric acid were added to 5 ml of methanolic extracts. The reddish brown color of interface indicated the presence of terpenoids.

Detection of saponins

Foam test: The extracts of 50 mg were first diluted with distilled water and volume was adjusted to 20 ml. The suspension was then shaken vigorously in a graduated cylinder for 15 minutes. A 2 cm layer of foam indicated the presence of saponins.

Detection of phenols

Ferric chloride test: 50 mg of extracts were dissolved in 5 ml of distilled water. To this, a few drops of neutral 5% ferric chloride solution was added. A dark green color indicated the presence of the phenol compound.

Detection of alkaloids

Solvent free extracts (50 mg) was stirred with 3 ml of diluted hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloids reagents. After that following (Mayer's test) was performed: To a few ml of filtrate, 1 or 2 drops of Mayer's reagent was added by the side of the test tube. Formation of white creamy precipitates indicated the presence of alkaloids.

Detection of glycosides

0.5g of solvent extracts were dissolved in 2 ml of glacial acetic acid containing one drop of ferric

chloride solution. It was then under laid with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of glycosides.

Detection of fixed oils and fats

A drop of concentrated extracts was pressed in between two filter papers and kept undisturbed. Oils stain on the paper indicated the presence of oils and fats.

Detection of carbohydrates

Fehling's test: Equal volume of Fehling A and Fehling B reagents are mixed together and 2 ml of it was added to crude extracts. It was boiled gently. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Detection of steroids

Cured extracts were mixed with 2 ml of chloroform and concentrated sulphuric acid was added sidewise. A red color produced in the lower chloroform layer indicated the presence of steroids.

Anti-microbial test

The antibacterial activity was determined by well diffusion method (Bauer *et al.* 1966). The medium was sterilized by autoclaving and was allowed to cool down at the room temperature. Filter discs (5.5/mm) were made and impregnated with the solution. The discs were dried at 37°C for 1 h. Muller Hinton's agar plates were used for *in vitro* antimicrobial testing. Bacterial strains were sub cultured from frozen stocks or from freshly obtained plates. Muller Hinton agar medium was seeded with 100 µl of inoculum (1×10^8 CFU/ml). The impregnated discs containing the test sample (100 µg/ml) were placed on the agar medium seeded with tested microorganisms. The plates were then incubated at 37°C for 24 hr to allow maximum growth of the microorganisms (Bauer

et al. 1966). The antibacterial activity of the test samples was determined by measuring the diameter of zone of inhibition expressed in millimeter. The assay was repeated twice and the mean of the three experiments was recorded. Controls were maintained where pure solvents were used instead of plant extracts.

Disc diffusion method given by Bauer was adopted for the determination of the antifungal activity of the extract residues. From the stock cultures of various test organisms, the inoculum was prepared by sub culturing each of the organisms on potato dextrose agar at 37°C. Seeding of potato dextrose agar plates was done using the 24 hr culture with a cotton swab under aseptic conditions. The discs loaded with extract residues were aseptically placed on top of the seeded medium and gently pressed to ensure contact. The plates were then incubated at 37°C. After overnight incubation, the plates were observed for zones of inhibition. Controls were maintained similar as for anti-bacterial test.

Minimum inhibitory concentration (MIC) measurements

A current definition of the minimum inhibitory concentration (MIC) is the lowest concentration which resulted in maintenance or reduction of inoculums viability (Carson *et al.* 1995). The MIC of the crude methanol extracts was determined by using serial dilution technique (Reiner, 1982). The serial tube dilution

technique was used to determine the MIC value of methanol extracts of these plants against bacteria and fungi. The extract (0.512 mg) was dissolved in 2 mL distilled water to obtain the stock solution. After preparation of suspensions of test organisms (10^7 organisms per mL), 1 drop of suspension (0.02 mL) was added to each broth dilution. After 24 h incubation at 37 °C, the tubes were then examined for growth. The MIC of the extract was taken as the lowest concentration that showed no growth. Growth was observed in those tubes where the concentration of the extract was below the inhibitory level and the broth medium was observed to be turbid. Experiments were done in triplicate and repeated twice.

RESULT AND DISCUSSION

The preliminary phytochemical analysis of *Pistia*, *Parthenium*, *Eichornia* and *Ipomoea*, when dissolved in methanol, is presented in table 1. Based on the result, we suggest that *Pistia* possesses alkaloids, carbohydrates, saponins and steroids. *Pistia* showed positive test for alkaloids, carbohydrates, flavonoids, saponin and steroid. *Parthenium* positively responded to alkaloid, flavonoid and steroid. Alkaloids and carbohydrates were screened in *Eichornia*. Whereas, *Ipomoea* was responsive to alkaloid test only. Phytochemical screening of various plants is reported by (Siddiqui *et al.* 2009; Chitradivu *et al.* 2009; Juna Beegum *et al.* 2014).

Table 1: Result of phytochemical screening of various invasive species found at around Paklihawa, 2016.

| Test | <i>Pistia</i> | <i>Parthenium</i> | <i>Eichornia</i> | <i>Ipomoea</i> | Blank |
|--------------------------|---------------|-------------------|------------------|----------------|-----------------------|
| Alkaloid (Mayer's test) | + | + | + | + | Colorless |
| Carbohydrate test | + | - | + | - | No red ppt. |
| Fixed oil and fat | - | - | - | - | No oil strain |
| Shinoda test (flavonoid) | - | - | - | - | Colorless solution |
| Alkaline reagent test | + | + | - | - | Colorless solution |
| Glycoside test | - | - | - | - | No ring formation |
| Phenol test | - | - | - | - | Colorless |
| Saponin test | + | - | - | - | No foam formation |
| Steroid test | + | + | - | - | No color |
| Terpenoid test | - | - | - | - | No color on interface |

Table 2: Zone of inhibition (mm) shown by various bacteria in response to selected plants extracts at Paklihawa, 2017.

| | <i>Staphylococcus aureus</i> | <i>Escherichia coli</i> | <i>Klebsiella pneumonia</i> | <i>Salmonella typhimurium</i> |
|-------------------|------------------------------|-------------------------|-----------------------------|-------------------------------|
| <i>Parthenium</i> | 13.7±0.06 ^d | 10.1±0.05 ^c | 11.27±0.09 ^c | 10.63±0.09 ^c |
| <i>Eichornia</i> | 8.6±0.06 ^e | 8.73±0.08 ^c | 8.67±0.12 ^d | 8.17±0.09 ^b |
| <i>Pistia</i> | 8.57±0.03 ^c | 9.2±0.1 ^d | 9.48±0.08 ^c | 9.1±0.11 ^d |
| <i>Ipomoea</i> | 7.46±0.13 ^b | 6.3±0.15 ^b | 8.27±0.13 ^b | 8.6±0.11 ^c |
| Control | 0 ^a | 0 ^a | 0 ^a | 0 ^a |
| Grand mean | 7.67±1.17 | 6.87±0.98 | 7.53±1.04 | 7.3±1.00 |
| CV | 13.04 | 14.56 | 13.27 | 13.69 |
| P value | 0.000 | 0.000 | 0.000 | 0.000 |

Zone of inhibition was found significantly different between the treatments. Maximum activity was seen in *Parthenium* for all the studied bacteria. *Eichornia* and *Pistia* were at par to each other for *Staphylococcus aureus*. *Ipomoea* was least responsive for *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* whereas *Eichornia* was least performer for *Salmonella typhimurium*.

The results also indicated that the extracts from the plants studied showed inhibition of growth against tested microorganisms with to various degrees. It revealed that the methanolic extract of various invasive species has broad spectrum antimicrobial activities as visualized by zone of inhibition formed. The result for antibacterial and antifungal activity is as shown in table 2 and table 3. A clear zone of growth inhibition was found around the wall because of diffusion of compounds. The diameter of the inhibition zone differed according to the relative susceptibility of the test microorganisms to a particular antimicrobial agent.

Table 3: Zone of inhibition (mm) shown by various fungi in response to selected plants extracts at Paklihawa, 2017

| | <i>Aspergillus niger</i> | <i>Candida albicans</i> | <i>Alternaria solani</i> | <i>Fusarium spp</i> |
|-------------------|--------------------------|-------------------------|--------------------------|-------------------------|
| <i>Parthenium</i> | 10.73±0.37 ^b | 16.56±0.29 ^c | 11.66±0.66 ^b | 9.03±0.03 ^c |
| <i>Eichornia</i> | 10.56±0.52 ^b | 14.9±0.49 ^b | 11.36±0.31 ^b | 7.66±0.04 ^b |
| <i>Pistia</i> | 10.3±0.50 ^b | 14.23±0.78 ^b | 11.60±0.30 ^b | 8.67±0.33 ^{bc} |
| <i>Ipomoea</i> | 10.50±0.43 ^b | 14.63±0.31 ^b | 12.53±0.53 ^b | 8.02±0.05 ^{bc} |
| Control | 0.00 ^a | 0.00 ^a | 0.00 ^a | 0.00 ^a |
| Grand mean | 8.42±1.14 | 12.06±1.62 | 9.42±1.27 | 6.68±0.9 |
| CV | 23.75 | 24.86 | 21.2 | 22.47 |
| P value | 0.000 | 0.000 | 0.000 | 0.000 |

No significant difference was noted in the zone of inhibition for *Aspergillus niger* and *Alternaria solani*, however, for *Candida albicans*, *Parthenium* showed maximum activity. In case of *Fusarium spp*, maximum activity was seen for *Parthenium*, however, it was as par with *Pistia* and *Ipomoea*.

Minimum inhibitory concentration (MIC) measurement:

The impact of aqueous methanol extract against the selected tested bacteria and fungi was assessed as Minimum Inhibitory Concentration (MIC) values of the extracts. The MIC values against the tested bacteria ranged from 11 to 19 µg mL⁻¹ and for the tested pathogenic fungi, from 10 to 20 µg mL⁻¹ (Table 4). The MIC assay indicated that *Parthenium*, *Eichornia*, *Pistia* and *Ipomoea* have antibacterial and antifungal activities against all tested bacteria and fungi at a lower concentration.

Table 4: Minimum inhibitory concentration (MIC) of various invasive plant leaves extract on pathogenic bacteria determined by modified agar well diffusion method

| Extract | <i>Staphylococcus aureus</i> | <i>Escherichia coli</i> | <i>Klebsiella pneumoniae</i> | <i>Salmonella typhimurium</i> |
|-------------------|------------------------------|-------------------------|------------------------------|-------------------------------|
| <i>Parthenium</i> | 11.06±0.03 ^a | 15.1±0.06 ^b | 17.1±0.06 ^b | 16.05±0.03 ^a |
| <i>Eichornia</i> | 16.14±0.08 ^c | 18.1±0.06 ^d | 18.09±0.06 ^c | 18.06±0.03 ^b |
| <i>Pistia</i> | 16.13±0.06 ^c | 16.1±0.05 ^c | 17.05±0.03 ^b | 16.05±0.04 ^a |
| <i>Ipomoea</i> | 14.16±0.08 ^b | 14.2±0.05 ^a | 16.09±0.06 ^a | 19.09±0.06 ^c |
| Grand mean | 11.5±1.61 | 12.68±1.73 | 13.66±1.83 | 13.85±1.87 |
| CV | 17.38 | 14.07 | 15.77 | 14.63 |
| P value | 0.000 | 0.000 | 0.000 | 0.000 |

The minimum inhibitory concentration (MIC) required for *Staphylococcus aureus* was found to be least in *Parthenium* (11.06±0.03) which was significantly lower than other extracts. For *Escherichia coli*, MIC was found to be least and statistically significant in *Ipomoea* extracts (14.16±0.08). For *Klebsiella pneumoniae*, MIC was lower in *Pistia* (17.05±0.03) but as par with *Parthenium* (17.1±0.06). In the case of *Salmonella typhimurium*, MIC was as par with both *Parthenium* (16.05±0.03) and *Pistia* (16.05±0.04).

Table 5: Minimum inhibitory concentration (MIC) of various invasive plant leaves extract on pathogenic fungi determined by modified agar well diffusion method

| Extract | <i>Aspergillus niger</i> | <i>Candida albicans</i> | <i>Alternaria solani</i> | <i>Fusarium spp</i> |
|-------------------|--------------------------|-------------------------|--------------------------|-------------------------|
| <i>Parthenium</i> | 17.04±0.03 ^b | 12.05±0.03 ^a | 14.03±0.04 ^b | 17.02±0.02 ^a |
| <i>Eichornia</i> | 14.04±0.03 ^a | 15.06±0.03 ^b | 14.03±0.03 ^b | 17.03±0.03 ^a |
| <i>Pistia</i> | 17.05±0.04 ^b | 17.06±0.03 ^c | 19.02±0.03 ^c | 16.99±0.01 ^a |
| <i>Ipomoea</i> | 17.08±0.06 ^b | 19.02±0.03 ^d | 10.22±0.22 ^a | 20.01±0.01 ^b |
| Grand mean | 13.04±1.77 | 12.63±1.79 | 11.46±1.7 | 14.21±1.92 |
| CV | 14.43 | 15.33 | 15.83 | 17.44 |
| P value | 0.000 | 0.000 | 0.000 | 0.000 |

The MIC for *Aspergillus niger* was least and significantly different in *Eichornia* extracts (14.04±0.03), *Candida albicans* in *Parthenium* extracts (12.05±0.03) and *Alternaria solani* in *Ipomoea* extracts (10.22±0.22). However, MIC results for *Fusarium spp* were as par with *Parthenium*, *Eichornia* and *Pistia*. These results of antimicrobial activity of different extracts of *E. crassipes* were similar to the results found by others authors (Jayanthi *et al.* 2011; Thamaraiselvi *et al.* 2012).

CONCLUSIONS

The usage of the selected plants for the medicinal purpose has been reported by several researchers. However, these are invasive in nature and are supposed to be toxic in nature so, further investigations are to be done before establishing them as standard medicinal plants or specific plant protection measures. The present study shows potential phytochemicals present in *Parthenium*, *Eichornia*, *Pistia* and *Ipomoea*. Moreover, these plants showed to have a good antibacterial and anti-fungal activity in the aerial parts of selected plants. The finding of this study also paves the way for further research to identify the specific bioactive compounds that are responsible for its claimed antimicrobial activity. Based on this study, it is concluded that under different circumstances the invasive plants might be used for modern therapeutics to prepare potential drugs and plant protection measures. Such study will explore other pharmacological activity of unexplored weeds inhabited in and around western Nepal. Detailed study, is however needed in this regard.

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