Phytochemicals screening and antimicrobial activities of selected medicinal plants of Khyberpakhtunkhwa Pakistan

Iqbal Hussain1*, Moneeb Ur Rehman Khattak1, Riaz ullah1,3, Zia Muhammad1, Naeem Khan1, Farhat Ali Khan2, Zahoor Ullah3 and Sajjad Haider3

1Department of Chemistry, Kohat University of Science and Technology Kohat-26000, Pakistan.
2Department of Pharmacy Sarhad University of Science and Information Technology Peshawar-25000, Pakistan.
3Department of Chemical Engineering, College of Engineering, King Saud University, P. O. BOX 800, Riyadh 11421, Saudi Arabia.

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The study was carried out to assess the phytochemical and antimicrobial bioassay of five medicinal plants, Lepidium sativum, Nerium oleander, Ranunculus repens, Tecoma stans and Urtica dioca. These plants are traditionally used as medicine in the Northwest Pakistan, therefore it is necessary to identify and estimate their alkaloid, flavonoid, saponin, phenol and tannin contents. Phytochemical investigation of plant samples determines that alkaloid (63.6%) and flavonoid (0.91%) were highest in N. oleander, saponin (11%) and phenol (0.031) in T. stans, tannin (0.61%) in L. sativum, All five species showed no significant antimicrobial activities.

Key words: Medicinal plants, phytochemical analysis, antimicrobial activities

INTRODUCTION

The world is fertile with natural and medicinal plants. Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmacological. The medicinal power of these plants lies in phytochemical constituents that cause definite pharmacological actions on the human body (Akinmoladun et al., 2007). Phytochemical, natural compound occur in plants such as medicinal plants, vegetables and fruits that work with nutrients and fibers to act against diseases or more specifically to protect against diseases.

The phytochemicals are grouped into two main categories (Krishnaiah et al., 2009) namely primary constituents which includes amino acids, common sugars, proteins and chlorophyll etc., and secondary constituents consisting of alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds etc. (Krishnaiah et al., 2007; Edeoga et al., 2005). Majority of phytochemicals have been known to bear valuable therapeutic activities such as insecticidals (Kambu et al., 1982), antibacterial, antifungal (Lemos et al., 1990), anti-constipative (Ferdous et al., 1992), spasmylotic (Sontos et al., 1998), antiplasmodial (Benolitval et al., 2001) and antioxidant (Varadar-unlu et al., 2003) activities etc. The plants thus find their medicinal value due to respective phytochemical constituents they contains.

Infectious diseases are the leading causes of death throughout the world that accounts for nearly one half of all death in the tropical countries, which are also becoming a serious problem in developed countries. It is calculated that infectious diseases are the main causes of death in 8% of the 9 deaths occurring in United States (Demissew and Dagne, 2001). In addition, antibiotics are sometime associated with adverse effects including hypersensitivity, immuno suppressant and allergic reactions. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants. Lepidium sativum, Nerium oleander, Ranunculus repens, Tecoma stans and Urtica dioca are medicinally very important plants and

*Corresponding author. E-mail: naseemyar@yahoo.com.
use extensively in pharmaceutical formulations and are also use by local practitioners for a variety of human diseases. Hence the aim of this study was to determine the phytochemical constituents and to investigate the antimicrobial properties so as to ascertain their uses in traditional medicines.

**MATERIAL AND METHODS**

**Preparation of sample**

The aqueous extract of each sample was prepared by soaking 10 g of powdered samples in 200 ml of distilled water for 12 h. The extract was filtered through Whatman filter paper. The phytochemicals in each sample was determined qualitatively and quantitatively (Krishnaiah et al., 2009; Mattila et al., 2007).

**Qualitative analysis of phytochemicals**

**Alkaloids**

The extracts were evaporated to dryness and the residues were heated with 2% Hydrochloric acid on a boiling water bath. The extract were cooled, filtered and treated with the Mayer’s reagent. The sample was then observed for the presence of yellow precipitation or turbidity (Tyler and Herbalgram, 1994; Harborne et al., 1973).

**Flavonoids**

1.5 ml of 50% methanol was added to 4 ml of extracts. Warmed the solution and metal magnesium was added. Then added 5 to 6 drops of concentrated hydrochloric acid to the solution and observed for red coloration (Tyler and Herbalgram, 1994; Harborne et al., 1973).

**Tannins**

To 0.5 ml of extract solution, 1 ml of distilled water and 1 to 2 drops of Ferric chloride solution was added, observed for blue or green black coloration (Tyler and Herbalgram 1994; Harborne et al., 1973).

**Phenol**

2 ml ethanol was added to the test solution and few drops of ferric chloride solution was observed for coloration (Tyler and Herbalgram, 1994; Harborne et al., 1973).

**Saponins**

2 ml of distilled water was added to 2 ml of the test solution and shaken well and observed for frothing (Tyler and Herbalgram, 1994; Harborne et al., 1973)

**Quantitative analysis**

**Alkaloids**

5 g of the plant sample was prepared in a beaker and 200 ml of 10% CH₃CO₂H in C₂H₅OH is added to the plant sample. The mixture is covered and allowed to stand for 4 h. The mixture was then filtered and the extract is allowed to become concentrated in a water bath until it reaches ¼ of the original volume. Concentrated ammonium hydroxide was added until the precipitation is complete. The whole solution is allowed to settle and the precipitate is collected and washed with dilute ammonium hydroxide and then filtered. The residue is alkaloid, which is then dried and weighed.

**Flavonoids**

Extracted 10 g of the plant sample with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered and the filtrate was then transferred into a water bath. The solution was evaporated to dryness and weighed to a constant weight (Williamson and Manach, 2005; Mattila and Hellström, 2007).

**Saponins**

20 g of each ground plant samples were put into a conical flask and 100 ml of 20% ethanol was added to the plant sample. The said sample is heated over a water bath for 4 h at about 55°C with continuous stirring. The extracted mixture is then filtered and the residue is then re-extracted again with 200 ml of 20% ethanol. The collective residues are reduced to 40 ml over a hot water bath. The concentrated is then transferred to a separating funnel and 20 ml of diethyl ether is added to the plant extract and the shaken vigorously. The aqueous layer was recovered while the organic layer was discarded and the process of purification was repeated. Sixty milliliter of n-Butanol was added and combined n-Butanol extract were washed twice with 10 ml of 5% sodium chloride. The remaining solution was then heated on water bath and after evaporation; the samples were dried in oven to a constant weight.

**Tannins**

500 mg of plant sample was weighed and transferred to 50 ml flask. Then added 50 ml of distilled water and stirred for 1 h. Sample was filtered into a 50 ml volumetric flask and the volume was made up to the mark. 5 ml of the filtered sample was pipette into test tube and then mixed with 2 ml of 0.1 M ferric chloride. The absorbance was measured using spectrophotometer at 395 nm wavelength within 10 min (Tyler and Herbalgram, 1994; Harborne et al., 1973).

**Phenols**

Plants sample was boiled for 15 min with 50 ml of (CH₃)₃CO₃O. 5 ml of the sample was pipette into 50 ml flask, and 10 ml of distilled water was added. Then 2 ml of NH₄OH solution and 5 ml of concentrated CH₂ (CH₃)₂CH₂OH was added to the mixture. The sample was made up to the mark and left to react for 30 min for color development and measured for 505 nm wave length using a spectrophotometer (Tyler and Herbalgram 1994; Harborne et al., 1973).

**Antimicrobial activity**

**Preparation of crude extract**

100 g of each of the coarsely powdered plant material was taken and extracted with ethanol, water and n-hexane. The extracts was filtered and sodium chloride solution was then added to the filtered extract to form precipitates. The precipitates were then separated, air dried and transferred to air tight amber glass container. The
crude extract was dissolved in chloroform and water to make the final concentration, which was kept in refrigerator till use (Harborne et al., 1973).

Preparation of standard bacterial suspension

The average number of viable, Bacillus subtilis (NCTC8236), Escherichia coli (ATCC25922), Proteus vulgaris (ATCC6380), Pseudomonas aeruginosa (ATCC27853), Salmonella typhimurium (ATCC0850) and Staphylococcus aureus (NCTC25953) organism per milliter of the stock suspension was determined by means of the surface viable counting technique. About (10$^8$ to 10$^9$) colony forming units per milliter was used. A fresh stock suspension was prepared each time (Lang et al., 1990; Bylka et al., 2004).

Test for antibacterial activity

The antimicrobial activity of the prepared extracts was determined by using well agar diffusion method. The standardized bacterial stock suspension (10$^8$ to 10$^9$) colony forming units per milliter was mixed with 60 ml of sterile nutrient agar thoroughly. 20 ml inoculated nutrient agar was poured into sterile Petri dishes. The agar was left to set and four well 10 mm in diameter was made in each of these plates using sterile cork borer No 8 and then agar discs were removed. The entire well was filled with 0.1 ml of each extracts using microtiter-pipette and allowed to diffuse at room temperature for 2 h. The plates were incubated at 37°C for 24 h. Two replicates were also performed for each extract against each of the test organism. Simultaneously addition of the respective solvent instead of extract was carried out as controls. After incubation, the zones of inhibition were measured and mean value was calculated (Hanna et al., 2008; Roberts and Wink, 1998).

RESULTS AND DISCUSSION

Phytochemicals are plant-derived chemical compounds which are non-essential nutrients, some of which show potential health-promoting properties.

Qualitative analysis

As can be seen from Table 1, alkaloids, flavonoids, saponins, tannins and phenols were present in studied plant samples.

Quantitative analysis

As can be seen from Table 2, high concentration 63.6% of alkaloids was found in N. oleander and less concentration of 0.40% was noted in L. sativum. The concentration of alkaloids in the rest of samples are as follow: 51.5% in T. stans, 0.80% in R. repens and 12.8% in U. dioca.

Table 1. Qualitative analysis of phytochemicals.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Alkaloid</th>
<th>Flavonoid</th>
<th>Saponin</th>
<th>Tannin</th>
<th>Phenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. sativum</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>N. oleander</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>R. repens</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>T. stans</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>U. dioca</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
</tbody>
</table>

Table 2. Quantitative analysis of phytochemicals.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Alkaloid (%)</th>
<th>Flavonoid (%)</th>
<th>Saponin (%)</th>
<th>Tannin (%)</th>
<th>Phenol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. sativum</td>
<td>0.40</td>
<td>0.42</td>
<td>2.8</td>
<td>0.61</td>
<td>0.004</td>
</tr>
<tr>
<td>N. oleander</td>
<td>63.6</td>
<td>0.91</td>
<td>9</td>
<td>0.01</td>
<td>0.003</td>
</tr>
<tr>
<td>R. repens</td>
<td>0.80</td>
<td>0.39</td>
<td>5</td>
<td>0.01</td>
<td>0.004</td>
</tr>
<tr>
<td>T. stans</td>
<td>51.5</td>
<td>0.53</td>
<td>11</td>
<td>0.19</td>
<td>0.031</td>
</tr>
<tr>
<td>U. dioca</td>
<td>12.8</td>
<td>0.60</td>
<td>3.1</td>
<td>0.21</td>
<td>0.006</td>
</tr>
</tbody>
</table>
in *U. dioica*. The use of alkaloid contains plants as dyes, spices, drugs or poisons can be traced back almost to the beginning of civilization (Roberts and Wink, 1998). They are well known for their CNS activities (Lewis and Elvin, 2003).

**Flavonoids**

Flavonoids are plant nutrients that when consumed in the form of fruits and vegetables are non-toxic as well as potentially beneficial to the human body; up till now, more than 2000 different flavonoids have been isolated from vegetables (Taiz and Ziegler, 2006). High percentage of flavonoids (0.91%) was determined in *N. oleander* followed by 0.60% in *U. dioica*, 0.53% in *T. stans*, 0.42% in *L. sativum* and 0.39% in *R. repens*.

**Saponins**

Pharmacological activities have been reported about saponins such as antibiotic, antifungal, antiviral, hepatoprotective anti-inflammatory and anti-ulcer (Oakentull, 1986; Zhang, 2001). Table 2 shows that the percentage of saponins (11%) was found very in *T. stans*, followed by 9% in *N. oleander* and 5% in *R. repens*. *U. dioica* contains 3.1% of saponins while in *L. sativum* the percentage was obtained in low concentration 2.8%.

**Tanins**

The concentration 0.61% was detected in *L. sativum* followed by 0.21% in *U. dioica*, 0.19% in *T. stans* while in the rest of samples, concentration of tannins was recorded 0.01%. Tannins are basically use for the treatment of inflammation, leucorrhoea, gonorrhoea, burn, piles, diarrhoea and as antidote in the treatment of alcaloidal poisoning (Buzzini et al., 2008). They are also used for tannin of animal hides to convert them to leather.

**Phenols**

Phenols are very wide spread in nature. They range from simple structures having a simple aromatic ring to highly complex polymeric structures and often exist in glycosidic forms (Williamson and Manach, 2005). Capsacin is found in the dried ripe fruit of different species of Capsicum. It had been used internally for dyspepsia and flatulence. Externally, it is frequently used as counterirritant (Mattila and Hellström, 2007). Table 2 shows very low concentration of phenols in the whole plant samples which range from 0.004 to 0.19%.

**Antimicrobial assay**

Sustainable amount of new antibiotic available in the market are obtained from natural or semi synthetic resources are obtained from about 20% of the plants present in world which were submitted to pharmaceutical or biological test. As can be seen from the analytical results obtained from the zone of inhibition of water extracts (Table 3) of five selected medicinal herbs

<table>
<thead>
<tr>
<th>Water extract of the plant</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. sativum</em></td>
<td>11</td>
<td>13</td>
<td>8</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td><em>N. oleander</em></td>
<td>14</td>
<td>9</td>
<td>12</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>R. repens</em></td>
<td>12</td>
<td>10</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>T. stans</em></td>
<td>10</td>
<td>8</td>
<td>12</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>U. dioica.</em></td>
<td>15</td>
<td>11</td>
<td>13</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

**Chloroform extract**

Table 4 shows high activity of 17 mm determined in the crude extract of *U. dioca* from chloroform extract against *B. subtilis* while the activity shown by the rest of the plant extracts were in between 9 to 15 mm. Activity of 16 mm was recorded in the extract of *L. sativum* against *P. vulgaris* and 11, 10, 7 and 7 mm activities were noted in *U. dioca*, *R. repens*, *N. oleander* and *T. stans*, respectively. *R. repens* extract showed 15 mm activity against *S. aureus* while less activity was found 9 mm in extract of *L. sativum*. Activity of 7 mm was recorded in *T. stans* extract against *E. coli*. 5 mm activity was recorded in both crude extracts of *N. oleander* and *U. dioca*. While 3 and 2 mm activities were also observed. Extracts of *N. oleander* and *L. sativum* were more active, 4 mm against *P. aeruginosa* while *T. stans* and *U. dioca* also showed same activity, 3 mm and no activity was seen of *R. repens* against same organism. Activity of 7 mm was shown by *L. sativum* against *S. typhi*, 5 mm by *R. repens*, 2 mm by *N. oleander*, 1 mm by *U. dioca* and no activity by *T. stans* against *S. typhi*.

Same antifungal activity of 2 mm was determined against *A. niger* by most of the plants except *R. repens* which was 1 mm. *T. stans* was noted more active at 4 mm against *C. albicans* followed by 2, 1 and 1 mm in *N. oleander*, *L. sativum* and *T. stans* respectively while no activity was shown by *R. repens*.

**REFERENCES**


