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Full Length Research Paper

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

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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 inveigation 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), spasmolytic (Sontos et al., 1998), antiplasmodial (Benoitvical et al., 2001) and antioxidant (Vardar-unlu et al., 2003) activities etc. The plants thus find their medicinal value due to respective phytochmical constituents they contains.

Infectious diseases are the leading causes of death through out 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

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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 Watt man 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 and 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 shaked 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 of10% CH_3CO_2H in C_2H_5OH 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 $(CH_3CH_2)_2O$. 5 ml of the sample was pipette into 50 ml flask, and 10 ml of distilled water was added. Then 2 ml of NH_4OH solution and 5 ml of concentrated CH_3 $(CH_2)_3CH_2OH$ 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

Table 1. Qualitative analysis of phytochemicals.

Sample code	Alkaloid	Flavonoid	Saponin	Tannin	Phenol
L. sativum	+ ve	+ ve	+ ve	+ ve	+ ve
N. oleander	+ ve	+ ve	+ ve	+ ve	+ ve
R. repens	+ ve	+ ve	+ ve	+ ve	+ ve
T. stans	+ ve	+ ve	+ ve	+ ve	+ ve
U. dioca	+ ve	+ ve	+ ve	+ ve	+ ve

Table 2. Quantitative analysis of phytochemicals.

Sample code	Alkaloid (%)	Flavonoid (%)	Saponin (%)	Tannin(%)	Phenol (%)
L. sativum	0.40	0.42	2.8	0.61	0.004
N. oleander	63.6	0.91	9	0.01	0.003
R. repens	0.80	0.39	5	0.01	0.004
T. stans	51.5	0.53	11	0.19	0.031
U. dioca	12.8	0.60	3.1	0.21	0.006

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* (ATCC0650) 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 milliters 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).

Preparation of standard fungal suspension

The fungal cultures, *Aspergillus niger* (ATCC 9763) and *Candida albicans* (ATCC7596) were maintained on saboraud dextrose agar, incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline and the suspension was stored in refrigerator till used (Hanna et al., 2008; Roberts and Wink, 1998).

Test for anti fungal activity

The method applied for determination of antifungal activity is the same as for antibacterial activity. The media used is sterile yeast and mould extract agar. *C. albican* is incubated for two days while *A. niger* for three days at 25°C.

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

Alkaloids

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%

Table 3. Zones of inhibitions of water extracts of selected medicinal plants.

Water extract of the plant	1	2	3	4	5	6	7	8
L. sativum	11	13	8	2	4	1	3	2
N. oleander	14	9	12	6	3	2	0	1
R. repens	12	10	7	5	2	4	2	1
T. stans	10	8	12	4	2	6	2	2
U. dioca.	15	11	13	6	2	0	3	1

Zones of inhibitions in millimeter: Gram positive bacteria: 1, *B. subtillis*; 2, *P. vulgaris*, 3, *S. aureus,* Gram negative bacteria: 4, *E. coli;* 5, *P. aeruginosa*; 6, *S.typhi.* Fungi: 7, *A.niger;* 8, *C.albicans.*

in *U. dioca*. 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. dioca,* 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, hepato-protective anti-inflammatory and anti-ulcer (Oakenfull, 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. dioca* 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. dioca*, 0.19% in *T. stans* while in the rest of samples, concentration of tannins was recoded 0.01%. Tannins are basically use for the treatment of inflammation, leucorrhoea, gonorrhea, burn, piles, diarrhea 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

Water extract

Table 3 shows high activity 15 mm recorded from the crude extract of *U. dioca* against *B. subtillis* while less activity. 10 mm was obtained from the crude extract of *T. stans* against *B. subtillrespectivelyis*. The high activity of *L. sativum* extract against *P. vulgaris* is 13 mm while 11 mm was recorded against the same bacteria in *U. dioca* of water extract. *R. repens* has showed 10 mm activity against *P. valgaris*. The activities of the other water extracts were relatively small; 9 and 8 mm was found in *N. oleander* and *T. stans* extracts. *T. stans* and *N. oleander* showed equal activity of 12 mm against *S. aureus* while less activity of 7 mm was recorded in *R. repens*. An equal activity of 6 mm was observed in *N. oleander* and *U. dioca* against *E. coli* followed by 5, 4 and 2 mm in *R. repens, T. stans and L. sativum,* respectively against the pathogen *E. coli*. High activity of 4 mm was shown by *L. sativum* against *P. aeruginosa* while 3 mm activity recorded in *N. oleander* against same bacteria and same relatively low activiat 6 mm against *S. typhi* while 4 mm activity was observed from *R. repens*. Low activity of 2 and 1 mm was shown by *N. oleander* and *L.*

Table 4. Zones of inhibitions of chloroform extracts of selected medicinal plants.

Chloroform extract of the plant	1	2	3	4	5	6	7	8
L. sativum	9	16	11	2	4	7	2	1
N. Oleander	11	7	12	5	4	2	2	2
R. repens	13	10	15	3	0	5	1	0
T. Stans	15	7	14	7	3	0	2	4
U. Dioca.	17	11	9	5	3	1	2	1

Zones of inhibitions in millimeter: Gram positive bacteria: 1, *B. subtillis*; 2, *P. vulgaris*, 3, *S. aureus,* Gram negative bacteria: 4, *E. coli;* 5, *P. aeruginosa*; 6, *S.typhi.* Fungi: 7, *A.niger;* 8, *C.albicans.*

sativum while *U. dioca* showed no activity. *L. sativum* and *U. dioca* showed high activity of 3 mm against *A. niger* while 2 mm activity was found from *R. repens* and *T. stans* extracts while *N. oleander* showed no activity against *A. niger*. High activity of 2 mm was noted in both *L. sativum* and *T. stans* against *C. albicans* whilet same 1 mm activity was recorded in the rest of the plant extracts.

Chloroform extract

Table 4 shows high activity of 17 mm determined in the crude extract of *U. dioca* from chloroform extract against *B. subtillis* while the activity shown by the rest of the plant extracts were in between 9 to15 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 found 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. oleonder*, 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*.

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