

# Chemical and biological evaluation of *Ranunculus muricatus*

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**Abstract:** *Ranunculus muricatus* is commonly known as spiny fruit buttercup and is used in the treatment of intermittent fevers, gout and asthma. Qualitative analysis of phytochemicals of *Ranunculus muricatus* indicated the presence of saponins, tannins, phenols, flavonoids and alkaloids. Saponins were present in high amount as compared with other chemicals. Inorganic and heavy metals constituents were determined. Heavy metals estimation in the sample showed that iron was present in high amount followed by zinc even then the concentration of these metals is below acceptable limit. The physical parameters, antioxidant and antimicrobial activities of the extracts were determined. Acetone extract fraction showed optimal antioxidant activity as compared to ethanol and chloroform fractions of the candidate plant. The antimicrobial and antifungal activities of the crude extract and extract fractions were determined by well agar diffusion method. Highest zone of inhibitions were observed for crude extract followed by acetone extract fraction against *Micrococcus luteus*. Antifungal activities were high for crude extracts against *Candida Albican*. Findings of this study show that *Ranunculus muricatus* has a good medicinal impact.

**Keywords:** *Ranunculus Muricatus*; phytochemicals; inorganic constituents; antioxidants; antibacterial; antifungal.

## INTRODUCTION

Medicines resulting from plants are commonly famous due to their protection, easy accessibility and low cost. Plant based products may contain whole parts of the plant or mostly prepared from leaves, roots, bark, seed and flowers. They are administered orally, inhaled or directly applied to the skin (Westh *et al.*, 2004). The uses of plants as medicinal agents are due to the presence of important phytochemicals like alkaloid, flavonoid, tannin, saponin and phenolic compound, which provides the basis of modern drugs known today (Crag and Newman, 2001; Krishnaiah, 2007)

Nowadays, multiple drug resistance has been developed due to the indiscriminate use of commercial antibacterial and antifungal agents used in the treatment of infectious diseases. These problems enforced scientists to investigate for new antimicrobial substances. Therefore, it is a needed to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Lee, 2003). Antimicrobial agents of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Byika *et al.*, 2003).

Anti-oxidants are chemical substances that help to inhibit the oxidation reactions caused by free radicals such as singlet oxygen peroxy radicals, hydroxyl radicals and peroxy nitrite thereby preventing or delaying damage to the cells & tissues. The mechanism of action include

scavenging reactive oxygen and nitrogen free radical species, decreasing the localized oxygen concentration thereby reducing molecular oxygen's oxidation potential, metabolizing lipid peroxides to non-radical products and chelating metal ions to prevents the generation of free radicals (Robinson and Maxwell, 1997).

Heavy metals in plants are the main constituents of the inorganic contamination. Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels and damage to blood composition, lungs, kidneys, liver and other vital organs. Long-term exposure may result in slowly progressing physical, muscular and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis. Allergies are rare and occur through repeated long-term contact with some metals or their compounds that may even cause cancer (Fletcher, 1991).

Health depends upon the organized state of elements like Na, K, Ca, Mg, Cl, F etc in the body and their imbalance causes diseases. The restoration of balance by drug can cure diseases (Farhat *et al.*, 2011). The remarkable progress that has been made in the science of Medical Elementology during the past few decades has not only opened avenues for research on human health related aspects but also aroused the interest of the pharmaceutical industries to reap the benefits of this research by formulations containing elements reported to be essential for human health. A variety of such formulations are available worldwide (Iqbal *et al.*, 2011).

*Ranunculus muricatus*, which is commonly known as spiny fruit buttercup is a member of Ranunculaceae

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family. About 22 genera and 114 species including *Ranunculus muricatus* are available in Pakistan that are of great concern in term of medicine and is used for varieties of ailments like intermittent fevers, gout and asthma (Chopra *et al.* 1986). Although *Ranunculus muricatus* is traditionally used by local practitioners for the treatment of various ailments but still it is not scientifically proved. Local practitioners and Hakeems are not fully aware of toxicity of heavy metal so it is needed to standardize plant materials in term of heavy metals and inorganic constituents. The therapeutic effect is mainly due to the phytochemicals present in different parts of plants. Keeping the need of these vital constituents, it is required to explore its biological activity and antioxidant activity, which will provide scientific database for further studies.

In this study the whole *Ranunculus muricatus* was subjected to extraction of various components. The various extracts were evaluated for phytochemicals, inorganic profile, antioxidant and biological activities.

## MATERIALS AND METHODS

The selected medicinal plant was collected in the month of March 2012 and transferred to the laboratory for taxonomical identification and confirmation (Voucher No: H.PCSIR 508) at the Pakistan Council of Scientific and Industrial Research (PCSIR) laboratories complex, Peshawar. The plant samples were rinsed with tap water followed by washing with de-ionized water. Roots stem and leaves were separated and dried in shady shelves. The dried materials were chopped, crushed and powdered with electrical grinder. The dried powdered samples were stored in polyethylene bottles for further use.

### *Phytochemical determination*

The stem, root and leaves aqueous extracts were prepared by soaking 10g of powdered samples in 200ml of distilled water for 12h. The extracts were then filtered using Whatman filter paper. The constituent phytochemicals in each sample were determined qualitatively and quantitatively using standard methods described in literature (Hanna, 2008; Lee *et al.*, 2003).

### *Determination of alkaloids*

For qualitative determination of alkaloids, the extracts were evaporated to dryness and the residues were heated with 2% HCl solution on a boiling water bath. The extracts were cooled, filtered and then treated with the Mayer's reagent. The appearance of yellow precipitation showed the presence of alkaloids (Hanna, 2008; Lee *et al.*, 2003).

For quantitative determination of alkaloids, 5g of each sample was put in a beaker and 200ml of 10% CH<sub>3</sub>COOH in C<sub>2</sub>H<sub>5</sub>OH was added to it. The mixture was covered and allowed to stand for 4h. The mixture was then filtered and

the extract was concentrated by heating on a water bath till 1/4<sup>th</sup> of its original volume. Concentrated NH<sub>4</sub>OH was added till the precipitation was completed. The mixture was allowed to settle. The precipitates were collected, washed with dilute ammonium hydroxide and filtered. The residue obtained was alkaloids, which were then dried and weighed (Hanna, 2008; Lee *et al.*, 2003).

### *Determination of total phenols*

To determine phenols qualitatively 2ml of ethyl alcohol was added to test solution and few drops of ferric chloride put in it and observed for yellow coloration (Hanna, 2008; Lee *et al.*, 2003).

For quantitative determination of phenols the plants sample was boiled for 15min with 50ml of (CH<sub>3</sub>CH<sub>2</sub>)<sub>2</sub>O. 5ml of the boiled mixture was taken into a 50ml flask, and 10ml of distilled water was added to it. Then 2ml of NH<sub>4</sub>OH solution and 5ml of concentrated CH<sub>3</sub>(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>OH were added to the mixture. The mixture was made up to the mark by adding distilled water and left to react for 30min for color development. The amount of phenols was determined spectrophotometrically at 505nm (Hanna, 2008; Lee *et al.*, 2003).

### *Determination of flavonoids*

For qualitative determination of flavonoids 1.5ml of a 50% aqueous methanol was added to 4ml of plant extracts, warmed gently and Mg metal was added to it. Then 5-6 drops of concentrated HCl was added to the resulting mixture and was observed for red coloration (Hanna, 2008; Lee *et al.*, 2003).

For quantitative determination of flavonoids, 10g of the plant sample was added to 100ml of 80% aqueous methanol and was kept at room temperature for 24 hours. The whole solution was filtered and the filtrates were dried by evaporation on water bath till constant weight (Hanna, 2008; Lee *et al.*, 2003).

### *Determination of saponins*

For qualitative determination of saponins, to a 2ml test solution was added 2ml of distilled water and was shaken. The appearance of foamy lather on the surface indicates the presence of saponins.

For quantitative determination of saponins, 20g grounded plant samples were mixed with 100ml of 20% aqueous ethanol. The mixture was heated on a water bath for 4h at 55°C with continuous stirring. The resulting mixture was filtered. The residue was then re-extracted with 100ml of 20% aqueous ethanol and was evaporated to 40ml of its volume by heating it on water bath at 90°C. The concentrates were transferred to a separating funnel and were shaken well with 20ml of diethyl ether. The aqueous layer was subjected to further purification while the organic layer was discarded. To the purified aqueous layer 30 ml of n-butanol was added and was washed twice with 10ml of 5% NaCl solution. The remaining solution

was then heated on a water bath till dryness. The dried residues were put in oven to get a constant weight (Hanna, 2008; Lee *et al.*, 2003).

#### Determination of tannins

For qualitative determination of tannins 0.5 ml extract solution was added with 1ml of distilled water and 1-2 drops of ferric chloride solution, observed for blue-black coloration.

For quantitative determination of tannins 500mg of powdered plant sample was taken into a 50 ml flask. 50ml of distilled water was added to it and stirred for 1h. The mixture was filtered into a 50ml volumetric flask and the volume was made up to the mark with distilled water. Pipette out 5ml of the filtered sample into test tube and was mixed with 2ml of 0.1M ferric chloride solution. The amount of tannins was determined using spectrophotometer at 395nm within 10min (Hanna, 2008; Lee *et al.*, 2003).

#### Antimicrobial activity

##### Preparation of Crude Extract

The crude extract was prepared by contacting 100g each of root, stem and leaves powder with 90% methanol and kept for two weeks with continuous stirring at regular intervals. After two weeks the mixtures were filtered from cloth and the liquid portions were dried in rotary evaporator. From crude extract aqueous and acetone fractions were prepared by dissolving it in distilled water and acetone respectively and dried using rotary evaporator.

##### Preparation of standard bacterial suspension

The average number of viable organism per ml of the stock suspensions containing *Bacillus subtilis* (NCTC8236), *Escherichia coli* (ATCC25922), *Micrococcus luteus* (ATCC6380), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella typhi* (ATCC0650) and *Staphylococcus aureus* (NCTC25953) were determined by means of the surface viable counting technique. About  $10^8$ - $10^9$  colony-forming units (CFU) per ml were used. Each time a fresh stock suspension was prepared (Sofowara, 1993; Chouhan *et al.*, 2002).

##### Antibacterial activity

The antimicrobial activities of the extracts were determined by well agar diffusion method (Sofowara, 1993). The standard bacterial stock suspension  $10^8$ - $10^9$  CFU/ml was mixed with 60ml of sterile nutrient agar thoroughly and 20ml from inoculated nutrient agar was poured into sterile petri dishes. These were left for some time to set. Then four wells (10mm in diameter) were made in each of these plates using sterile cork borer No. 8 and agar discs were removed. The entire wells were filled with 0.1ml of root, stem and leave extracts using micro titer-pipette and allowed to diffuse at room temperature for 2h. The plates were incubated at 37°C for 24h. Two

replicates were also performed for each extract against the test organisms. Simultaneously, addition of the respective solvent instead of extract was carried out as controls. After incubation, the zones of inhibitions were measured in millimeters and mean values were calculated (Sofowara, 1993; Chouhan *et al.*, 2002).

##### Preparation of standard fungal suspension

The fungal cultures, *Aspergillus niger* (ATCC 9763) and *Candida albicans* (ATCC7596) were incubated on sabouraud dextrose agar for four days at 25°C. The cultures were harvested and washed with sterile saline solution and the suspension was stored in refrigerator for further use (Sofowara, 1993; Chouhan *et al.*, 2002).

**Table 1:** Qualitative phytochemical analysis of *Ranunculus muricatus*

| Constituent | Root | Stem | Leaves |
|-------------|------|------|--------|
| Alkaloids   | +ve  | +ve  | +ve    |
| Flavonoids  | +ve  | +ve  | +ve    |
| Tannins     | +ve  | +ve  | +ve    |
| Saponins    | +ve  | +ve  | +ve    |
| Phenols     | +ve  | +ve  | +ve    |

**Table 2:** Quantitative phytochemical analysis of *Ranunculus muricatus* (%)

| Constituent | Root   | Stem   | Leaves |
|-------------|--------|--------|--------|
| Alkaloids   | 0.967  | 0.801  | 0.456  |
| Flavonoids  | 0.403  | 0.699  | 0.313  |
| Tannins     | 0.013  | 0.015  | 0.009  |
| Saponins    | 4.730  | 7.110  | 7.350  |
| Phenols     | 0.0038 | 0.0044 | 0.0031 |

##### Anti-fungal activity

Well agar diffusion method was used to determine the antifungal activities of the prepared water, methanolic and acetone extracts. The 0.6ml standard fungal stock suspension  $10^8$ - $10^9$  CFU/ml were mixed with 60ml of sterile yeast and mould extract agar thoroughly. 20ml of inoculated yeast and mould extract agar mixture was poured into sterile petri dishes. Allowed the agar to settle and four wells (10mm in diameter) were made in each of these plates using sterile cork borer No 8. Then agar discs were removed. The entire wells were filled with 0.1ml of root, stem and leave extracts using micro titer-pipette and allowed to diffuse at room temperature for 2h. The plates were incubated at 25°C for four days. Simultaneously, addition of water, methanol and acetone instead of extract was carried out as controls (Sofowara, 1993; Chouhan *et al.*, 2002). After incubation, the zones of inhibition in millimeter were measured and mean values are presented in table 6.

##### Determination of physical parameters

The pH of extracts was determined by pH meter (InoLab pH 720). The pH meter was first calibrated with two point

**Table 3:** Zones of inhibitions of water extracts of *Ranunculus muricatus* in millimeters of bacterial strains

| Plant's part | B. Subtillis | S. Aureus | M. Luteus | E. Coli | P. Aeruginosia | S. Typhi |
|--------------|--------------|-----------|-----------|---------|----------------|----------|
| Root         | 5            | 6         | 7         | 2       | 3              | 5        |
| Stem         | 3            | 3         | 5         | 2       | 3              | 2        |
| Leaves       | 6            | 8         | 8         | 4       | 3              | 3        |

**Table 4:** Zones of inhibitions of methanolic extracts of *Ranunculus muricatus* in millimeters of bacterial strains

| Plant's part | B. Subtillis | S. Aureus | M. Luteus | E. Coli | P. Aeruginosia | S. Typhi |
|--------------|--------------|-----------|-----------|---------|----------------|----------|
| Root         | 12           | 10        | 13        | 7       | 5              | 10       |
| Stem         | 7            | 11        | 10        | 5       | 7              | 7        |
| Leaves       | 13           | 12        | 13        | 8       | 9              | 8        |

**Table 5:** Zones of inhibitions of acetone extracts of *Ranunculus muricatus* in millimeters of bacterial strains

| Plant's part | B. Subtillis | S. Aureus | M. Luteus | E. Coli | P. Aeruginosia | S. Typhi |
|--------------|--------------|-----------|-----------|---------|----------------|----------|
| Root         | 8            | 9         | 10        | 5       | 5              | 7        |
| Stem         | 5            | 9         | 6         | 5       | 4              | 7        |
| Leaves       | 10           | 12        | 11        | 6       | 4              | 7        |

**Table 6:** Zones of inhibitions of water, methanolic and acetone extracts of *Ranunculus muricatus* in millimeters of fungal strains

| Plant's part | Water extract |            | Methanolic extract |            | Acetone extract |            |
|--------------|---------------|------------|--------------------|------------|-----------------|------------|
|              | A. Niger      | C. Albican | A. Niger           | C. Albican | A. Niger        | C. Albican |
| Root         | 0             | 1          | 2                  | 2          | 1               | 1          |
| Stem         | 0             | 1          | 1                  | 2          | 0               | 1          |
| Leaves       | 1             | 1          | 1                  | 2          | 1               | 1          |

buffers (pH= 4 and 9). The sample in form of juice was taken in 50ml beaker and pH electrode was inserted in it. The pH was noted from screen when stabilized. After each reading the electrode was wiped out with small piece of cotton soaked in distilled water.

Hardness was measured through complex metric titration with EDTA while electrical conductivity was measured using conductivity meter (HI 99300 HANNA).

**Determination of heavy metals**

For the determinations of heavy metals 1g of root, stem and leaves samples were charred in a crucible for 4 to 6 minutes. After charring, ashing was done by putting plant samples in a furnace for 5h at 600°C in a crucible. After ashing, it was cooled in the desiccators. The cooled contents were dissolved in 2.5ml of 6M HNO<sub>3</sub>. The samples were filtered and the filtrates were kept in plastic bottles for further use. The heavy metals in the samples were determined by atomic absorption spectrophotometer (Hitachi, Model Z-8000 Japan) analysis.

**Determination of inorganic constituents**

The collected plant was dried at 120°C to get a constant weight. It was grinded to fine powder and was subjected to drying ashing. For drying ashing pre-cleaned silica crucible was heated at 600°C to a constant weight. The powdered sample material was heated in a muffle furnace at 600°C till there was no evolution of smoke. The

mixture was cooled at room temperature and was added with deionized water to keep it overnight. The undissolved particles were filtered and the volume of the filtrate was made up to 100 ml. The filtrates were used as sample solution for the determination of inorganic constituents (Pendias, 1986).

Sodium and potassium were determined by flame photometer (Coning-40). Calcium and magnesium were determined by complexometric titration while phosphate was determined by calorimetric method using ammonium dihydrogen phosphatase standard solution and molybdate as complexing agent. The sulphate and bicarbonate were determined by titrimetric method and chloride contents were determined by the standard argentometric method using potassium chromate indicator (Farhat *et al.*, 2011).

**DPPH radical-scavenging activity**

Hydrogen donating or radical scavenging ability of acetic, chloroform and ethanolic fractions of the extracts of *Ranunculus muricatus* stem, leaves and root were measured by using the table DPPH method (Hanato *et al.*, 1988). The different extracts were diluted and 1ml of each diluted extract was added to 0.25ml of a 0.2 mmol/l DPPH methanolic solution. The mixtures were placed in dark at room temperature for 30min. Using UV-Visible spectrophotometer the absorbance of the resulting solution was examined at 517nm. Using the following

equation, the ability to scavenge the DPPH radical was calculated:

$$\text{DPPH scavenging effect (\%)} = \left[ \frac{A_0 - A_1}{A_0} \right] \times 100$$

Where  $A_0$  is the absorbance of the control at 30 min, and  $A_1$  is the absorbance of the sample at 30 min. All samples were analyzed in triplicate.

## RESULTS

### Phytochemicals analysis

The qualitative phytochemical analysis showed the presence of alkaloids, flavonoids, tannins, saponins and phenols. The results of qualitative phytochemical analysis are given in table 1. The qualitative phytochemical analysis results were further confirmed by quantitative phytochemical analysis (table 2).

### Antimicrobial activities

The zones of inhibitions of water, methanolic and acetone extracts in millimeter for 6 different bacterial strains are summarized in tables 3-5 and for 2 fungal strains are given in table 6.

**Table 7:** Physical parameters of *Ranunculus muricatus*

| Names of Parameter | Root  | Stem  | Leaf  |
|--------------------|-------|-------|-------|
| pH                 | 10.35 | 10.4  | 9.9   |
| EC                 | 1142  | 847   | 576   |
| TDS                | 867.8 | 818.3 | 523.4 |
| Hardness           | 210   | 70    | 120   |

**Table 8:** Concentration (mg/Kg) of selected heavy metals in *Ranunculus muricatus*

| Heavy metal | Root  | Stem | Leaves |
|-------------|-------|------|--------|
| Cu          | 0.38  | 0.08 | 0.24   |
| Fe          | 20.05 | 8.19 | 42.40  |
| Pb          | 0.0   | 0.0  | 0.07   |
| Zn          | 1.12  | 0.50 | 0.80   |

**Table 9:** Concentration (mg/Kg) of selected cations and anions in *Ranunculus muricatus*

| Name of Parameter   | Root   | Stem  | Leaves |
|---------------------|--------|-------|--------|
| $\text{HCO}_3^{-1}$ | 130    | 130   | 230    |
| $\text{Ca}^{+2}$    | 12     | 16    | 20     |
| $\text{Mg}^{+2}$    | 43.74  | 7.29  | 17.01  |
| $\text{Cl}^{-1}$    | 441.60 | 338.4 | 115.20 |
| $\text{NO}_3^{-1}$  | 6.1    | 12.1  | 4.1    |
| $\text{SO}_4^{-2}$  | 13     | 12    | 33     |
| $\text{Na}^{+1}$    | 270    | 280   | 170    |
| $\text{K}^{+1}$     | 24     | 33    | 12     |
| $\text{F}^{-1}$     | 0.28   | 0.17  | 0.11   |

### Physical parameters

The various physical parameters are given in table 7.

### Heavy metal contents

The heavy metals contents (mg/Kg) in *Ranunculus muricatus* are given in table 8.

### Inorganic constituents

Table 9 shows the quantitative determination of inorganic constituents of *Ranunculus muricatus*.

**Table 10:** DPPH anti scavenging activities (%) for chloroform, ethanolic and acetone extracts of *Ranunculus muricatus*.

| Plant's Part | Chloroform extract | Ethanolic extract | Acetone extract |
|--------------|--------------------|-------------------|-----------------|
| Root         | 49.57              | 51.5              | 61.3            |
| Stem         | 43.9               | 49.9              | 54.3            |
| Leaves       | 52.6               | 53.5              | 66.9            |

### Antioxidant activities

To evaluate the antioxidant activities of fruits and vegetables numerous analyses such as total antioxidant activity, DPPH and ABTS assays, ROS quenching assay, metal chelating, reductive potential,  $\beta$ -carotene linoleate system and linoleic acid method are the most commonly used.

The antioxidant activities of *Ranunculus muricatus* are given in table 10.

## DISCUSSION

### Phytochemicals analysis

Alkaloids are cyclic organic compound having nitrogen in a negative oxidation state. They have limited distribution in living organism (Hodnick *et al.*, 1988). Alkaloids are used as dyes, spices, drugs or poisons. They are well known for their CNS activities (Cook and Samman, 1996; Yamamoto and Gaynor, 1980). High concentration of alkaloids was found in root followed by stem and comparatively less amount in leaves.

Flavonoids are a group of polyphenolic compounds possessing low molecular weight that exhibit a common benzo-pyrone structure. They show anti-allergic (Cushnie and Lamb, 2005), anti-inflammatory (Xu *et al.*, 2005), antimicrobial and anticancer activities (Oakenfull, 1986). Flavonoids also referred as bioflavonoids, are polyphenol antioxidants found naturally in plants. They are secondary metabolites that have no direct involvement with the growth or development of plants. The effect of flavonoids on plants growth is indirect and associated with the action of auxins. Flavonoids can improve the blood circulation and reduces the blood pressure (Zhang *et al.*, 2001). High amount of flavonoids, as is evident from table 2 was there in stem followed by root and least in leaves.

The saponins are naturally occurring surface- active glycosides. Many pharmacological activities have been

reported about saponins such as antibiotic, antifungal, antiviral, hepatoprotective, anti-inflammatory and anti-ulcer (Hostettmann and Marston, 1995; Ireland and Dziedzic, 1986). Saponins have been reported to have important biological activities in humans including hypocholesterolemia, hemolytic, immunostimulatory and antitumorigenic activities (Fukuda *et al.*, 1995) as well as chemo protective activities (Woldemichael and Wink, 2001). Steroid and triterpenoid saponins with a single sugar chain were found to have strong hemolytic activity, whereas those with two sugar chains showed less activity (Sindambiwe *et al.*, 1998). Some saponins and sapogenins have been shown to be capable of deactivating viruses for example purified saponin mixture from *maesa lanceolata* (Buzzini *et al.*, 2008). The comparatively high percentage of saponins was observed in root, stem and leaves as compared to other phytochemical constituents.

Tannins are basically used for the treatment of inflammation, leucorrhoea, gonorrhoea, burn, piles, diarrhea and as antidote in the treatment of alkaloidal poisoning (Williamson and Manach, 2005). They are also used for tanning of animal hides to convert them to leather. High concentration was there in stem followed by root and leaves.

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 (Mattila and Hellström, 2007). Capsaicin is found in the dried ripe fruit of different species of *Capsicum*. It has been used internally for dyspepsia and flatulence. Externally it is frequently used as counterirritant (Cragg *et al.*, 1999). From table 2 it is clear that the phenols concentration is low in the whole plant. Comparatively high concentration of phenols was there in stem and root.

#### **Antimicrobial activities**

Sustainable amount of new antibiotic available in the market are obtained from natural or semi synthetic resources which are obtained from about 20% of the plants present in world which were submitted to pharmaceutical and biological tests. The chemical compounds with antimicrobial activities isolated from plants have vast remedial power and are useful in the cure of infectious diseases. Several plants byproducts possess antimicrobial activities against pathogenic bacteria and fungi (Bylka *et al.*, 2004).

As can be seen from table 3, high activity 8 mm was recorded for leaves water extracts against *S. Aureus* and *M. Luteus* while less activity 3mm was seen against *P. Aeruginosia* and *S. Typhi*. The leaves extracts showed highest inhibition zones as compared to root and stem extracts. Same trend was observed for methanolic and acetone extracts tables 4 and 5). Methanolic extracts exhibit highest zones of inhibitions followed by acetone extracts as compared to water extract.

From table 6 it is evident that methanolic extracts showed highest zones of inhibition for the two fungal strains followed by acetone extracts. Water extracts are comparatively less potent against the fungal strains.

#### **Physical parameters**

The pH of root, stem and leaves are at alkaline side with highest 10.35 for root extract. The electrical conductivity was highest for root extract followed by stem extract. Highest TDS value was recorded for root extract followed by stem extract. Hardness was high for root followed by leaf extract.

#### **Heavy metal contents**

Some of the heavy metals are needed for the normal growth of plants and animals while others are toxic to both of them. From table 8 it is evident that iron is present in highest concentration ( $42.40\text{mgKg}^{-1}$ ) in leaves as compared to root and stem. Iron is an essential trace element required by all forms of life. In man it is required for the synthesis of haem proteins which function in the process of oxygen transport and oxidative metabolism. The total body iron for an adult male has been estimated to be about 4g and for the female 2.5g. The requirements for growth have been estimated to be 30mg/kg body weight. In rats an oral administration of 60-100mg/kg body causes toxicological effects. The iron contents present in the samples are below the toxic level (Odell and Sunde, 1997).

Zinc is an essential element in the nutrition of man, animals and plants. It acts as an integral part of numerous enzymes. Because of its essentiality, zinc is present in all plant and animal tissues. The total body zinc for a 70kg individual has been estimated to be 2.3g. Zn contents were very low as compared to iron contents. Highest concentration of Zn  $1.12\text{mgKg}^{-1}$  was recorded in root followed by leaves and stem. The Food and Nutrition Board of the United States recommendations for dietary allowances for zinc are as follows: infants 0-0.5 years, 2 mg and 0.5-1.0 years, 5 mg; children 1-10years, 10mg; men and women 11-51+ years, 15mg; pregnant women, 20mg and lactating women, 25mg. Similar figs have been recommended by WHO/FAO (Odell and Sunde, 1997; WHO, 2004).

Lead is toxic to both plants and animals. Its maximum acceptable limit in foodstuffs is  $1\text{mgKg}^{-1}$ . In root and stem samples the concentration of lead was beyond the detectable limit. However in leaves its concentration was  $0.07\text{mgKg}^{-1}$  which is below the acceptable limit for food stuffs (Odell and Sunde, 1997).

Copper is an essential component of certain enzymes and is required for normal growth and development of plants and animals. The essential role of copper in maintaining normal health in both animals and humans has been recognized for many years. The average daily dietary

requirement for copper in the adult human has been estimated at 2 mg and for infants and children at 0.05mg/kg body weight (WHO, 2004).

It can be toxic at excessive level. Phytotoxicity can occur at a concentration higher than 20mgKg<sup>-1</sup> of the dry weight of the plant. The concentration of copper in *Ranunculus muricatus* is below the phytotoxic level.

#### **Inorganic constituents**

All inorganic constituents shown in table-9 are vital to growth of the plant and animals. Some of them are growth initiators while others are enzyme activators. Calcium strengthens the bones and its importance almost becomes double during pregnancy. It also helps in blood coagulation. Potassium activates certain enzymes, whereas iron is integral part of hemoglobin. Inorganic constituents are essential in one way or the other to life and their importance cannot be neglected at any level.

Bicarbonates and Ca<sup>+</sup> were present in leaves while high concentration of Cl<sup>-1</sup> and Mg<sup>+2</sup> were observed in root of the plant. NO<sub>3</sub><sup>-1</sup> and SO<sub>4</sub><sup>-2</sup> amount were high in stem and leaves respectively. Na<sup>+1</sup> and K<sup>+1</sup> content were high in stem while highest F<sup>-1</sup> concentration was recorded for root.

#### **Antioxidant activities**

DPPH is a free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.*, 1997). The reduction capability of DPPH radical was determined by the decrease in absorbance induced by plant antioxidants (Ksouri *et al.*, 2009). BHT was a synthetic phenolic used as positive standard with a maximum absorption at 517nm that can readily undergo scavenging as an antioxidant (Lu and Yeap Foo, 2001), this pure antioxidant has been widely used to evaluate the antiradical activity of various samples.

From table 10 it is evident that highest scavenging can be obtained for acetone extracts followed by ethanolic. In case of leaves highest antioxidant activity was recorded for chloroform extract. Same trend was recorded for ethanolic and acetone extracts.

#### **CONCLUSION**

In the present study root, stem and leaves of *Ranunculus muricatus* were analyzed for phytochemicals and minerals. The presence of saponins, tannins, phenols, flavonoids and alkaloids were confirmed by qualitative and quantitative phytochemical analysis. Saponins were present in high amount as compared to alkaloids, tannins, phenols and flavonoids. Inorganic and heavy metals constituents were also determined. High amount of iron followed by zinc was observed in the sample plant. The

lead concentration in the samples was below the acceptable limit for the food stuffs (1mgKg<sup>-1</sup> body weight). The physical parameters, antioxidant and antimicrobial activities of the extracts were also determined. Highest antioxidant activities was observed for acetone extract fraction as compared to ethanolic and chloroform extract fractions. Highest zone of inhibitions were observed for methanolic extract followed by acetone extract fraction against *micrococcus luteus*. Antifungal activities were high for methanolic extracts against *Candida Albican*. Phytochemicals, antioxidant, antimicrobial and minerals analysis in the present study showed that *Ranunculus muricatus* can be used as medicine due to its high antioxidant, antimicrobial activities, presence of essential phytochemical and inorganic constituent.

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