

Full Length Research Paper

Phytochemical analysis of selected medicinal plants

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Four medicinal plants including *Ranunculus arvensis*, *Equisetum raven*, *Carthamus lanatus* and *Fagonia critica* were used for the study. All the plants were biologically active and were used for different types of ailments. Keeping in view their importance, this work was carried out to investigate the quantitative determination of their crude phytochemicals, vitamins and protein contents. The quantitative determination of crude phytochemicals (alkaloids, total phenols, flavonoids and saponins) vitamins (riboflavin, vitamin C, niacin and pectin) and protein were determined in the aforementioned herbs. The phytochemicals including alkaloids, total phenols, flavonoids and saponins were determined quantitatively using literature methods. Vitamins were measured using a UV/ visible spectrophotometer (UV- 1601 Shimadzu) and the protein was determined by the Micro Kjeldahl's method (Horwitz et al., 2000). The studied plants showed variable amounts of phytochemicals, vitamins and protein contents. The study is very important, in that it intended to show the contents of the studied medicinal herbs and also provide a scientific data base line which is of particular importance for the local practitioners as well as for the local people using these herbs for a variety of body disorders.

Key word: Phytochemical analysis, medicinal plants, Pakistan.

INTRODUCTION

In Pakistan, many herbaceous plants are used as food and medicine. These herbaceous plants have been traditionally used for medicinal properties since a long time ago. Fruits and vegetables values have been greatly known for their numerous healthful properties. Among the various evidence revealing that medicinal and culinary herbs have some endemic species, a diet rich in fruits and vegetables and phytochemicals which decrease the risk of cardiovascular diseases and some forms of cancer are of particular interest (Javanmardi et al., 2003).

These herbaceous plants including *Fagonia critica* and

Carthamus lanatus have a wide range of biological, health care and diseases protection activities such as pharmacological, anti-bacterial and anti-fungal activities (Okwu et al., 2003). Extracts of fruits, herbs, vegetables and other plants' part rich in flavonoids, phenolic acid, vitamins and other phytochemicals, are used in the preparation of traditional medicine in the treatment of liver cirrhosis, hepatitis and are also used to improve the quality of nutritional foods (Ogu and Agu, 1995).

The active constituents of these plants and herbs suggest the presence of phytochemicals, vitamins, minerals and anti-microbial constituents in their tissues.

Many herbaceous and medicinal plants contain important phytochemicals and vitamins such as alkaloids, flavonoids, tannins, cyanogenic glycosides, phenolic compounds, saponins, lignins and lignans and vitamin C,

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vitamin E and carotenoid which are utilized both by humans and animals as important components of diets (Okwu et al., 2005).

The potential of the phytochemicals have large scale pharmacological and biological activities such as antioxidant constituents (hydrolysable tannins, phenolic acid and flavonoids) of the plant materials for the care of health and protection from coronary heart diseases, cancer, anti-carcinogenic and anti-mutagenic effects (Loliger, 1991). Varieties of herbaceous vegetables are protective against various diseases, particularly cardiovascular diseases. These herbaceous plants and species are harmless sources for obtaining natural antioxidants. Antioxidant constituents can delay or inhibit the oxidation of lipids and other compounds by inhibiting the propagation of oxidation chain reaction (Kim et al., 1994).

Primarily, antioxidant effect is due to phenolic compounds such as phenolic acid, flavonoids and phenolic diterpenes and their mode of action for antioxidant compounds is due to its redox reaction properties which can absorb and neutralize free radicals by quenching singlet and triplet oxygen (Krauss et al., 2000).

Vitamins are organic substances necessary for metabolism. Human diet does not always contain the required amount of vitamins for the normal growth and maintenance of the body function and as such cannot produce enough quantity for their body metabolism, so it can be obtained from fruits, vegetables and foods. Deficiency of vitamins can cause serious human health diseases and sometimes, very small concentrations are required for maintenance of good human health (Hussian et al., 2006).

Keeping in view the importance of phyto-chemicals, vitamins and other important constituents of these medicinal plants and their wide use by the local community and practitioners for a variety of ailments, it is therefore very vital to discern their active constituents and to provide a scientific data base line which may play a significant role in knowing the quantities of these phytochemicals, vitamins and other constituents.

MATERIALS AND METHODS

Plant material

Four different medicinal plant samples such as *Ranunculus arvensis*, *Equisetum ravenis*, *Carthamus lanatus* and *Fagonia critica* were collected from Peshawar (Pakistan). The plant samples were rinsed with tap water and then with de-ionized water. It was dried, chopped, crushed and powdered with electrical grinder and then the dried powdered samples were stored in polyethylene bottles for further processes.

Phytochemical determination

Determination of alkaloids

For alkaloids determination, 5 g of each sample was weighed into a

250 ml beaker, and 200 ml of 20% acetic acid in ethanol was added and was covered to stand for 4 h. This was filtered and the extract was concentrated using a water bath to evaporate one-quarter of the original volume. The concentrated ammonium solution was added drop-wise to the extract until the precipitation was completed. The entire solution was allowed to settle and the precipitate was collected by filtration, after which it was weighed (Obadoni et al., 2001).

Determination of total phenols

To determine the total phenols, 5 g of the plant sample was weighed into a 250 ml titration flask and 100 ml n-hexane was added twice for 4 h each; the filtrates were discarded for fat free sample preparation. Then, 50 ml diethyl ether was added twice, was heated for 15 min each, was cooled up to room temperature and was filtered into a separating funnel. About 50 ml of the 10% NaOH solution was added twice and shook well each time to separate the aqueous layer from the organic layer. It was washed three times with 25 ml de-ionized water. The total aqueous layer was acidified up to pH 4.0 by adding 10% HCl solution and 50 ml dichloro methane (DCM) twice to acidify the aqueous layer in the separating flask. Consequently, the organic layer was collected, dried and then weighed.

Determination of flavonoids

To determine flavonoids, 5 g of each plant sample was weighed in a 250 ml titration flask, and 100 ml of the 80% aqueous methanol was added at room temperature and shaken for 4 h in an electric shaker. The entire solution was filtered through Whatman filter paper no. 42 (125 mm) and again, this process was repeated. The filtrate as a whole was later transferred into a crucible and evaporated to dryness over a water bath and weighed (Boham et al., 1994).

Determination of saponins

For the saponins determination, 5 g of each plant samples was weighed and was dispersed in 100 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The filtrate and the residue were re-extracted with another 100 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and about 30 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated in percentage (Obadoni et al., 2001).

Determination of riboflavin

To determine riboflavin, 5 g of the sample was extracted with 100 ml of 50% ethanol solution and shaken for 1 h. This was filtered into a 100 ml flask, while 10 ml of the extract was put into 50 ml volumetric flask. 10 ml of the 5% potassium permanganate and 10 ml of the 30% H₂O₂ were added and allowed to stand over a hot water bath for about 30 min. Subsequently, 2 ml of the 40% sodium sulphate was added. This was made up to 50 ml mark and the absorbance was measured at 510 nm in a UV/visible spectro-

Table 1. Phytochemicals composition of the four plant samples on dry weight basis expressed as mg/100 g dry weight.

Plant name	Alkaloid	Phenol	Flavonoid	Saponin
<i>R. arvensis</i>	0.2579 ± 0.007	0.848 ± 0.0072	1.7698 ± 0.022	2.491 ± 0.02
<i>E. ravens</i>	0.3985 ± 0.013	1.068 ± 0.018	1.034 ± 0.008	1.607 ± 0.012
<i>C. lanatus</i>	0.176 ± 0.0013	1.251 ± 0.017	0.5617 ± 0.0013	2.516 ± 0.016
<i>F. critica</i>	0.226 ± 0.0068	1.438 ± 0.013	0.9863 ± 0.0026	0.823 ± 0.004

photometer (UV- 1601 SHIMADZU) (Okwu et al., 2006).

Determination of ascorbic acid

Accurately, 1 g of each sample was weighed in a 25 ml conical flask. Then 10 ml of the oxalic acid (0.05 M)-EDTA (0.02 M) solution was added and placed in the sample for 24 h, to provide the required reaction time. After 24 h, the samples were filtered through 0.45 µm filter paper. Then 2.5 ml of each sample was transferred to a separate 25 ml volumetric brown flask, after which 2.5 ml of the oxalic acid (0.05 M)-EDTA (0.02 M) solution was added. Subsequently, meta phosphoric acid was added separately with acetic acid (0.5 ml), sulphuric acid (5% v/v) solution (1 ml) and ammonium molybdate solution (2 ml) in each volumetric brown flask and the volume was made up to 25 ml with distilled water. The absorbance was measured at 760 nm on a UV/visible spectrophotometer (Bajaj et al., 1981; Hussian et al., 2006; Zeng et al., 2005).

Determination of niacin

5 g of the sample were treated with 50 ml of 1 N sulphuric acid and was shaken for 30 min. Three drops of the ammonia solution were added to the sample and was then filtered. Afterwards, 10 ml of the filtrate was added into a 50 ml volumetric flask and 5 ml of 0.02 N H₂SO₄ absorbance was measured in the spectrophotometer at 470 nm (Okwu et al., 2006).

Determination of pectin

To determine pectin, 5 g of the plant sample was dispersed in 40 ml water in a beaker, and was boiled gently for one hour. The content of the beaker was transferred to a 50 ml volumetric flask and diluted up to the mark with distilled water. The contents of the flask were shook well and filtered into a 50 ml volumetric flask, after which 10 ml of the filtrate was taken into 80 ml beaker, where 30 ml de-ionized water and 1 ml sodium hydroxide were added to it along with a constant string. It was then allowed to stand overnight.

To this solution, 5 ml of 1 N acetic acid was added with continuous stirring and was allowed to stand for 5 min. Then 1 N and 2.5 ml of the calcium chloride solution were allowed to stand for one hour and the solution was boiled for one minute. The solution was filtered through Whatman No 41 filter paper and washed with hot de-ionized water until the solution become free from chloride. The residue was transferred to the previously weighed watch glass. The watch glass, along with the residue, was placed on a water bath and dried in the oven at 100°C until a constant weight was obtained (Horwitz et al., 2000).

Determination of protein

Protein was determined by the Micro KJELDAHL'S method (Horwitz et al., 2000).

RESULTS AND DISUSSION

The phytochemical quantitative composition of *R. arvensis*, *E. ravens*, *C. lanatus* and *F. critica* are shown in Table 1. Alkaloids were observed in higher quantity in *E. ravens* (0.399 mg/100 g) than the other three plant samples, while *C. lanatus* (0.176 mg/100 g) was found to contain the least amount of alkaloids. Phenolic constituents were found to be higher in concentration than the alkaloids and flavonoids, in that the phenolic contents were detected to have high concentration in *F. critica* (1.438 mg/100 g) and less concentration in *R. arvensis* (0.848 mg/100 g). Alkaloids and flavonoids values in these medicinally important plants were observed in small concentration than the phenolic and saponins constituents.

High quantity of flavonoid was detected in *R. arvensis* (1.769 mg/100 g), while *C. lanatus* had the least flavonoid (0.562 mg/100 g). The amount of saponins (2.516 mg/100 g) was recorded to be high for *C. lanatus*, while *F. critica* contained the least amount of saponins (0.823 mg/100 g).

The biological function of alkaloids and their derivatives are very important and are used in analgesic, anti-spasmodic and bactericidal activities. However, alkaloids are mainly observed in large amount in flowering plants and they have an important physiological effect on mankind. Morphine, quinine, ephedrine, nicotine and strychnine are the major types of alkaloids. In these types, morphine and codeine are narcotic analgesics as well as are anti-tussive agent (Stary et al., 1998).

Flavonoids are water soluble phytochemical and an important plant phenolic. They show antioxidant activities and they have the property of preventing oxidative cell damage and carcinogenesis. They have anti cancer, anti inflammatory activities and a large effect in lower intestinal tract and heart disease. Flavonoids as antioxidants from *R. arvensis*, *E. ravens*, *C. lanatus* and *F. critica* provide anti-inflammatory action (Farquar et al., 1996).

These plants show high constitutions of phenols and phenolic compounds which imply that they may be used as anti-microbial agents. Table 1 shows that *C. lanatus* and *F. critica* have high amount of phenol. This is the reason why these plants are being used for skin diseases. Phenols and phenolic compounds are greatly used in skin infections and other wounds treatment and also for healing, when compared to other bactericides (Okwu et al., 2001). Phenolic compounds have an electron donor

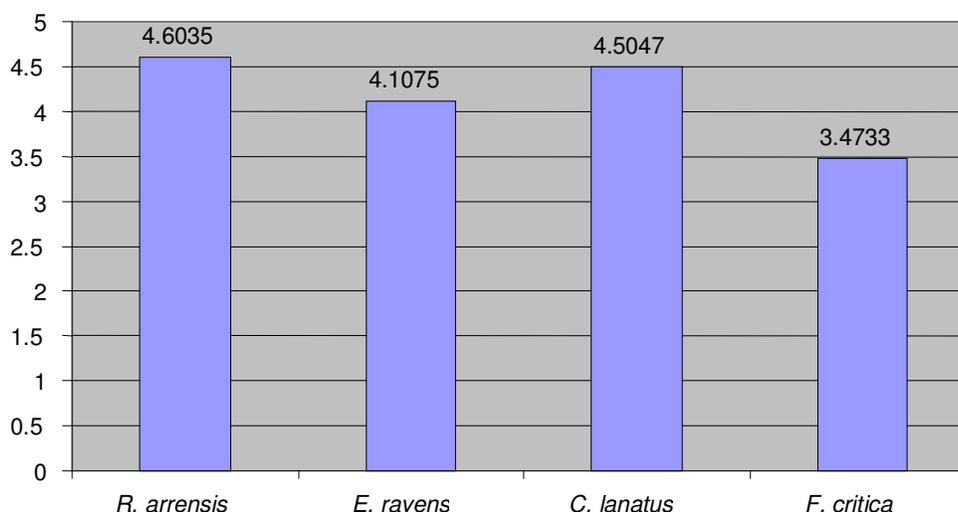


Figure 1. Total amount of phytochemical in the four plant samples.

Table 2. Vitamins constituents of the four plant samples on dry weight basis expressed as mg/100 g.

Plant name	Riboflavin (B-2)	Niacin (B-5)	Ascorbic acid
<i>R. arvensis</i>	0.4586 ±0.002	0.5371 ±0.0028	15.415 ±0.183
<i>E. ravens</i>	0.3939 ±0.0013	0.2849 ±0.001	9.326 ±0.036
<i>C. lanatus</i>	1.0726 ±0.0016	1.0061 ±0.0022	17.587 ±0.13
<i>F. arabica</i>	0.9143 ±0.0024	0.0847 ±0.0017	12.644 ±0.07

Table 3. Pectin and protein constituents of the four plant samples on dry weight basis expressed as mg/100 g.

Plant name	Pectin	Protein
<i>R. arvensis</i>	0.0815 ±0.000	0.3518 ±0.0013
<i>E. ravens</i>	0.1137 ± 0.0003	0.4025 ±0.0014
<i>C. lanatus</i>	0.0259 ±0.0008	0.2857 ±0.0015
<i>F. critica.</i>	0.0402 ±.0007	0.5338 ±0.0019

capability; moreover, due to this ability, phenolic compounds are readily oxidized to form phenolate ion or quinone, which is an electron acceptor. Phenols and phenolic compounds modify the prostaglandin pathways and due to this action, they prevent platelets from clumping and have the ability to block specific enzymes that cause inflammation; antioxidant, immune enhancers, anti clotting and hormone modulators.

The presence of saponins in higher concentration in the four plants as compared to other phytochemicals, gave a justification why the extracts from these plants are used in wound healing and bleeding treatment. Saponins have properties of precipitating and coagulating red blood cells and they also have cholesterol binding properties, formation of foams in aqueous solutions and hemolytic activity (Sodipo et al., 2000). The comparative values of

the total amount of phytochemicals in these plants are shown in Figure 1. It is clear from the figure that they are very much rich in phytochemicals such as alkaloids, phenols, flavonoids and saponins. As discussed earlier, the presence of these phytochemical constituents showed that these plants may be used as a basic medicinal agent such as analgesic, antispasmodic, anti-bacterial, anti cancer, anti inflammatory and anti oxidant.

The result of vitamins analysis obtained from these plant samples are shown in Table 2. It showed that these plants were rich in vitamins. Vitamin C (ascorbic acid) was found in higher concentration than riboflavin (B2) and niacin (B5). The concentrations of vitamins C was detected to be high in *C. lanatus* (17.587 mg/100 g), while lower concentration of ascorbic acid (vitamin C) was found in *E. arvensis* (9.326 mg/100 g).

The content of riboflavin (B2) was found to be high (1.07 mg/100 g) in *C. lanatus* followed by *F. critica* (0.91 mg/100 g). The concentration of other vitamins was also found to be high, except for niacin trace amount in all the studied plant samples. The concentration of niacin was recorded as 1.01 mg/100 g in *C. lanatus* and 0.0847 mg/100 g in *F. critica*. It can be deduced from the analysis in Table 2 that *C. lanatus* is rich in riboflavin, niacin and ascorbic acid, while *E. ravens* has a small amount of these vitamins. The amounts of pectin and protein obtained in these plants are shown in Table 3.

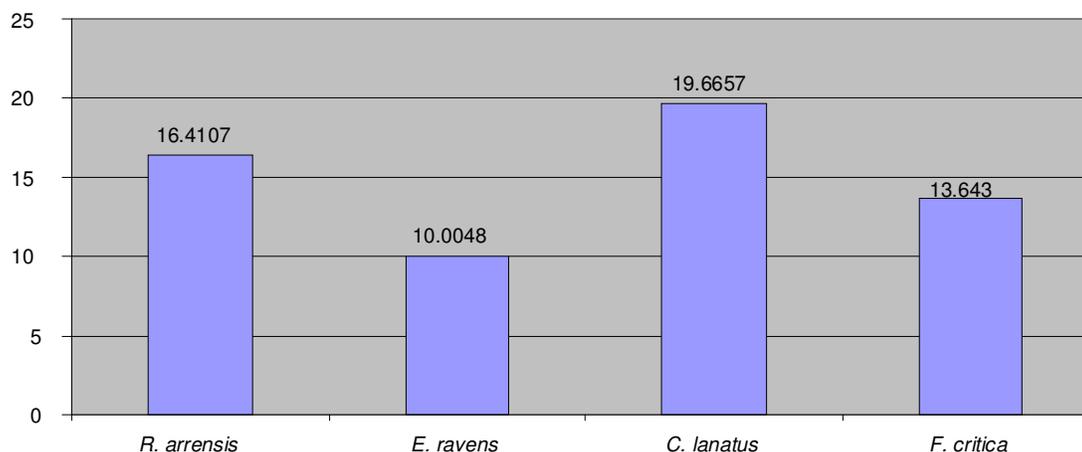


Figure 2. Total amount of vitamins in the four plant samples.

High quantity of pectin was observed in *E. ravens* (0.11 mg/100 g), while lesser amount of pectin was detected in *C. lanatus* (0.03 mg/100 g). On the other hand, protein was detected in high concentration than pectin. However, *F. critica* showed a large amount of protein (0.53 mg/100g), while less concentration was observed in *C. lanatus* (0.29 mg/100 g).

Vitamins are important and their deficiencies cause adverse effects on the metabolism of the human body and even in a trace amount, they are very essential for the body metabolism. The combined comparative values of vitamins which are shown in Figure 2, indicated that *C. lanatus* had high quantity of vitamins (19.67 mg/100 g) and it justified the ability to use the extract as a source of these vitamins.

Riboflavin, niacin and ascorbic acid are the water soluble vitamins. Vitamin C (ascorbic acid, ascorbate, sodium ascorbate and calcium ascorbate) is synthesized in the liver in some mammals, while humans and some other species are unable to synthesize vitamin C (Jain et al., 1988).

Sufficient amount of vitamin C in the diet is important for the body, in that its deficiency causes scurvy disease. In the scurvy disease, defective collagen is formed, leading to a disease of connective tissue. The ascorbates have also anti-oxidant ability to prevent the formation of carcinogenic substances. Carcinogenic compounds, such as nitroso compounds, are formed from oxidation of nitrate which is oxidized to nitrite and the nitrites react with certain amino compounds to form nitroso compounds; ascorbate can therefore prevent the oxidation of nitrate (Robert et al., 1997).

Plants are a good source of riboflavin, niacin and ascorbic acid. Niacin is a constituent in two pyrimidine nucleotide coenzymes, NAD and NADP, and it is active in preventing pellagra disease in humans and 'black' tongue disease in dogs (Jain et al., 1988).

It can be seen from the results of Table 3 that *C. lanatus* gave the least amount of pectin and protein

among the studied plants. Generally, pectin was higher in *E. ravens* while protein was higher in *F. critica*.

Protein is a complex nitrogen containing organic compounds which are found in all animal and plant cells, characterized by the presence of peptide bonds and formed by the polymerization of amino acids. On the other hand, pectin is a heterosaccharide and is found in plant cells (Farquar et al., 1996). Pectin is used for the treatment of constipation and diarrhea and it is an anti-atherogenic and anti-obesity agent which also reduces the risks of heart and artery diseases (Mulder et al., 1923).

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