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Full Length Research Paper

Analysis of chemical constituents in medicinal plants of selected districts of Pakhtoonkhwa, Pakistan

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Alkaloids, flavonoids and saponins were determined in the *Teraxacam officinale*, *Cichorium intybus* and *Figonia tritica*. Quantative determinations of crude alkaloids, flavonoids and saponins (g kg^{-1}) and their percentages (%) were determined in *C. intybus*, *T. officinale* and *F. critica* collected from Kohat, Mardan, Nowshera and Peshawar regions. Thin layer chromatography (TLC) study of the alkaloids, flavonoids and saponins were carried out with different solvent systems and color of the spot and Rf value of each constituent was determined. Among the samples of *C. intybus*, relatively higher contents of alkaloids (14 g kg^{-1}) were found in the sample collected from Mardan region, followed by the sample gathered from Nowshera which is equal to 13.8g kg^{-1}. Higher contents of flavonoids were found in *C. intybus* collected from Kohat region which was 23.48 g kg^{-1}. The other remaining samples of *C. intybus* ranged in the decreasing order of 19.7, 17.2 and 10.0 g kg^{-1} for the ones collected from Nowshera, Mardan and Peshawar, respectively. The overall saponin contents remained almost low as compared to the alkaloid and flavonoid contents.

Key words: Alkaloids, flavonoids, saponins, TLC study, medicinal plants.

INTRODUCTION

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms (Huffman, 2003). The name is derived from the word alkaline and is used to describe any nitrogen-containing base and organic compounds with one or more of the following features: a heterocyclic compound containing nitrogen, with an alkaline pH and a marked physiological action on animal physiology. However, there are exceptions to each of these criteria. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, animals and part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid-base extraction method. Some alkaloids are toxic to other organisms. They often have pharmacological effects and are used for medications as recreational drugs. Examples are the local anesthetic, stimulant cocaine and caffeine, nicotine, analgesic morphine and antimalarial drug quinine. Most alkaloids have a bitter taste. Low-molecular weight alkaloids (nicotine, spartine, coniine and phenethylamine) are often liquid at room temperature. The basicity of alkaloids depends on the lone pairs electrons on their nitrogen atoms. As organic bases, alkaloids form salts with mineral acids such as hydrochloric acid, sulfuric acid and organic acids such as tartaric acid or maleic acid. These salts are usually more water-soluble than their free

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base form.

Flavonoids are widely distributed in plants, fulfilling many functions including producing yellow or red/blue pigments in flowers and protection from attacks by microbes and insects. The widespread distribution of flavonoids, their variety and relatively low toxicity as compared to other active plant compounds (for instance alkaloids) mean that many animals, including humans, ingest significant quantities in their diet. Flavonoids have been referred to as "nature's biological response modifiers" because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses and carcinogens. They also show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity.

Saponins are glycosides which occur in a wide variety of plants and are characterized by the foaming action in aqueous solution. It is this property that accounts for their main uses. There are two classes of saponins: triterpenoids and the steroidal, which differ in the structure of the sapogenin portion of the glycoside (Cindy and Houghton, 2002). The commercially important saponins are derived from soapbark, soaproot, soapberries or soapnuts and soapwort. The free saponin is a yellowish white, amorphous and very hygroscopic powder that causes sneezing and irritates the mucous membranes (Jann, 2004; Hutchings et al., 2003). It forms a colloidal solution in water, slightly soluble in cold ethyl alcohol, more soluble in hot ethyl alcohol and readily soluble in methanol. It is insoluble in ether, chloroform and benzene (Vijver et al., 2001). The triterpenoid saponins are used in the manufacture of acoustic tile, photographic plates, films and papers, ceramics, foam fire extinguishers and toothpaste; they are used to produce foam in beverages (soft drinks and beer), shampoos, liquid soaps and cosmetic preparation; and to emulsify oils for fruit tree sprays. They are also used in the determination of oxygen in blood (Broyer and Paull, 1997; Aggarwal et al., 2007; Malani and Ichikawa, 1998). In plants, saponins may serve as anti-feedants and protect the plant against microbes and fungi (Malani and Ichikawa, 1998; Aggarwal et al., 2007). Some plant saponins (from oat and spinach) may enhance nutrient absorption and aid in animal digestion. Saponins have also been used as adjuvant in vaccines (Ron and Pia, 2001; Shridhar and Girish, 2001).

MATERIALS AND METHODS

Sampling area

All the three plants, *T. officinale*, *C. intybus* and *F. critica* were collected from different areas of N.W.F.P including Nowshera, Peshawar, Kohat and Mardan. After collecting the plants, they were washed with distilled water to remove the dusts, dried in shade, crushed and stored in closed bottles for further processing. The powdered plant materials were tested for their phytochemical properties (alkaloids, saponins and flavonoids). The plant samples showed variable amount of alkaloids, flavonoids and saponins.

Determination of alkaloids

200 ml of 20% acetic acid in ethanol was added to 5 g of the plant sample. After 24 h, it was filtered and the process was repeated three times. The filtrate was concentrated to 40 ml by rotary apparatus and ammonium hydroxide (NH_4OH) was added drop by drop for precipitation purposes. The precipitate was collected on the pre-weighted filter paper and the weight of the crude alkaloids was determined.

TLC study of alkaloids

1 g of each sample of the powdered plant materials was wet with a half diluted NH_4OH and placed in EtOAc solvent for 24 h at room temperature. The organic phase was separated from the acidified filtrate and basified with NH_4OH (pH 11 to 12). It was extracted with chloroform three times, concentrated by evaporation and used for chromatography. The alkaloid spots were separated using the solvent mixture chloroform and methanol (15:1). The colour and Rf values of the separated alkaloids were recorded both under ultraviolet at 254 and 359 nm and visible light after spraying with Dragendorff's reagent.

Determination of flavonoids

10 g of the plant sample was put in 80% aqueous methanol (200 ml) for 24 h. The same process was repeated thrice. The solution was filtered and concentrated using rotary apparatus. The concentrated filtrate was put in the vials (of known weight) and dried on the water bath at 70°C. The weights of crude flavonoids were calculated.

TLC study of flavonoids

1 g of each sample of the powdered plant materials was extracted with 10 ml methanol on water bath (60°C/5 min). The filtrate was concentrated, and a mixture of water and EtOAc (10:1 ml) was added. The EtOAc phase thus retained was used for thin layer chromatography. The flavonoids spots were separated using chloroform and methanol (19:1) solvent mixture and the spots were recorded under ultraviolet (359 nm).

Determination of saponins

Plant sample of 10 g was put in 100 ml of 20% aqueous ethanol for 24 h. The whole solution was filtered and further, 100 ml of 20% ethanol was added to the plant residue and put on the water bath at 60°C for 30 min. The solution was filtered, and concentrated to 40 ml using rotary apparatus. Then, 20 ml of di-ethyl ether was added and shaken vigorously. Two layers were produced. Di-ethyl layer was discarded and 60 ml of n-butanol was added to the aqueous layer and after shaking, n-butanol layer was recovered. Then, 10 ml of 5% aqueous sodium chloride solution was added to the combined n-butanol extracts. The n-butanol layer containing saponins were concentrated to dryness by rotary apparatus. The weight of crude saponins was calculated.

TLC study of saponins

1 g of each sample of the powdered plant materials was extracted with 10 ml of 70% EtOH by refluxing for 10 min. The filtrate was condensed, enriched with saturated *n*-BuOH and thoroughly mixed. The butanol was retained, condensed and used for chromatography.

Table 1. Quantitative determinations of crude alkaloids (g kg^{-1}) in *C. intybus, T. officinale* and *F. critica* collected from Kohat, Mardan, Nowshera and Peshawar regions.

S/N	Plant code	Weight of plant (g)	Weight of crude alkaloid (g)	Percentage
1	C-K	5	0.39	7.8
2	C-M	5	0.7	14
3	C-N	5	0.69	13.8
4	C-P	5	0.63	12.6
5	T-K	5	0.14	2.8
6	T-M	5	0.69	13.8
7	T-N	5	0.19	3.8
8	T-P	5	0.30	06
9	F-N	5	0.24	4.8

C, *Cichorium intybus*; T, *Taraxacum officinale*; F, *Figonia critica*; K, Kohat; M, Mardan; N, Nowshera; P, Peshawar.

Table 2. Quantitative determination of crude flavonoids (g kg^{-1}) in *C. intybus*, *T. officinale* and *F. critica*, collected from Kohat, Mardan, Nowshera and Peshawar regions.

S/N	Plant code	Weight of plant (g)	Weight of crude flavonoid (g)	Percentage
1	C-K	10	2.35	23.48
2	C-M	10	1.72	17.2
3	C-N	10	1.97	19.7
4	C-P	10	1.0	10
5	T-K	10	1.56	15.6
6	T-M	10	1.88	18.8
7	T-N	10	1.47	14.7
8	T-P	10	1.12	11.2
9	F-N	10	1.65	16.5

C, *Cichorium intybus*; T, *Taraxacum officinale*; F, *Figonia critica*; K, Kohat; M, Mardan; N, Nowshera; P, Peshawar.

The saponins were separated using solvent mixture of chloroform, glacial acetic acid, methanol and water (64:16:12:8). The colour and RF values of these spots were recorded by exposing chromatogram to the iodine vapors.

RESULTS AND DISCUSSION

The alkaloids, flavonoids and saponins determined in *T. officinale*, *C. intybus* and *F. tritica* are given in the Tables 1 to 3. The TLC study of alkaloids, flavonoids and saponins are given in Tables 4 to 6. The graphical representation of crude alkaloids, flavonoids and saponins in *C. intybus, T. officinale* and *F. critica* collected from Kohat, Mardan, Nowshera and Peshawar regions is shown in Figures 1 to 3.

In Table 1, high contents of alkaloids (14.0 g kg^{-1}) among the samples of *C. intybus* plant was found in the sample collected from Mardan region, followed by the sample gathered from Nowshera which is equal to 13.8 g kg^{-1}. The *C. intybus* from Peshawar yielded 12.50 g kg^{-1} which is higher than the *C. intybus* collected from Kohat (7.8 g kg^{-1}). *T. officinale* secured from Mardan yielded 13.8 g kg^{-1} which was higher than same samples collected from Peshawar, Nowshera and Kohat; the corresponding yields were 6.0, 3.8 and 2.8 g kg^{-1}, respectively. *F. critica* from Nowshera region yielded alkaloids equal to 4.8 g kg^{-1}.

In Table 2, higher contents of flavonoids were found in *C. intybus* collected from Kohat region which was 23.48 g kg^{-1}. The other remaining samples of *C. intybus* ranged in the decreasing order of 19.7, 17.2 and 10.0 g kg^{-1} from Nowshera, Mardan and Peshawar, respectively. The *T.*

Table 3. Quantitative determination of crude saponins (g kg^{-1}) in *C. intybus, T. officinale* and *F. critica* collected from Kohat, Mardan, Nowshera and Peshawar regions.

S/N	Plant code	Weight of plant (g)	Weight of crude saponin (g)	Percentage
1	C-K	10	0.9	09
2	C-M	10	0.5	05
3	C-N	10	0.4	04
4	C-P	10	0.4	04
5	T-K	10	0.4	04
6	T-M	10	0.2	02
7	T-N	10	0.3	03
8	T-P	10	0.3	03
9	F-N	10	0.6	06

C, *Cichorium intybus*; T, *Taraxacum officinale*; F, *Figonia critica*; K, Kohat; M, Mardan; N, Nowshera; P, Peshawar.

Table 4. TLC study of alkaloids.

S/N	Name of plant	Color of the spot	R_f value(s)	Plant parts tested
1	*Cichorium intybus*	-	-	All
2	*Teraxacum officinale*	-	-	All
3	*Figonia critica*	Light green	0.91	All

Table 5. TLC study of flavonoids.

S/N	Name of plant	Color of the spot	R_f value(s)	Plant parts tested
1	*C. intybus*	-	-	All
2	*T. officinale*	-	-	All
3	*F. critica*	Blue	0.94	All

Table 6. TLC study of saponins.

S/N	Plant name	Color of the spot	R_f value(s)	Plant parts tested
1	*C. intybus*	Red	0.87	All
		Yellow	0.80	
		Light Green	0.6	
		Pink	0.53	
2	*T. officinale*	Deep yellow	0.8	All
3	*F. critica*	Red	0.98	All
		Green	0.72	
		Green	0.46	

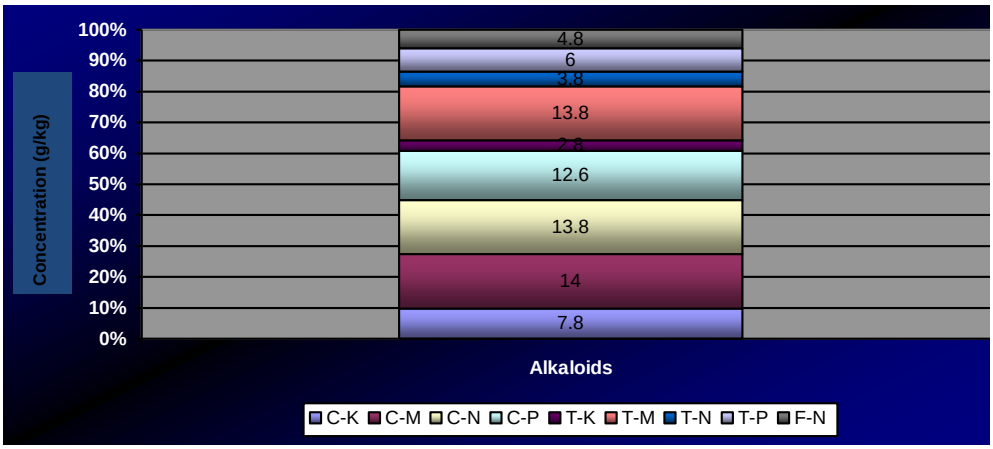

Figure 1. Graphical representation of crude alkaloids (g kg^{-1}) in *C. intybus, T. officinale* and *F. critica* collected from Kohat, Mardan, Nowshera and Peshawar regions.

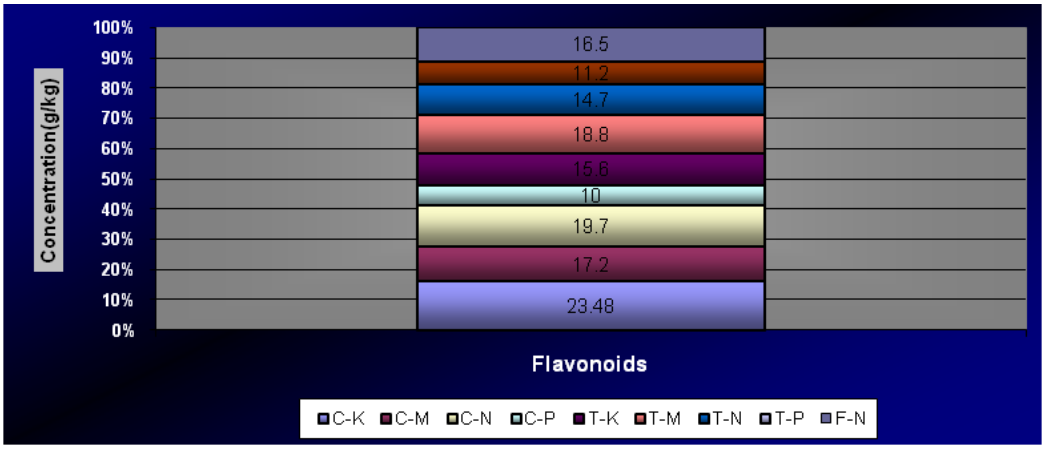

Figure 2. Graphical representation of crude flavonoids (g kg^{-1}) in *C. intybus, T. officinale* and *F. critica* collected from Kohat, Mardan, Nowshera and Peshawar regions.

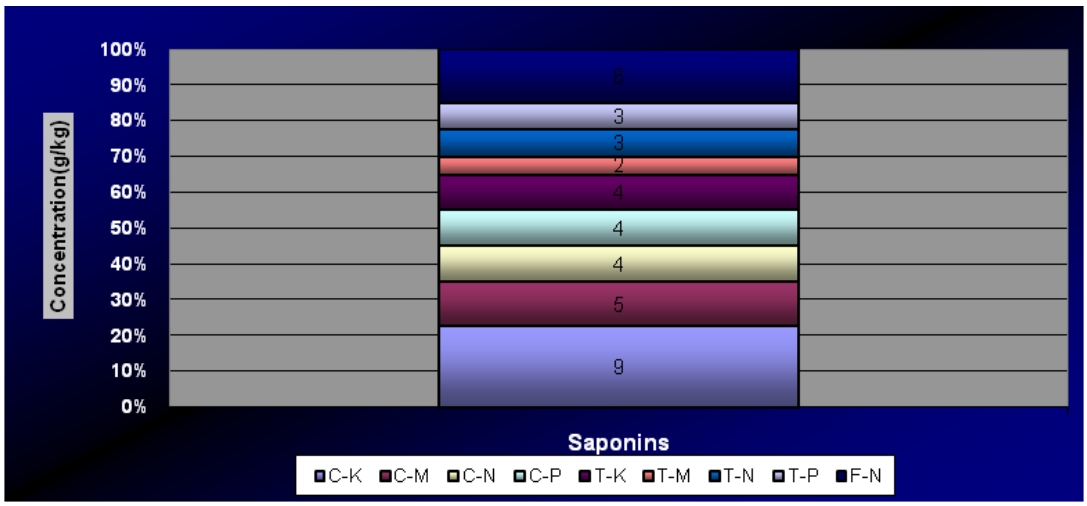

Figure 3. Graphical representation of crude saponins (gm kg^{-1}) in *C. intybus, T. officinale* and *F. critica* collected from Kohat, Mardan, Nowshera and Peshawar regions.

officinale secured from Mardan showed flavonoids contents equal to 18.80 g kg^{-1} which was higher than same samples collected from Kohat, Nowshera and Peshawar and the concentrations were 15.6, 14.7 and 11.2 g kg^{-1}, respectively. *F. critica* yielded up to 16.5 g kg^{1}.

From Table 3, the overall saponin contents remained almost low as compared to the alkaloid and flavonoids contents discussed earlier. In the *C. intybus*, the saponin contents were higher in the sample collected from Kohat which was 9.0g kg^{-1}, followed by the sample from Mardan. The remaining two samples of *C. intybus* collected from Peshawar and Nowshera yielded almost the same saponin contents (4.0 g kg^{-1}).

T. officinale sample obtained from Kohat region yielded 4.0 g kg^{-1} of saponin, followed by the *T. officinale* sample collected from Peshawar and Nowshera which both yielded 3.0 g kg^{-1} of saponin. The *T. officinale* sample secured from Mardan region had 2.0 g kg^{-1} saponin.

From the discussion, it is clear that different environmental conditions affect plants; even the same plant species collected from different areas showed different quantities of phytochemicals. The obtained results were interesting when the effects of various environmental factors on the yield of the plants crude phytochemicals were compared.

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