The Journal of Animal & Plant Sciences, 22(2): 2012, Page: 283-288
ISSN: 1018-7081

CHEMICAL EVALUATION OF WEED SEEDS MIXED WITH WHEAT GRAINS AT HARVEST

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ABSTRACT

Phytochemical potential of fifteen weeds associated with wheat crop was evaluated. Qualitative and quantitative phytochemical analysis of weed seed extracts was made. Alkaloids, saponins, glycosides, terpenoids, anthraquinine, steroids, flavonoids and tannins were detected from the weed seeds. Tannins and alkaloids were in high concentration. Tannins ranged from 7.97 to 24.17%, alkaloids 0.88 to 4.00%, saponins 0.54 to 1.29% and flavonoids 3.91 to 15.55%. Wheat weeds care medicianlly important but their phytochemical potential needs to be further investigated.

Key words: Alkaloids, Biodiversity, Laxative, Tannins, Predator-Prey.

INTRODUCTION

Field of ethno-medicine is still unexplored in Pakistan. The country has a wealth of 5,700 species of medicinal plants of which 372 are endemic and 456 are in active trade for the manufacture almost 350 classical formulations to treat various ailments. Medicinal weeds play a key role in traditional health care system for human and animals. Most of allopathic drugs also comprise extracts taken from medicinal plants. The traditional and indigenous medical knowledge of plants, both oral and codified, is however eroding rapidly from our system (Rashid and Arshad, 2002; Ahmad *et al.*, 2003; Hussain *et al*., 2006; Baquar1989; Mujtaba and Khan, 2007).

In spite of their ethno-botanical importance, weeds associated with wheat are exterminated from the crop to increase per hectare yield. A wide variety of weeds is associated with wheat in our country that not only adds phytomorphic diversity to the crop but also offers feeding, sheltering, refuge and breeding niches to beneficial arthropods. These weeds have been extensively used in herbal medicine (Table 1) but no such study is available from Pakistan.

Keeping in mind this dearth of knowledge, the present study was designed to investigate the scientific bases for the use of some weeds associated with wheat by defining and quantifying their percentage of crude phytochemical constituents. We focused our effort towards the characterization of *Vicia sativa, Galium aparine, Rumex dentatus, Avena fatua, Lathyrus aphaca, Phalaris minor, Carthamus oxycantha, Convolvulus arvensis, Chenopodium album, Coronopus didymus, Melilots indica, Cichorium intybus, Anagallis arvensis, Chenopodium murale* and *Euphorbia sp.*

MATERIALS AND METHODS

Seeds of wheat weeds (*Vicia sativa, Galium aparine, Rumex dentatus, Avena fatua, Lathyrus aphaca, Phalaris minor, Carthamus oxycantha, Convolvulus arvensis, Chenopodium album, Coronopus didymus, Melilots indica, Cichorium intybus, Anagallis arvensis, Chenopodium murale* and *Euphorbia sp.*) that had already been taxonomically identified and stored in the "Seed Bank, Ayuab Agriculture Research Institute, Faisalabad Pakistan" were collected from and were packed in sterilized polythene bags.

Preparation of extracts. The seeds were crushed into fine powder using a mill grinder and 25 g of each weed seed powder was dissolved separately in 100 ml of commercially available pure ethanol. The solution was kept at room temperature for seven days to allow the extraction of compounds from seeds. The solution for each weed variety was stirred after every 24 h using sterile glass rod. After seven days, the solution was filtered through Whatman filter paper no. 1 and a greenish filtrate was obtained. The solvent was evaporated and a black sticky substance was obtained that was stored in the refrigerator and suspended in 10% dimethyl sulfoxide prior to use.

Phytochemical screening. Chemical tests were carried out both on the ethanolic extract and on the powdered specimens using standard procedures to identify the constituents as described by Harborne (1973), Trease and Evans (1989) and Sofowara (1993). The specific procedure involved for the evaulation of a particular group of chemicals is mention below.

1. **Tannins.** One ml of water and 1-2 drops of ferric chloride solution were added in 0.5 ml of extracted solution. Blue colour was observed for gallic tannins

and green black for catecholic tannins (Iyengar, 1995).

2. **Saponins.** Foam test: Small amount of extract was shaken with little quantity of water. If foam produced persists for ten minutes it indicates the presence of saponins (Roopashree, *et al.,* 2008).
3. **Flavonoids (Alkaline Reagent Test).** Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids (Roopashree, *et al.,* 2008).
4. **Steriods.** Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2S0_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.
5. **Terpenoids (Salkowski test).** Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show the presence of terpenoids.
6. **Cardiac glycosides (**Keller-Killani test**):** Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.
7. **Alkaloids.** Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added (Siddiqui and Ali, 1997).
8. **Anthraquinones** Borntrager's test was used for detecting the presence of anthraquinones. In this case 0.5 g of the plant extract was shaken with benzene layer separated and half of its own volume of 10% ammonia solution added. A pink, red or violet colouration in the ammoniacal phase indicated the presence of anthraquinone.

Quantitative determination of the chemical constituency

Preparation of fat free sample: Two g of the sample were defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 h.

1. **Alkaloid.** Harborne (1973) method was used to determine alkaloid. Five gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.
2. **Tannin.** Van-Burden and Robinson (1981) method was used to determine Tannin. For this 500 mg of the sample was weighed into a 50 ml plastic bottle. Fifty ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 605 nm within 10 min.
3. **Saponin.** Obadoni and Ochuko (2001) method was used to determine Saponin. The samples were ground and 20 g of each were put into a conical flask and 100 Cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 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 separatory 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 60 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 as percentage.
4. **Flavonoids.** Bohm and Kocipai-Abyazan (1994) method was used to determine flavonoid. For This Ten g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Statistical analysis. All values have been expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Self growing plants (weeds) in association with wheat crop plants have ethno-botanical importance. They can be used as medicinal plants assessing the need to evaluate the constituents of these weed plants. The present study carried out on the seeds of weeds viz. *Vicia*

sativa, Galium aparine, Rumex dentatus, Avena fatua, Lathyrus aphaca, Phalaris minor, Carthamus oxycantha, Convolvulus arvensis, Chenopodium album, Coronopus didymus, Melilots indica, Cichorium intybus, Anagallis arvensis and *Chenopodium murale.* The chemical analysis revealed the presence of medicinally active constituents. The phytochemical screening and quantitative estimation of the crude yields of chemical constituents of the plant seeds studied showed that the seeds were rich in alkaloids, flavonoids, tannins, cardiac glycosides and saponins. They were known to show medicinal activity as well as exhibiting physiological activity (Sofowara, 1993; Ajayi, *et al.,* 2011).

The phytochemical characteristics of the fifteen wheat weeds have been summarized in the Table 2. Flavinoids, tannins and alkaloids were present in all the weed plants. Anthraquinones and terpenoids were absent in both the *R. dentatus and C. murale* whearse steroids and glycosides were absent in *G. aparine*. Flavinoids was found in high concentration in all seeds of weeds occurring in wheat crop may be responsible for their use in herbal medicine for cure of various ailments like capillary and vascular weakness (varicose veins, dysfunction, blood cloting and oedema) (Olivere- Bever, 1986; Rogar, 2002). Flavinoids were also useful for sexual stimulation (Benson, *et al.,* 2008). Flavonoids had the record of being powerful water soluble free radical scavengers and wonderful antioxidants which prevented oxidative cell damage, had potent anticancer activity and inhibited tumor growth (Stauth, 1993; Sharma, *et al.,* 2007). A study showed that flavonoids could inhibit the development of fluids that resulted in diarrhea by targeting the intestinal cystic fibrosis trans-membrane conductance regulator (Schuier, *et al*, 2005).

Table 1. Brief Review of the Various Selected Weeds Used For the Present Study of Wheat Crop

Family	Botanical Name	Local Name	Traditional Uses	Reference
Fabaceae	*Vicia sativa*	Rivari	Antiseptic	Dwivedi, 2008;Jabeen *et al.,* 2009
Fabaceae	*Lathyrus aphaca*	Jangali matar	Famine food.	Dwivedi, 2008. Jabeen *et al*., 2009; Jabeen *et al.,* 2009
Rubiaceae	*Galium aparine*	Betistraw	Diuretic, Dropsies, Gravel and Calculi, Tonic Lymphatic alterative, Anti-inflammatory, Astringent, Anti-neoplastic	Proving Report 2006;
Polygonaceae	*Rumex dentatus*	Jangli palak	Treatment for pneumonia, Cough, Bscesses, stomach-ache and smallpox	Hussain *et al.,* 2006; Kumar, 2010
Poaceae	*Avena fatua*	Jangli jai	Seeds are nerve tonic, stimulant and laxative.	Islam *et al.,* 2006;
Poaceae	*Phalaris minor*	Dumbi setti	The seed can be ground into a flour and used in making bread, cakes etc.	Moerman, 1998.
Asteraceaae	*Carthamus oxycantha*	Pohli	Antihyperlipidemic, Antihyperglycemic, Antinephrolithiatic and Hepatoprotective.	Bukhsh *et al.,* 2007; Jabeen *et al.,* 2009
Convolvulaceae	*Convolvulus arvensis*	Lehli	Roots are purgative and possess blood coagulating properties due to presence of vitamin K like substances, Used as animal feed.	Khan, 2004; Gorsi and Rizwan, 2002
Chenopodiaceae	*Chenopodium album*	Batho	Antipruritic and Antinociceptic.	Yadav *et al.,* 2007; Jabeen *et al*., 2009; Qureshi, *et al*., 2009
Chenopodiaceae	*Chenopodium murale*	Krund	The leaves are used in salads.	Yadav *et al.,* 2007;
Brassicaceae	*Coronopus didymus*	Jangli halon	Antiallergic, Antipyretic, Hypoglycemic and hepatoprotective.	Qureshi *et al.,* 2009; Busnardo, *et al.,* 2009
Leguminosae	*Melilots indica*	Zaed sangi	The leaves and flowers are added to bathwater for the external treatment for warts, Rheumatism, and wounds. Ocular infections are also treated.	Shinwari,2000; Jabeen *et al.,* 2009
Asteraceae	*Cichorium intybus*	Kasni	liver tonic, cardiotonic, diuretic, stomachic, cholagogue, depurative, emmenagogue, hepatomegaly, cephalalgia, inflammations, anorexia, dyspepsia, flatulence, colic, jaundice, splenomegaly, amenorrhea dysmenorrhea, and asthma, etc.	Sala, 1994; Jabeen *et al.,* 2009
Primulaceae	*Anagallis arvensis*	Billi boti	Skin diseases: infected wounds.	Akerreta *et al.,* 2007; Jabeen, *et al*., 2009; Kumar, 2010
Euphorbiaceae	*Euphorbia sp.*	Chatri dohdhk	Stem pound and mother liquor used (Mtakalang'onyo) to expel retained Placenta.	Komwihangilo *et al.,* 1995; Guèye,2002

Table 2. Qualitative phytochemical components of seeds of selected weed plants relevant to wheat crop.

Weed Plants	Flavinoids	Saponins	Tannins	Steroids	Glycosides	Alkaloids	Anthrequinones	Terpenoids
Vicia sativa	+	+	+	+	+	+	+	+
Galium aparine	+	+	+	-	-	+	-	+
Rumex dentatus	+	+	+	+	-	+	-	-
Avena fatua	+	+	+	-	+	+	-	+
Lathyrus aphaca	+	-	+	-	+	+	+	+
Phalaris minor	+	+	+	-	+	+	+	+
Carthamus oxycantha	+	+	+	-	+	+	+	+
Convolvulus arvensis	+	+	+	-	+	+	+	+
Chenopodium album	+	+	+	+	+	+	-	+
coronopus didymus	+	+	+	+	+	+	+	+
Melilots indica	+	-	+	+	+	+	+	+
Cichorium intybus	+	-	+	+	+	+	+	+
Anagallis arvensis	+	+	+	+	+	+	+	-
Chenopodium murale	+	+	+	+	+	+	-	-
Euphorbia sp.	+	-	+	+	+	+	-	+

Table 3. Qualitative (percent) Phytochemical components of seeds of selected weed plants

Weed Plants	Alkaloids	Saponins	Flavinoids	Tannins
Vicia sativa	2.27±0.14	0.85±0.04	4.62±0.06	10.41±0.08
Galium aparine	2.76±0.03	0.99±0.03	6.36±0.03	16.96±0.01
Rumex dentatus	1.81±0.07	0.56±0.03	10.94±0.05	15.18±0.04
Avena fatua	2.19±0.01	0.87±0.06	3.91±0.08	18.53±0.05
Lathyrus aphaca	4.00±0.04	0.00±0.00	4.93±0.07	15.56±0.03
Phalaris minor	1.21±0.03	0.70±0.04	11.85±0.11	7.97±0.02
Carthamus oxycantha	2.61±0.03	0.48±0.06	4.14±0.28	15.58±0.03
Convolvulus arvensis	2.63±0.07	0.76±0.04	13.88±0.11	20.26±0.06
Chenopodium album	2.12±0.02	0.76±0.04	8.80±0.04	16.09±0.03
coronopus didymus	2.25±0.02	0.74±0.02	15.55±0.13	15.17±0.03
Melilots indica	2.62±0.07	0.00±0.00	13.52±0.09	21.97±0.02
Cichorium intybus	2.13±0.01	0.00±0.00	14.13±0.13	14.53±0.02
Anagallis arvensis	2.21±0.02	1.29±0.03	4.43±0.10	9.56±0.03
Chenopodium murale	0.88±0.06	0.54±0.01	3.92±0.02	24.17±0.02
Euphorbia sp.	2.46±0.07	0.00±0.00	9.20±0.54	20.86±0.05

The biological functions of flavonoiods include protection against inflammatory allergies, free radical scavenging, ulcers, microbes, hepatoxins, platelets aggregation, viruses and tumors (Okwu and Omodamiro, 2005; Okwu and Emenike, 2006).This accounts for the natural antioxidants by acting against oxidative stress related disease such infections as diabetics, cancer and coronary heart diseases (Burits and Bucar, 2002). So the people that are suffering from such infection can feed on wheat weed seeds as source of natural antioxidants. Tannins were reported by many researchers that the molecule has high molecular weight water soluble polycyclic aromatic compounds widely distributed through the plant kingdom and almost found in every plant part and is one of active component of seeds cotyledon (Haslam, 1989; Kaur and Arora, 2009; Okoli and Okere, 2010). Enzo (2007) reported that tannins were also responsible for different anti-diarrheal activities.

Quantitative estimation of percent crude chemical constituents in these wheat weeds determined has been given in Table 3. *C. murale* contained highest percentage of crude tannins (24.17%), *P. minor* contained the lowest yield of tannins (7.97%) but the highest yield of flavinoids was found in *C. didymus* (15.55%) and the lowest was found in *C. murale* (3.92%). *L. aphaca* was found to contain highest yield of alkaloids that is (4.00%) and the lowest yield was found in *C. murale* (0.88%). Alkaloids might be found in all plant parts but tended to accumulate in storage organs of plants. Alkaloids act as stimulators, inhibitors and growth terminators (Rowson, 1958; Waller, 1978; Nazrullaev, *et al.,* 2001)

Saponins were also obtained from the seeds of weeds but the yield recorded were minimal ranging from 1.29% in *A. arvensis* and 0.48% in the *C. oxycantha.* Saponin has been reported as antinutrient, found in different plant parts and in low quantitiy in seeds and

posses both beneficial (i.e. Cholesterol lowering) as well as deleterious properties and reveal structure dependent biological activity (Savage, 1993; Akubugwo *et al.,* 2007).

The analyzed seed cotyledons of weeds contained steroidal compounds which were of importance and interest in pharmacy due to their relationship with compounds as sex hormones (Okwu, 2001). Presence of terpenoids in the studied seeds is in line with with the results of Goto, *et al*. (2010), who stated that Terpenoids were present in many herbal plants, and several terpenoids had been shown to be available for pharmaceutical applications, for example, artemisinin and taxol as malaria and cancer medicines, respectively. Accordingly various terpenoids were available in many plants for not only herbal but also for dietary use.

Conclusion: Seeds from all the fifteen weed plants associated with wheat crops possessed a significant amount of phytochemicals. The presence of phytochemicals like alkaloids, tannins, saponins etc. in these weed plants might be responsible for their curative effects. Moreover, it revealed a strong hope for the development of more novel chemotherapeutic agents from such weed plants which in future may serve for the production of synthetically improved therapeutic agents. Further studies are recommended to explore other parts of weed frequently occurring in our cropland.

Acknowledgement: All the field and laboratory facilities were provided under the Research Project No. 20-813/R and D/HEC. The Authors highly acknowledge Higher Education Commission, Islamabad, Pakistan for funding the same to accomplish this work.

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