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Original article

Phytochemical screening and evaluation of antioxidant, total phenolic and flavonoid contents in various weed plants associated with wheat crops

Triagem fitoquímica e avaliação dos teores de antioxidantes, fenólicos totais e flavonoides em várias plantas daninhas associadas a culturas de trigo

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Abstract

This study explores the antioxidant activity, phytochemical screening, total phenolic and flavonoids contents in the extracts of four locally available weeds plants namely *Convolvulus arvensis*, *Chenopodium murale, Avena fatua* and *Phalaris minor* with different solvents. The antioxidant activities of these extracts were determined via various in-vitro methods such as total antioxidant activity (TAA), reducing power (RP), DPPH (2,2-Diphenyl-1-Picrylhydrazyl) free radical scavenging and hydrogen peroxide scavenging assays. Phytochemical screening was performed both qualitatively as well as quantitatively. Total phenolic content (TPC) and total flavonoid content (TFC) were determined through Folin- Ciocalteu reagent and aluminium chloride methods respectively. Methanol-chloroform solvent showed the presence of a high amount of TPC in milligram of gallic acid equivalent per gram of dry weight (mg of GAE/g of DW) in the extracts of all weeds. Their descending sequence was *Avena fatua* (74.09) > *Phalaris minor* (65.66) > *Chenopodium murale* (64.04) > *Convolvulus arvensis* (61.905), while, chloroform solvent found to be best solvent for the extraction of TFC. Methanol-chloroform solvent was also found to be best solvent for TAA (Total antioxidant activity assay) which showed values in milligram of ascorbic acid equivalent per gram of dry weight (mg of AAE /g of DW), for DPPH scavenging activity, reducing power (antioxidant activity) and hydrogen peroxide scavenging activity. Phytochemical screening indicated the presence of polyphenols, flavonoids, tannins, saponins, alkaloids and glycosides in these weeds.

Keywords: antioxidant activity, DPPH, flavonoids, phytochemical screening, total phenolic contents.

Resumo

Este estudo investiga a atividade antioxidante, a triagem fitoquímica, os teores de fenólicos totais e de flavonoides nos extratos de quatro plantas daninhas disponíveis localmente, quais sejam, *Convolvulus arvensis*, *Chenopodium murale*, *Avena fatua* e *Phalaris minor* com diferentes solventes. As atividades antioxidantes desses extratos foram determinadas por meio de vários métodos *in vitro*, tais como atividade antioxidante total (TAA), poder redutor (RP), sequestro de radicais livres DPPH (2,2-Difenil-1-Picril-hidrazil) e ensaios de sequestro de peróxido de hidrogênio. A triagem fitoquímica foi realizada tanto qualitativamente quanto quantitativamente. O teor de fenólicos totais (TPC) e o teor de flavonoides totais (TFC) foram determinados pelos métodos do reagente de Folin-Ciocalteu e do cloreto de alumínio, respectivamente. O solvente metanol-clorofórmio mostrou a presença de elevada quantidade de TPC em miligramas de ácido gálico equivalente por grama de peso seco (mg de GAE/g de DW) nos extratos de todas as plantas daninhas. Sua sequência descendente foi *Avena fatua* (74,09) > *Phalaris minor* (65,66) > *Chenopodium murale* (64,04) > *Convolvulus arvensis* (61,905), enquanto o solvente clorofórmio foi o melhor solvente para a extração de TFC. O solvente metanol-clorofórmio também foi considerado o melhor solvente para AAT (ensaio de atividade antioxidante total), que apresentou valores em miligramas de equivalente de ácido ascórbico por grama de peso seco (mg de AAE/g de DW), para atividade sequestrante de DPPH, RP (atividade antioxidante) e atividade de sequestro de peróxido de hidrogênio. A triagem fitoquímica indicou a presença de polifenóis, flavonoides, taninos, saponinas, alcaloides e glicosídeos nessas plantas daninhas.

Palavras-chave: atividade antioxidante, DPPH, flavonoides, triagem fitoquímica, teores fenólicos totais.

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1. Introduction

Free radicals are atoms, molecules or ions that have odd, unpaired valence electrons. Oxygen in the body splits into single atoms with unpaired electrons and these atoms are called free radicals. If this continues to happen, it begins a process called oxidative stress which causes damage to cells, proteins and DNA, which may lead to illnesses and the aging process (Khan et al., 2018; Metin et al., 2013). During normal metabolic processes in the human body, large number of free radical species (hydroxyl $OH^{\bullet}$, perhydroxyl radical $HO_2^{\bullet}$, superoxide anion $O_2^{\bullet -}$) and some non-radical species (the precursors of free radicals) such as hydrogen peroxide H_2O_2, ozone O_3, singlet oxygen $^1O_2$ are frequently formed (Keser et al., 2014). Beside these reactive oxygen species (ROS), the reactive nitrogen species (RNS) are also produced which include nitric oxide $NO^{\bullet}$, nitrogen dioxide $NO^{\bullet}_2$ and dinitrogen trioxide N_2O_3 and nitrous acid HNO_2 etc. Sometimes immune systems of the body purposefully create free radicals to neutralize viruses and bacteria.

Antioxidants oppose oxidative effects of these free radicals by mechanisms of scavenging free radical species, suppressing free radical species formation by inhibiting some enzymes or chelating trace metals involved in free radicals production and prevailed system of antioxidant defense (Mucha et al., 2021). Various types of natural secondary metabolites such as phenols, flavonoids, phenolic glycosides and saponins are formed in various parts of the plants which act as natural antioxidants and possess typical pharmacological actions (Jan et al., 2021). Phenolic compounds possess significant antioxidant characteristics, protect oxidative damages and safeguard health from different diseases (Li et al., 2013). Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are now-a-day being avoided owing to its in-vivo carcinogenic effects (Saeed et al., 2016). Synthetic antioxidants are now replaced by natural antioxidants of plant origin due to their excellent nontoxic properties, detoxifying free radicals, lower prices as well as fewer side effects (De Britto et al., 2011)

Phenolics are chemically consisting of hydroxylated aromatic rings; the OH group is bonded to phenyl group, substituted phenyl or other aryl group. They are usually secondary metabolites of plants and present naturally (Rahman, 2019). These phenolic substances are normally associated with defense reactions in the plant and they also perform a significant role in various ways, for example adding attractive compounds to promote pollination, coloring for camouflage and protect against herbivores, antibacterial and antifungal activities (Aherne and O'Brien, 2002; Robards et al., 1999). Hydroxyl groups and aromatic or phenolic rings are responsible for antioxidant activities of phenolic substances. Their antioxidant activity is usually due to their ability of scavenging of free radicals, giving hydrogen atoms, electrons or chelating of metal ions (Aherne and O'Brien, 2002; Robards et al., 1999). In plants, phenolic compounds can be classified into 4 classes viz (1) Phenolic acids (Gallic, Protocatechuic, Caffeic, as well as Rosmarinic acids), (2) Diterpenes (Carnosol as well as Carnosic acids), (3) Flavonoids (Quercetin as well as Catechin) as well as (4) Volatile oils (Eugenol, Carvacrol, Thymol as well as Menthol) (Shan et al., 2005). Number as well as the position of OH groups in polyphenols significantly affected their antioxidant activity. Numerous in-vitro investigations showed greater antioxidant activity by phenolic compounds as compared to Vitamins and Carotenoids (Chen et al., 2020; Re et al., 1999).

Flavonoids are a very important group of natural products of plant secondary metabolites possessing polyphenolic structure. Flavonoids based upon the flavone skeleton of C_6- C_3 -C_6 are the varied groups of polyphenol. They are found in plants, weeds, fruits, vegetables and specific beverages (Shah and Smith, 2020). Flavonoids are used in different nutraceutical, pharmaceutical, medicinal and cosmetic industries. This is due to their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic characteristics (Panche et al., 2016)

This study was performed in order to determination of antioxidant activity, total phenolics contents and phytochemical screening of *Convolvulus arvensis, chenopodium murale, Avena fatua and Phalaris minor* extracts, while using various solvents such as methanol, acetone, chloroform, water, methanol-acetone, methanol-chloroform as well as methanol-water.

2. Materials and Methods

2.1. Materials

Ascorbic acid (Vitamin-C), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent and gallic acid were purchased from Sigma-Aldrich (Germany). Hydrogen peroxide (H_2O_2), rutin and dimethyl sulphoxide (DMSO) were purchased from Sigma Aldrich (St. Louis, MO, USA). Ferrous sulphate was obtained from Biochemicals (Lahore, Pakistan) and Potassium ferricyanide, Aluminum chloride, Ammonium molybdate were from Merck (Darmstadt, Germany).

2.2. Collection and preparation of plants extracts

All the weed plants were identified by Dr. Shah Khalid, incharge of herbarium, department of botany, Islamia College Peshawar. The specimens are kept for record in the herbarium. The plants were identified at this herbarium as: *Convolvulus arvensis,* ICP-000133, *Chenopodium murale,* ICP-001133, *Avena fatua,* ICP-000433 and *Phalaris minor,* ICP-005133

Four weed plants, *Convolvulus arvensis, Chenopodium murale, Avena fatua and Phalaris minor* self-grown in wheat crops were collected. After collection, whole plants were shade dried in the room. After drying, the whole weed plants were grinded into smaller pieces with the help of pestle and mortar and stored.

About10 grams of crushed pieces of each weed plant was added to 200 ml of solvent and shaken vigorously. The content was stirred every day for 7 days at room temperature and filtered through Whatman filter paper No 1 to obtain filtrate. Filtrate was evaporated to remove solvent until a black color sticky substance was obtained which was stored in the refrigerator. Solvents used for

extraction were: 1- methanol, 2- acetone, 3- chloroform, 4- water, 5-methanol-acetone (50:50 V/V), 6- methanol-chloroform (50:50 V/V), 7- methanol-water (50:50 V/V).

2.3. Determination of Antioxidant activity

A. Antioxidant activity by DPPH (2,2-Diphenyl-1-Picrylhydrazyl) free radical scavenging

Antioxidant activity via DPPH free radicals scavenging activity of various solvents extracts of weed plants were measured by method of Kulisic et al. (2004). A portion of 50 µl of extract of different concentrations (1 µg/ml to 133 µg/ml) was added to 950 µl of methanolic solution of DPPH (3.943 mg/100 ml). The reaction mixture was then kept at 37 ºC for 1 hour in dark. Disappearance of the purple color revealed free radical scavenging capacity of an extract. Absorbance of the resulting mixture was measured at a wavelength of 517 nm. Blank was prepared in which extract was replaced by DMSO. Ascorbic acid solutions of the same concentration (1µg/ml to 133µg/ml) as standard were prepared and followed the same procedure of an antioxidant activity as positive control. Antioxidant activity of various solvents extracts for DPPH free radical scavenging was performed in triplicate and calculated by the Equation 1.

$$\% \text{ scavenging of DPPH} = \left[\frac{\text{Absorbance}^{\text{Control}} - \text{Absorbance}^{\text{Sample}}}{\text{Absorbance}^{\text{Control}}}\right] \times 100 \quad (1)$$

Then IC_{50} value was calculated via linear regression analysis.

B. Total Antioxidant Activity by phosphomolybdate method

The total antioxidant activities by phosphomolybdate assay of various solvents extracts of weeds plants were determined according to the method of Umamaheswari and Chatterjee with minor changes (Umamaheswari and Chatterjee, 2007). About 20 mg of various solvents extracts were dissolved in 5 ml of DMSO. And 0.1 ml of extract was mixed in 1 ml of phosphomolybdate reagent solution (composed of 1 ml of 0.6 M of sulfuric acid, 1 ml of 28 mM sodium phosphate and 1 ml of 4 mM of ammonium molybdate). The mixture was incubated in a water bath at 95 °C for 90 min. After cooling to room temperature, the absorbance was measured at 695 nm. Blank was prepared in which extract was replaced by DMSO. Standard curve was constructed with ascorbic acid in the concentration range of 50 µg/ml to 750 µg/ml, following the same procedure to develop a straight line equation. The mg of AAE/g of extract was determined from equation $y = 0.002x - 0.012$ ($R^2 = 0.995$) where y is the absorbance and x is the µg/ml of ascorbic acid (Ahmed et al., 2012). All experiments were performed in triplicate and the average values were noted.

2.4. Determination of total phenolic contents

The total phenolic contents of various solvents extracts of weed plants were determined according to the method used by Singleton and Rossi (Singleton and Rossi, 1965).

About 20 mg of various solvent extracts were dissolved in 5 ml of DMSO. About 40 µl of extract was mixed with 3.16 ml of distilled water and then added 200 µl of 10% Folin-Ciocalteau reagent. After incubation for 8 minutes, 600 µl 20% of Na_2CO_3 was added. The mixture was again incubated for ½ h at 40 °C and absorbance was measured at 760 nm. Blank was prepared in which plant extract was replaced by DMSO. Gallic acid standard curve in range of 50 µg to 750 µg/ml was constructed following the above procedure to make straight line equation $y = 0.0008209 x + 0.0130633$ ($R^2 = 0.997$) where "y" is the absorbance and "x" is the µg/ml of gallic acid. The results were calculated as mg of GAE/g of dry weight (Ahmed et al., 2012)

2.5. Determination of total flavonoid contents

The total flavonoid contents of various solvents extracts of weeds plants were determined according to the aluminum chloride colorimetric method as discussed by Chang et al. (2002) with minor changes. About 20 mg of various solvents extracts were dissolved in 5 ml of DMSO.

About 0.5 ml of extract solution was taken, and 1.5 ml of DMSO, 0.1 ml of 10% aluminum chloride, 0.1 ml of potassium acetate and 2.8 ml of water were added to it. The resulting mixture was incubated at room temperature for 30 minutes and absorbance was measured at 415 nm. Blank was also prepared in which extract was replaced by DMSO. Standard calibration curve of rutin trihydrate was constructed in the range of 50 µg to 750 µg/ml following the same procedure. Rutin trihydrate equivalent (RTE) was calculated from line equation $y = 0.002 x + 0.048$ ($R^2 = 0.998$) where y is the absorbance and x is the concentration in µg/ml of rutin. The results were expressed as mg of RTE/g of dry weight. All experiments were performed in triplicate and the average values were noted.

2.6. Reducing Power Assay (RPA)

The reducing power of various solvents extracts of weed plant was performed by method of Oyaizu et al. with minor changes (Oyaizu, 1986). About 4 mg of various solvents extracts were dissolved in 1 ml of DMSO to prepare the stock solution. Different dilutions were made to obtain a series of concentrations (0.015625 mg/ml to 4 mg/ml).

Then 0.5 ml of above extract was mixed with 1.25 ml of 0.2 M phosphate buffer at pH 6.6, and 1.25 ml of 1% (W/V) potassium ferricyanide. The mixture was incubated in a water bath at 50°C for 20 minutes. After cooling at room temperature, 1.25 ml of 10% of trichloroacetic acid was added to it. Following centrifugation at 3000 rpm for 10 minutes, supernatant was collected. About 1.25 ml of supernatant and 1.25 ml of distilled water was mixed and then 0.25 ml of 0.1% of ferric chloride was added to it. This mixture was kept at room temperature for 10 minutes. Absorbance was measured at wavelength 700 nm. Blank was prepared in which extract was replaced by DMSO. Ascorbic acid was used as positive control with the same concentration (0.015625 mg/ml to 4 mg /ml). Then IC_{50} value was calculated via linear regression analysis.

2.7. Hydrogen peroxide (H_2O_2) scavenging assay

An ability of various solvents extracts of weeds plants for the scavenging of hydrogen peroxide was performed by technique used by Ruch et al. (1989). About 4 mg of various solvents extracts were dissolved in 1 ml of DMSO to make a stock solution. Then further dilutions were made with different concentrations (0.015625 mg/ml, 0.03125 mg/ml, 0.0625 mg/ml, 0.125mg/ml, 0.25 mg/ml, 0.5 mg/ml, 1 mg/ml, 2 mg/ml and 4 mg/ml).

A portion (0.1 ml) of above diluted solutions was added to 0.3 ml of 50 mM phosphate buffer of pH 7.4 and 0.6 ml of 2 mM H_2O_2 solution. The resulting mixture was vortexed and after 10 minutes, the absorbance was measured at 230 nm. Blank was also prepared, in which extract was replaced by DMSO. Ascorbic acid was used as positive control, having the same concentration (0.015625 mg/ml to 4 mg/ml. H2O2 scavenging activity can be calculated by Equation 2.

$$H_2O_2 \text{ scavenging activity } (\%) = \left[\frac{A_0 - A_1}{A_0}\right] \times 100 \qquad (2)$$

Where A_0 = Absorbance of control, A_1 = Absorbance of test. Then IC_{50} Value was calculated via linear regression analysis.

2.8. Phytochemical screening

Various chemical tests were performed for different solvents extracts of weeds plants namely *Avena fatua, Chenopodium murale, Convolvulus arvensis and Phalaris minor*. Different chemical tests were performed for qualitative measurement on an ethanolic concentrate by employing reference protocols in order to differentiate the constituents (Siddiqui and Ali, 1997).

The following tests were performed for determination of the chemical constituents:

a. ***Tannins***: In a test tube first of all solution of extract 0.5 ml or 500 µl was taken and then 1 ml of water and 1 to 2 drops of ferric chloride solution ($FeCl_3$) was added. Gallic tannins produced blue color whereas catecholic tannins formed green black color (Iyengar, 1995);
b. ***Saponins: (Foam Test)***: For saponins, foam test was used by vigorous shaking of a small quantity of an extract with small amount of water for 10 minutes which formed foam and keep it for 10 minutes which denoted saponins in the extract (Roopashree et al., 2008);
c. ***Flavonoids:*** For an existence of flavonoids, alkaline reagent test was employed in which first of all small amount of extract was taken in a test tube and then added to it minute droplets of NaOH solution which produced deep yellow colouration which disappeared on mixing with dilute acid, which indicated an existence of flavonoids in the extract (Roopashree et al., 2008);
d. ***Alkaloids (Mayer's reagent test):*** For the confirmation or presence of alkaloids in the extracts samples Mayer's reagent test were used. Alkaloids are actually basic nitrogenous compounds with specific pharmacological and physiological functions. The various solvent extract was evaporated to dryness and the residue was heated in a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. Alkaloidal solution formed white yellowish precipitates or turbidity on addition of a petty droplets of Mayer's reagents (1.36 g of mercuric chloride was dissolved in 60 ml water and 5 g of potassium iodide dissolved in 10 ml of distilled water, solution were mixed and diluted to made up the volume upto 100ml i.e potassium mercuric iodide) (Siddiqui and Ali, 1997).. Statistical analysis was performed and the data are expressed as mean ± SD from three separate observations. For different contents assays one way ANOVA test ($P < 0.05$) was used to analyze the differences among EC50. A probability of $P < 0.05$ was considered as significant.

3. Results and Discussion

As shown in Table 1, the methanol-chloroform solvent was found to be the best solvent for the extraction of total phenolic contents (TPC) in all four weeds plants. Their TPC values in descending order are; *Avena fatua* (74.09 mg of GAE/g of DW) > *Phalaris minor* (65.66 mg of GAE/g of DW) > *Chenopodium murale* (64.04 mg of GAE/g of DW) > *Convolvulus arvensis* (61.905 mg of GAE/g of DW). Greater amount of TPC was found in *Avena fatua* extract whereas the lowest quantity TPC was found in *Convolvulus arvensis* extract. Other solvents extracts showed good values of TPC but it was less as compared to methanol-chloroform solvent. These results are in agreement with literature that to methanol-chloroform solvent has strong extraction capabilities (Akhtar et al., 2018).

Our results (Table 1) showed that by using chloroform as solvent there was a significant increase in the extraction of total flavonoid contents (TFC) in all 4 weeds plants as compared to other solvents. Their TFC values were found to be (in descending order); *Avena fatua* (257.46 mg of RTE/g of DW) > *Chenopodium murale* (239.04 mg of RTE/g of DW) > *Convolvulus arvensis* (215.875 mg of RTE/g of DW) > *Phalaris minor* (140.2925 mg of RTE/g of DW). Highest amount of TFC was found in chloroform extract of *Avena fatua* and the minimum amount was found in *Phalaris minor* extract. Other solvents systems such as methanol, acetone, methanol-acetone, and methanol-chloroform showed significant values of TFC in all these plants but it was less than values obtained from chloroform extract.

Similarly, methanol-chloroform (50:50 V/V) was also found to be a suitable extraction solvent for the assay of total antioxidant activity (TAA) in all four plants of weeds. The sequence of total antioxidants activity in four plants extracts were to be; *Chenopodium murale* (190.46 mg of AAE/g of DW) > *Avena fatua* (180.33 mg of AAE/g of DW) > *Phalaris minor* (161.2925 mg of AAE/g of DW) > *Convolvulus arvensis* (84.8 mg of AAE/g of DW). The highest amount of total antioxidant activity was observed in methanol-chloroform extract of *Chenopodium murale* and lowest amount was found in the extract of *Convolvulus arvensis*. These findings are in agreements with Nosheen Akhtar *et al*., they found maximum total antioxidant activity when chloroform-methanol extract of Manilkara zapota (4171.25 mg GAE/g of DW) were used (Akhtar et al., 2018).

All samples of plants extracts with different solvent systems had shown DPPH scavenging activity which is

Table 1. Various activities of *Convolvulus arvensis, Chenopodium murale, Avena fatua and Phalaris minor* weed plants, when extracted with different solvents.

Name of weeds and solvents	TPC (mg of GAE/g DW)	TFC (mg of RTE/g DW)	TAA (mg of AAE/g DW)	DPPH assay IC_{50} AA 55.004 µg/ml	RPA IC_{50} AA 6.89 µg/ml	HPSA IC_{50} AA 148.48 µg/ml
Methanol						
Convolvulus arvensis	45.36	113.58	84.55	69.428	168.44	185.02
Chenopodium murale	50.64	115.21	140.625	61.266	211.42	228.79
Avena fatua	45.16	33.6675	127.92	82.25	204.21	204.16
Phalaris minor	43.53	29.625	109.125	241.868	401.25	233.49
Acetone						
Convolvulus arvensis	61.515	167.54	75	90.088	144.38	212.36
Chenopodium murale	56.32	137.71	169.25	43.857	186.73	179.74
Avena fatua	49.11	149.92	179.54	73.211	306.87	174.28
Phalaris minor	46.78	115.96	141.625	183.345	426.01	208.9
Chloroform						
Convolvulus arvensis	27.49	215.875	83.75	82.853	856.26	191.45
Chenopodium murale	18.86	239.04	95.58	45.296	503.99	257.92
Avena fatua	55.61	257.46	180.25	60.922	240.39	166.36
Phalaris minor	42.31	140.293	161.11	149.758	460.87	180.46
Water						
Convolvulus arvensis	2.8225	1.8325	9.33	73.629	327.42	290.49
Chenopodium murale	15.21	8.5	27.96	108.739	420.37	301.74
Avena fatua	43.43	17.92	63.96	120.785	334.21	232.89
Phalaris minor	37.64	10.5	100.125	118.477	292	273.79
Methanol-Acetone						
Convolvulus arvensis	52.26	147.58	84.65	63.584	140.43	176.6
Chenopodium murale	50.33	166.75	131.33	62.313	239.14	243.16
Avena fatua	55.92	76.92	144.67	66.022	215.11	221.36
Phalaris minor	51.24	42.125	143.25	223.148	270.25	195.28
Methanol-Chloroform						
Convolvulus arvensis	61.905	180.958	84.8	62.531	125.62	165.01
Chenopodium murale	64.04	187	190.46	42.653	185.69	168.65
Avena fatua	74.09	111.375	180.33	59.901	182.01	165.91
Phalaris minor	65.66	55.33	161.293	117.441	246.99	179.74
Methanol-Water						
Convolvulus arvensis	61.255	34	70.55	66.825	130.25	210.68
Chenopodium murale	38.96	36.5	85.83	98.425	302.46	273.5
Avena fatua	33.48	20	83.62	128.942	258.14	221.1
Phalaris minor	40.89	14	103.17	188.28	248.05	241.15

TPC = Total Phenolic Contents, TFC = Total Flavonoids Content, TAA = Total Antioxidant Activity, RPA = Reducing Power Assay, HPSA = Hydrogen Peroxide (H_2O_2) Scavenging Assay, RTE = Rutin Trihydrate Equivalent.

evident by their IC 50 values (Figure 1). Methanol-chloroform extract of *Chenopodium murale* exhibited highest antioxidant activity (IC 50 = 42.653 µg/ml) followed by *Avena fatua* (59.901) < *Convolvulus arvensis* (62.531) and < *Phalaris minor* (117.414). Their IC 50 values were compared to the IC 50 of ascorbic acid as reference (55. 0004 µg/ml) (Table 1).

Methanol-chloroform solvent seemed to be good solvent for reducing power assay of all plants extracts. Decreasing order of reducing power of methanol-chloroform extracts were also shown as *Convolvulus arvensis* (IC_{50} 125.62) > *Avena fatua* (IC_{50} 182.01 µg > *Chenopodium murale* (IC_{50}185.69) > *Phalaris minor* (IC_{50} 246.59). Their IC_{50} were compared with the IC_{50} values of ascorbic acid as references. These results showed that antioxidant activity via reducing power assay of extracts of *Convolvulus arvensis* with different solvent systems exhibited higher antioxidant

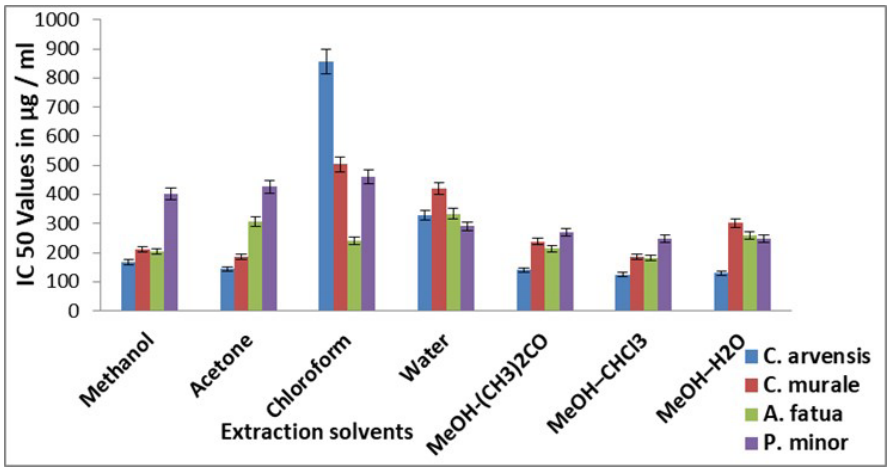

Figure 1. DPPH Assay for *Convolvulus arvensis, Chenopodium murale, Avena fatua, Phalaris minor* extracts in different solvents.

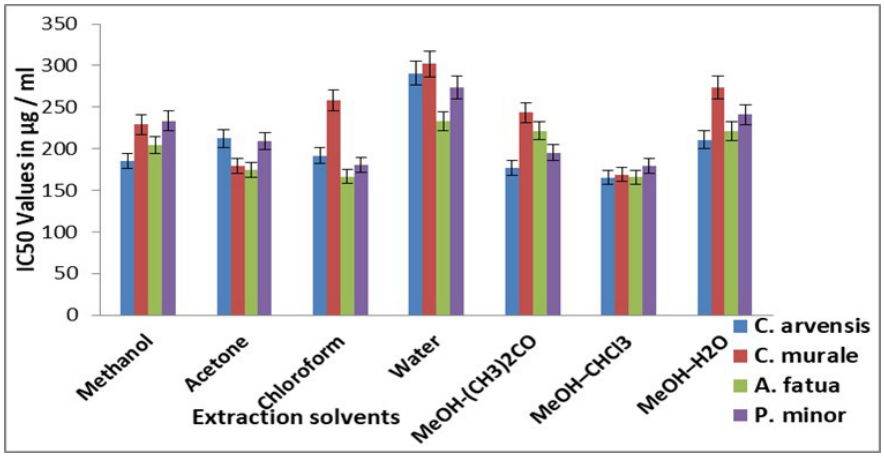

Figure 2. Reducing power assay for *Convolvulus arvensis, Chenopodium murale, Avena fatua, Phalaris minor* extracts in different solvents.

activity when compared with reducing power assay of extracts of other 3 plants with various solvents (Figure 2).

Similarly, methanol-chloroform extract of the plants showed very good results for scavenging of hydrogen peroxide (Figure 3). The extract of *Convolvulus arvensis* showed the highest scavenging of hydrogen peroxide (IC_{50} = 165.01 µg/ml) followed by hydrogen peroxide scavenging activity > *Avena fatua* (IC_{50} = 165.91) > *Chenopodium murale* (IC_{50} = 168.65) > *Phalaris minor* (IC_{50} = 179.74). Their IC_{50} values were compared with the IC_{50} value of ascorbic acid as reference (148.48 µg /ml) (Table 1).

Qualitative phytochemical screening study showed that flavonoids, saponins tannins, glycosides and alkaloids were present in all the selected four weeds plants namely *Convolvulus arvensis, Chenopodium murale, Avena fatua* and *Phalaris minor* (Table 2). The terpenoids were also found in all of the above mentioned weeds plants except *Chenopodium murale*. Steroids were absent in *Avena fatua, Phalaris minor* as well as in *Convolvulus arvensis* but they were present in *Chenopodium murale*. Anthraquinones were found in *Phalaris minor* and *Convolvulus arvensis* but were absent in *Avena fatua and Chenopodium murale*. The quantitative phytochemical screening examination revealed that *Avena fatua* has highest percentage of flavonoid contents (19.99% ± 0.09) followed by *Chenopodium murale* (17.99% ± 0.01), *Convolvulus arvensis* (15.78% ± 0.10) and *Phalaris minor* (10.65% ± 0.12) respectively (Table 3). Similarly *Chenopodium murale* (25.76% ±0.01) produced highest percentage of crude tannins in contrast to *Convolvulus arvensis* (21.31% ± 0.07), *Avena fatua* (19.33% ± 0.06) and *Phalaris minor* (8.11% ± 0.03) individually. High percentage of alkaloids were yielded by *Convolvulus arvensis* (3.71% ± 0.08) followed by *Chenopodium murale* (3.01% ± 0.05) as well as *Avena fatua* (2.29% ± 0.02) and *Phalaris minor* (1.31% ± 0.02) respectively whereas saponins percentage was less in all these plants of weeds but *Avena fatua* produced higher percentage of saponins (0.95% ± 0.05) succeeded by *Phlaris minor* (0.90% ± 0.05), *Convolvulus arvensis* (0.85% ± 0.03) and *Chenopodium murale* (0.68% ± 0.01) respectively (Table 3).

All plant extracts with different solvent systems had shown reasonable TPC, TAAC, DPPH scavenging activity, reducing power capacity and hydrogen peroxide scavenging

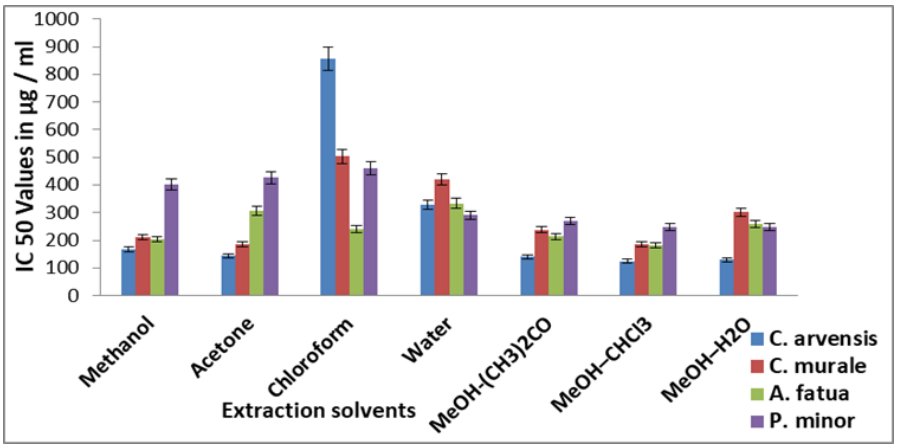

Figure 3. H_2O_2 Scavenging assay for *Convolvulus arvensis, Chenopodium murale, Avena fatua, Phalaris minor* extracts in different solvents.

Table 2. Confirmation of different components in selected four weeds plants.

Weed Plants	Flavo-noids	Saponi-ns	Tanni-ns	Stero-ids	Glycosi-des	Alkal-oids	Anthreq-uinones	Terpen-oids
Avena fatua	+	+	+	-	+	+	-	+
Phalaris minor	+	+	+	-	+	+	+	+
Convolvulus arvensis	+	+	+	-	+	+	+	+
Chenopodium murale	+	+	+	+	+	+	-	-

- shows absence of particular component while, + shows presence of particular component.

Table 3. Quantitative (%) phytochemical components of selected weed plants associated with wheat crops.

Weed Plants	Alkaloids	Saponins	Flavonoids	Tannins
Avena fatua	2.29±0.02	0.95±0.05	19.99±0.09	19.33±0.06
Phalaris minor	1.31±0.02	0.90±0.05	10.65±0.12	8.11±0.03
Convolvulus arvensis	3.71±0.08	0.85±0.03	15.78±0.10	21.31±0.07
Chenopodium murale	3.01±0.05	0.68±0.01	17 .99±0.01	25.76±0.01

activity, however, methanol-chloroform solvent was found the best solvent for all of the above assays. While, the chloroform solvent showed the highest value for TFC.

4. Conclusion

In this study different weeds namely *Convolvulus arvensis, Chenopodium murale, Avena fatua and Phalaris minor*, extracted with different solvents (e.g methanol, acetone, chloroform, water, methanol-acetone, methanol-chloroform and methanol-water) were studied for TPC, TFC and antioxidant activity. For this purpose different methods were used such as, Folin-Ciocalteau Reagent, Aluminium Chloride Colorimetric, phosphomolybdate, DPPH, reducing potential and H_2O_2 scavenging assays.

Our findings show that methanol-chloroform extract of *Convolvulus arvensis, Chenopodium murale, Avena fatua and Phalaris minor* has the highest amount of TPC, TAAC and exhibited lowest IC 50 values in DPPH and H_2O_2 scavenging assays (Figure 3), which in turn indicating greater antioxidant activities respectively. These plant extracts may be administered by the people to treat different illnesses such as infectious diseases and it can serve as dietary source of natural antioxidants. The phytochemical screening of these weeds confirmed the presence of alkaloids, flavonoids, saponins, steroids and tannins. The antioxidants activity shown by these weeds can be attributed to the presence of specific quantities of polyphenols as well as flavonoids. So, these weeds can be the best sources of natural antioxidants. Up to my knowledge, no work has been done before for investigation of best solvent to extract these weeds plants for determination of TPC, TFC, TAAC as well as reducing potentials of weeds plants. Further study should be formed to know the interaction of various solvent with bioactive components found in these weeds. This study would be helpful in selection of best solvents for extraction of natural antioxidants, phenolic, flavonoids, in order to get maximum benefits from natural sources of bioactive components.

The future perspective of this work is that different bioactive components extracted via different solvents can be evaluated for their in vivo activity in animal models. And they can be used for different applications especially the antioxidants can be used for protection against free radicals, cosmetics product, as anti-aging, in heart diseases, cancer and other diseases.

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Erratum

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