

Full Length Research Paper

Antioxidant and antimicrobial activities of Chowlai (*Amaranthus viridis* L.) leaf and seed extracts

Muhammad Javid Iqbal^{1*}, Sumaira Hanif¹, Zahed Mahmood¹, Farooq Anwar² and Amer Jamil¹

¹Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan.

²Department of Chemistry, University of Sargodha, Sargodha-40100, Pakistan.

Accepted 19 June, 2012

The present study was conducted to evaluate the phenolics, antioxidant and antimicrobial activities of leaf and seed extracts from an edible herb namely *Amaranthus viridis* L. The extract yields of active components, produced using pure and aqueous methanol, from the leaves and seeds ranged from 5.4 to 6.0 and 2.4 to 3.7%, respectively. The extracts contained appreciable levels of total phenolic contents (1.03 to 3.64 GAE, g/100 g) and total flavonoid contents (18.4 – 5.42 QE, g/100 g) and also exhibited good 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity as revealed by IC₅₀ (14.25 - 83.43 µg/ml). Besides, the tested extracts showed considerable antimicrobial activity against selected bacterial and fungal strains with minimum inhibitory concentrations (MIC) ranging from 179-645 µg/ml. Of the parts tested, the seed extracts exhibited superior antioxidant and antimicrobial activity. It was concluded that *A. viridis* leaf and seed can be explored as a potential source for isolation of antioxidant and antimicrobial agents for uses in functional food and pharmaceuticals.

Key words: *Amaranthus viridis*, phytochemical constituents, minimum inhibitory concentrations (MIC), total phenolics, total flavonoids, 1,1-diphenyl-2-picrylhydrazyl (DPPH), radical scavengers.

INTRODUCTION

Amaranthus viridis L. (Amaranthaceae), commonly known as "Chowlai", is a fast growing herb mainly cultivated in Asia, Africa and Latin America (Amin et al., 2006). Being resistant to drought, hot climate and pests, and with little requirements for its cultivation, this pseudo-cereal has attracted much attention as an important food commodity (Sexna et al., 2007). In the last decade, the use of amaranth has expanded not only in the common diet, but also in diet of people with celiac disease or allergies to typical cereals (Berti et al., 2005). Reactive oxygen species (ROS) and reactive nitrogen species produced as a result of oxidation have been shown to be linked with different degenerative disorders such as aging, inflammation, cancer, cardiovascular complications, and osteoporosis (Wilcox, 2004). Interest in search for new natural antioxidants has grown over the

past few years because of their preventive role in protecting from oxidative-stress related chronic diseases (Halvorsen et al., 2002).

Existence of microorganisms causes food spoilage and results in deterioration of the quality and quantity of processed food products. Some plant-based biologically active compounds isolated from herbs have been explored for the growth inhibition of pathogenic microbes because of their antimicrobial potential (Abubakar et al., 2008). The medicinal value and multiple biological functionalities of several plants are defined by their phytochemical constituents (Fallah et al., 2005). Many herbal species being a promising source of bioactive compounds such as phenolics, anthocyanins, flavonoids, and carotenoids, are usually used to impart flavor and enhance the shelf-life of dishes and processed food products, recently reported work was (Nisar et al., 2010a, b, 2011; Qayum et al., 2012; Zia-Ul-Haq et al., 2008, 2011a, b, 2012). Due to their high antioxidant potency, the consumption of many such plants species is recommended (Ozsoy et al., 2009). Antioxidant

*Corresponding author. E-mail: imjavid@gmail.com or amerjamil@yahoo.com.

properties of green leafy vegetables and herbs including different amaranth species have been preliminarily studied (Ozbucak et al., 2007).

The main aim of the present study was to evaluate the phenolic compounds, antioxidant and antimicrobial activities of pure and aqueous methanol extracted components from leaves and seeds of locally grown *Amaranthus viridis* plants to explore their potential pharmaceutical and/or functional food uses.

MATERIALS AND METHODS

Collection and pretreatment of plant material

The leaves and seeds of fully matured *A. viridis* L. were collected during June to July 2009, from the local fields of Faisalabad, Pakistan, and identified by the Department of Botany, GC University Faisalabad, Pakistan. Collected specimens were dried at room temperature and stored in polyethylene bags at 4°C.

Chemical and reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, Folin-Ciocalteu reagent, sodium nitrite, butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Co (St. Louis, USA) and anhydrous sodium carbonate, methanol and ethanol used were obtained from Merck (Darmstadt, Germany). All culture media antibiotic, discs and sterile solution of 10% (v/v) DMSO in water were purchased from Oxoid (Hampshire, UK).

Preparation of *A. viridis* extracts

Ground (80 mesh) leaf and seed samples (100 g each) were extracted separately with 1000 ml absolute methanol and 80% methanol (80:20, methanol: water, v/v) using an orbital shaker (Gallenkamp, UK) for 12 h at room temperature. The extracts were separated from solids by filtering through Whatman No. 1 filter paper. The residues were extracted thrice and the extracts were collected. The solvent was removed under vacuum at 45°C, using a rotary vacuum evaporator (N-N Series, Eyela, Rikakikai Co. Ltd. Tokyo, Japan) and stored at 4°C till further analysis.

Phytochemical screening

The methanol extracts of the tested plant material were screened for the presence of various phytoconstituents such as phlobatannins, tannins, alkaloids, terpenoids, glycosides, flavonoids, and phenolic compounds (Abubakar et al., 2008).

Antioxidant activity

Determination of total phenolic contents (TPC)

Total phenolic contents (TPC) were determined using the Folin-Ciocalteu reagent method and gallic acid was used as gallic acid equivalent (GAE) (Amin et al., 2006).

Determination of total flavonoid contents (TFC)

The total flavonoid contents (TFC) in the leaf and seed extracts

were determined following the modified procedure of Edeoga et al. (2005), and quercetin was used as standard as quercetin equivalent (QE).

DPPH radical scavenging assay

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical assay was carried out spectrophotometrically (Miliauskas et al., 2004). The percent inhibition was calculated as:

$$I (\%) = 100 \times (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}})$$

Where A_{blank} is absorbance of the control reaction (containing all reagents except the test sample), and A_{sample} is the absorbance of test samples. Extract concentrations providing 50% inhibition (IC_{50}) values were calculated from the plot of percentage scavenging versus extracts concentration.

Antimicrobial activity

Microbial strains

The *A. viridis* leaf and seed extracts were individually tested against a panel of microorganisms (locally isolated), including two bacteria, *Staphylococcus aureus* and *Escherichia coli*, and two pathogenic fungi, *Fusarium solani* and *Rhizopus oligosporus*. The selected strains have strong pathogenic activities against plant and animals that lead to a significant loss of lives and food. The pure bacterial and fungal strains were obtained from the Bioassay section, Protein Molecular Biochemistry laboratory, Department of Chemistry and Biochemistry, University of Agriculture Faisalabad, Pakistan. The bacterial strains were cultured at 37°C overnight, while fungal strains were cultured overnight at 28°C in an incubator (Memmert, Germany).

Disc diffusion method

The antimicrobial activity of the leaf and seed extracts was determined by using disc diffusion method (CLSI, 2007). The discs (6 mm in diameter) were impregnated with 20 µg/ml sample extracts (20 µg/disc) and placed on inoculated agar. Rifampicin (20 µg/disc) (Oxoid) and fulconazole (20 µg/disc) (Oxoid) were used as positive reference for bacteria and fungi, respectively. Antimicrobial activity was evaluated by measuring the inhibition zones (in mm) by zone reader.

Minimum inhibitory concentration (MIC) assay for determination of antimicrobial activity

Incubator at 35 and 37°C; pipettes of various sizes (Gilson); sterile tips, 100, 200, 500 and 1000 µL; 5 ml multi-channel pipette; centrifuge tubes; vortex mixer; centrifuge (Fisons); Petri-dishes, sterile universal bottles; UV-spectrophotometer (Shimadzu) and sterile resazurin tablets (BDH Laboratory Supplies) were used. Isosensitest medium was used throughout this assay, as it is pH buffered. Although the use of Mueller Hinton medium was recommended for susceptibility testing (NCCLS, 2000), the isosensitest medium had comparable results for most of the tested bacterial strains.

Use of standardized bacterial colony numbers

The method wherein turbidity is compared to McFarland standards

usually 0.5, is not able to give a standardized number of CFU for bacterial strains only because this is operator-driven and is thus subjective. It also makes it difficult to compare different bacterial species as they have differing optical densities. A final concentration of 5×10^5 CFU/ml was adopted for this assay. Thus, different strains and different bacterial species could be compared.

Preparation of microbial culture

Using aseptic techniques, a single colony was transferred into a 100 ml bottle of isosensitest broth capped and placed in incubator overnight at 35°C. After 12 to 18 h of incubation, using aseptic preparation and the aid of a centrifuge, clean samples of bacteria and fungi were prepared. The broth was spun down using a centrifuge at 4000 rpm for 5 min with appropriate aseptic precautions. The supernatant was discarded into an appropriately labeled contaminated waste beaker. The pellet was re-suspended using 20 ml of sterile normal saline and centrifuged again at 4000 rpm for 5 min. This step was repeated until the supernatant was clear. The pellet was then suspended in 20 ml of sterile normal saline and was labeled as Bs. The optical density of the Bs was recorded at 500 nm, and serial dilutions were carried out with appropriate aseptic techniques until the optical density was in the range of 0.5 to 1.0. The actual number of colony forming units was calculated from the viability graph. The dilution factor needed was calculated and the dilution was carried out to obtain a concentration of 5×10^6 CFU/ml (Winn and Koneman, 2006).

Preparation of resazurin solution

The resazurin solution was prepared by dissolving a 270 mg tablet in 40 ml of sterile distilled water. A vortex mixer was used to dissolve tablet and homogenous solution formation.

Preparation of the plates

Plates were prepared under aseptic conditions. A sterile 96 well plate was labeled. A volume of 100 μ L of test material in 10% (v/v) dimethylsulphoxide (DMSO)/sterile water (10 mg/ml for crude extracts) was pipetted into the first row of the plate. To all other wells, 50 μ L of nutrient broth or normal saline was added. Serial dilutions were performed using a multichannel pipette. Tips were discarded after use such that each well had 50 μ L of the test material in serially descending concentrations. To each well, 10 μ L of resazurin indicator solution was added. Furthermore, using a pipette, 30 μ L of 3.3 \times strength isosensitised broths was added to each well to ensure that the final volume was single strength of the nutrient broth. Finally, 10 μ L of bacterial suspension (5×10^6 CFU/ml) was added to each well to achieve a concentration of 5×10^5 CFU/ml. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each plate had a set of controls: a column with a broad-spectrum antibiotic as positive control (usually ciprofloxacin in serial dilution), a column with all solutions with the exception of the test compound, and a column with all solutions with the exception of the bacterial solution adding 10 μ L of nutrient broth instead. The plates were prepared in triplicate, and placed in an incubator set at 37°C for 18 to 24 h. The color change was then assessed visually. Any color change from purple to pink or colorless was recorded as positive. The lowest concentration at which color change occurred was taken as the MIC value. The average of three values was calculated and that was the MIC for the test material and microbial strain.

Statistical analysis

Values are given as means \pm standard deviation (SD) of each

measurement. Where appropriate, the data were tested by one-way ANOVA using Minitab 15. Pearson correlation coefficients and p -values were used to show correlations and their significance. Differences of $P < 0.05$ were considered significant (Steel et al., 1997).

RESULTS AND DISCUSSION

Extract yields

Yields (g/100 g) of *A. viridis* leaf and seed extracts produced using different extraction solvents are given in Table 1. Maximum extract yields of antioxidant components from leaves and seed were obtained with 80% methanol (6.0 g/100 g) and 100% methanol (3.7 g/100 g), respectively. The present results demonstrated a significant ($P < 0.05$) variations for the seed extract yields, but non-significant ($P > 0.05$) for the leaves extracts yields between the solvents used. The present extraction yields are lower than those reported by Ozsoy et al. (2009) in water, methanol and ethyl-acetate extracts of *Amaranthus* species (13.09 to 20.46 g/100 g). Kyung et al. (2006) also reported the strong antioxidant and antimicrobial activity of *Amaranthus cruentus* in diabetic rat.

Phytochemical constituents

In plants, the secondary metabolites function to attract beneficial and repel harmful organisms, serve as phytoprotectants and respond to environmental changes. In humans, however, the compounds have beneficial effects including antioxidant, anti-inflammatory, modulation of detoxification enzymes, stimulation of the immune system, modulation of steroid metabolism and antibacterial and antiviral effects (Lipkin et al., 2004). Table 2 shows the qualitative data for the presence of different phytochemicals in leaves and seeds of *A. viridis*. The results from the current study indicate that *A. viridis* leaves and seed extracts contained varied types of pharmacologically active compounds with antimicrobial potential. The commonly identified components in this species included flavonoids, cyanogenic glycoside, saponins, tannin, and phlobatannins. The results, especially for alkaloids, indicate levels comparatively similar to many medicinal plants (Elizbith et al., 1999; Okwu and Josiah, 2006), thus supporting the medicinal uses of this species.

Antioxidant activity

DPPH radical scavenging assay

We investigated DPPH free radical scavenging activity of *A. viridis* leaves and seeds extracts produced through

Table 1. Percentage yield extracts from leaves and seeds of *Amaranthus viridis*.

Plant used	Extract	Percent yield (g/100 g)
Leaves	100% Methanol	5.4 ± 0.13 ^a
	80% Methanol	6.0 ± 0.14 ^a
Seeds	100% Methanol	3.7 ± 0.11 ^{ab}
	80% Methanol	2.4 ± 0.04 ^b

Values are mean ± SD of three samples analyzed individually in triplicate. Different letters in superscript indicate significant and non-significant differences with solvents.

Table 2. Phytochemical constituents of leaf and seed extract of *Amaranthus viridis*.

Phytochemical constituent	<i>Amaranthus viridis</i>	
	Leaf	Seed
Tannins	+	+
Phlobatannins	+	+
Saponins	-	-
Flavonoids	+	+
Terpenoids	-	-
Cardiac glycosides	+	+

+Represents presence of the phytoconstituents; represents absence of the phytoconstituents.

Table 3. Antioxidant activity of *Amaranthus viridis* leaf and seed methanolic extracts.

Plant used	Extracts	DPPH, IC ₅₀ (µg/ml)	TP contents*	TF contents**
Leaves	100% Methanol	14.25 ± 0.712 ^a	1.03 ± 0.5 ^a	2.78 ± 0.20 ^b
	80% Methanol	83.43 ± 3.84 ^a	2.89 ± 0.2 ^a	18.4 ± 0.30 ^a
Seeds	100% Methanol	46.50 ± 2.97 ^a	3.64 ± 0.4 ^a	5.42 ± 0.20 ^a
	80% Methanol	75.91 ± 3.03 ^a	3.20 ± 0.39 ^a	2.51 ± 0.07 ^b

Values are mean ± SD of samples analyzed in triplicate. *, Total phenolic contents in gallic acid equivalent; **, Total flavonoid contents in quercetin equivalent.

extraction by pure and aqueous methanol. The free radical scavenging capacity increased with increasing extracts concentrations. The leaves and seed extracts showed good hydrogen-donating ability in the presence of DPPH stable radicals (Table 3), with IC₅₀ (the extract concentration providing 50% inhibition) values ranging from 14.25 – 83.43 and 46.50 – 75.91 µg/ml, respectively. When compared with the synthetic antioxidant BHT (15.7 µg/mL), the tested extracts offered slightly lower activity except 100% methanol leaf extract (14.25 µg/ml).

These results were consistent with previous observation that *Amaranthus* varieties contained radical scavenging agents that could directly react with and quench stable DPPH radicals (Oboh, 2005). The ability of an *Amaranthus paniculatus* extracts to act as a free radical scavenger or hydrogen donor has also been

reported previously (Amin et al., 2006). The betalians from plants in the family Amaranthaceae exhibit strong antiradical activity, with IC₅₀ values ranging from 3.4 to 8.4 µM, and representing a new class of dietary antioxidants (Cai et al., 2005). These results showed that the methanol leaf extract contained strongest DPPH free radical scavenging compounds; the efficacy of those was quite comparable with the positive control BHT (15.7 µg/ml).

Total phenolic and total flavonoid contents

The total phenolic content (TPC) and total flavonoid content (TFC) of *A. viridis* leaf and seed extracts are presented in Table 3. The differences in the amount of TP and TF may be due to varied efficacy of the extracting

Table 4. Antimicrobial activity and minimum inhibitory concentration of *Amaranthus viridis* leaf and seed methanolic extracts against the selected strains of bacterial and fungal strain.

Part of plant used	Extracts	<i>S. aureus</i>		<i>E. coli</i>		<i>F. solani</i>		<i>R. oligosporus</i>	
		Zone size	MIC	Zone size	MIC	Zone size	MIC	Zone size	MIC
Leaves	100% Methanol	24 ± 1.9 ^{ab}	179 ± 1.28 ^{ab}	16 ± 1.4 ^a	398 ± 1.26 ^a	17 ± 1.8 ^{ab}	436 ± 1.46	19.0 ^c ± 1.4	302 ± 1.36 ^c
	80% Methanol	23 ± 2.4 ^{ab}	182 ± 1.68 ^{ab}	12 ± 0.3 ^b	603 ± 2.36 ^b	15 ± 2.2 ^b	491 ± 1.76 ^b	18.0 ^b ± 0.9	352 ± 2.42 ^b
Seeds	100% Methanol	16 ± 2.1 ^c	428 ± 1.36 ^c	11 ± 1.5 ^b	639 ± 1.26 ^b	13 ± 1.7 ^c	602 ± 1.89 ^c	15.0 ^{bc} ± 1.0	482 ± 1.27 ^{bc}
	80% Methanol	18 ± 1.5 ^c	403 ± 2.36 ^c	10 ± 1.2 ^b	645 ± 1.48 ^b	10 ± 1.5 ^c	641 ± 2.38 ^c	13.0 ^c ± 1.6	547 ± 2.38 ^c
Control	Methanol	26 ± 3.1 ^a	141 ± 1.31 ^a	17 ± 1.7 ^a	381 ± 2.39 ^a	18 ± 1.9 ^a	391 ± 2.48 ^a	16 ^a ± 2.1	436 ± 2.17 ^a

Values are mean ± SD of three samples analyzed individually in triplicate. Diameter of inhibition zone (mm) including disc diameter of 6 mm and MIC in µg/ml. Controls used are rifampicin and fulconazole for bacterial and fungal strains, respectively. Different letters in superscript indicate significant differences within solvents.

solvents to dissolve endogenous compounds. The ability of different solvents to extract TP and TF contents was of the order: for leaf 80% > 100% methanol and for seed 100% > 80% methanol. These values were higher than the reported values of *A. cruentus* (0.3 g/100 g) (Nisimba et al., 2007). Gorinstien et al. (2007) showed from the values obtained in their work, less phenolic content compared to the four reported *Amaranthus* varieties (107 g/kg). Amaranth plants have been reported as one of many vegetables rich in antioxidant compounds (Obadoni and Ochuko, 2001). The other reported total phenolic contents (TPC) for *Amaranthus* species ranged from 2.95 - 3.75 GAE, mg/100 g (Pasko et al., 2009).

Antimicrobial activity

The antimicrobial activity for *A. viridis* leaf and seeds extracts against food-borne and pathogenic microorganisms is depicted in Table 4. The extracts showed considerable antimicrobial activity against all the strains tested, particularly against Gram-positive bacterium. The results from MIC indicated that

E. coli was most sensitive microbe tested, showing the largest inhibition zones (24 mm) for leaves and minimum (18 mm) for seeds extracts. The least activity is inhibited by 80% methanol seeds extract against *R. oligosporus*, with the smallest zone (13 mm).

In general, the antimicrobial activity of the tested *A. viridis* leaves and seeds extracts was comparable with the standard drugs, streptomycin and mecanozol. In support to our present data, in a previous study, isolation of the antifungal peptide from the *A. viridis* seed extracts has been done (Lipkin et al., 2004). Resazurin is an oxidation-reduction indicator used for the evaluation of cell growth. The effectiveness of resazurin oxidation compound is higher for the leaves extracts of selected Amaranth species. The current results support the earlier findings which demonstrate the presence of antimicrobial activity in seeds of Amaranthaceae (Cia et al., 2005).

Conclusion

The results from the current study indicate that

A. viridis leaves and seeds extracts contained varied types of pharmacologically active compounds with antioxidant and antimicrobial activities which differed between the two parts and extraction solvents used. Further research work involving more detailed *in vitro* and *in vivo* investigations to establish which component of the extracts offer best antioxidant and antimicrobial activity is needed. Detail toxicological studies are also recommended to explore the uses this plant extracts as natural food preservative. The production of bioactive components from such indigenous resources and their utilization as potential natural food preservatives could be of high economic value.

REFERENCES

- Abukakar MG, Ukwuani AN, Shehu RA (2008). Phytochemical screening and antibacterial activity of *Tamarindus indica* Pulp extract. J. Biochem., 3: 134-138.
- Amin I, Norazaidah Y, Hainida E (2006). Antioxidant activity and phenolic content of raw and blanched *Amaranthus* species. J. Food. Chem., 94: 47-52.
- Berti C, Riso P, Brusamolino A, Porrini N (2005). Effect on appetite control of minor cereal and pseudocereal products. British. J. Nutr., 94: 850-858.
- Cai YZ, Sun M, Croke H (2005). Characterization and

- application of betalain pigment of plant *Amaranthaceae*. *Trend. Food. Sci. Technol.*, 16: 370-376.
- Edeoga HO, Okwu DE, Mbaebie BO (2005). Photochemical constituents of some Nigerian Medicinal plants. *Afr. J. Biotechnol.*, 4: 685-688.
- Elizabeth M, Johnson AS, Warnock DW (1999). Comparison of E-test and broth microdilution methods for antifungal drug susceptibility testing of molds. *J. Clin. Microbiol.*, 37: 1480-1483.
- Fallah HSM, Alavian HR, Heydari MR, Abolmaali K (2005). The efficacy of Liv-52 on liver cirrhotic patients: a randomized, double blind, placebo-controlled first approach. *J. Phytomed.*, 12: 619-624.
- Gorinstien S, Vargas OJM, Jaramillo NO, Salas IA, Ayala ALM, Avila PA, Toledo F, Katrich E, Trekhenberg S (2007). The total polyphenols and antioxidant potential of some selected cereals and pseudo-cereals. *J. Europ. Food. Res. Technol.*, 225: 321-328.
- Halvorsen BL, Holte K, Myhrstad MC, Barikmo I, Hvattum E, Remberg SF (2002). A systematic screening of total antioxidants in dietary plants. *J. Nutr.*, 132: 461-471.
- Kyung KH, Jeong KM, Yon CH, Eun-Ki K, Hoon SD (2006). Antioxidant and antidiabetic effects of Amaranth (*Amaranthus cruentus*) in diabetic rat. *Cell Biochem. Funct.*, 24: 1.
- Lipkin A, Veronika A, Aleksandra N, Aleksey B, Eberhardt K, Mikhae LB, Eugene G, Tsezi E (2004). An antimicrobial peptide Ar-AMP from amaranth (*Amaranthus retroflexus* L.) seeds. *J. Sci. Direct.*, 34: 93-95.
- Miliauskas PR, Venskutonis TM, Van-Beek TA (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *J. Food. Chem.*, 85: 231-237.
- National Committee for Clinical Laboratory Standards (2000). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically-. Fifth Edition Approved standard M7-A4. NCCLS, Wayne, PA
- Nisar M, Kaleem WA, Qayum M, Hussain A, Zia-UI-Haq M, Ali I, Choudhary MI (2011). Biological screening of *Zizyphus oxyphylla* Edgew stem. *Pak. J. Bot.*, 43 (1) 311-317.
- Nisar M, Kaleem WA, Qayum M, Zia-UI-Haq M, Ali I, Choudhary MI (2010b). Biological screening of *Zizyphus oxyphylla* edgew leaves. *Pak. J. Bot.*, 42(6): 4063-4069.
- Nisar M, Qayum M, Shah MR, Kaleem WA, Ali I, Zia-UI-Haq M (2010a). Antimicrobial screening of *Impatiens bicolor royle*. *Pak. J. Bot.*, 42(1): 523-526.
- Nisimba R, Hiroe KY, Yotaro K (2007). Antioxidant activity of various extracts and fractions of *Chenopodium quinoa* and *Amaranthus viridis* specie. Seeds. *J. Food. Chem.*, 51: 2144-2155.
- Oboh G (2005). Effect of blanching on the antioxidant properties of some tropical green leafy vegetables. *J. Food. Sci. Tech.*, 38: 513-517.
- Okwu DE, Josiah C (2006). Evaluation of the chemical composition of two Nigerian Medical plants. *Afr. J. Biotech.*, 5: 357-361.
- Ozbucak TB, Ergen AO, Yalcin S (2007). Nutritional content of some wild edible plants in Central Black region of Turkey. *J. Nat. Eng. Sci.*, 1: 11-13.
- Ozsoy N, Tilmaz OK, Yanardag CR (2009). *In-vitro* antioxidant activity of *Amaranthus lividus* L. *J. Agric. Food. Chem.*, 116: 867-872.
- Pasko P, Henryk B, Gorinstein ZP, Folta SM, Zachwieja Z (2009). Anthocyanins, total polyphenols and antioxidant activity in Amaranth and Quinoa seeds and sprouts during their growth. *J. Agric. Food. Chem.*, 115: 994-998.
- Qayum M, Nisar M, Shah MR, Zia-UI-Haq M, Kaleem WA, Marwat IK (2012). Biological screening of oils from *Impatiens bicolor* Royle. *Pak. J. Bot.*, 44: 355-259.
- Saxena R, Venkaiah K, Anitha P, Venu L, Raghunath M (2007). Antioxidant activity of commonly used plant foods of India: Contribution of their phenolic content. *Int. J. Food. Sci. Nutr.*, 58: 250-260.
- Steel FGD, Torrie JH, Dickey DA (1997). Principles and procedures of A Biometrical approach 3rd edition. W.C. MCM Graw Hill, New York.
- The Clinical Laboratory Standards Institute CLSI (2007). Agar Dilution and Disk Diffusion Susceptibility Testing of *Campylobacter* spp. *J. Clin. Microbiol.*, 45: 2758-2759.
- Willcox JK, Ash SL, Catignani GL (2004). Antioxidants and prevention of chronic diseases. *Crit. Rev. Food. Sci. Nutr.*, 44: 275-295.
- Winn WC, Koneman EW (2006) *Methods in Microbiology*. Lippincott Williams & Wilkins, 355: 1200-1390.
- Zia-UI-Haq M, Ahmad M, Mehjabeen, Jehan N, Ahmad S, Qayum M, Marwat IK (2011c). Antimicrobial screening of selected flora of Pakistan. *Arch. Biol. Sci.*, 63(3): 691-695.
- Zia-UI-Haq M, Shahid SA, Ahmad S, Qayum M, Khan I (2012). Antioxidant potential of various parts of *Ferula assafoetida* L. *J. Med. Plants. Res.*, 6: 3254-3258.