Screening of *Aerva javanica* and *Linum ustitatissimum* for their anti-diabetic and anti-oxidant activity

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Abstract: The present study investigates anti-diabetic and anti-oxidant potentials of the aerial parts of *Aerva javanica* and seeds of *Linum ustitatissimum*. Our results revealed that maximum antioxidant activity was found in hexane fractions of both plants. *Linum ustitatissimum* recorded maximum antioxidant value of 15.77mmol/L while *Aerva javanica* had antioxidant activity of 16.07mmol/L. Anti-diabetic activities were also evaluated using normal rats, induced diabetic (untreated) rats and treated diabetic rats. *Aerva javanica* revealed a significant potential in decreasing blood glucose level to 77.08mg/dl and body weight 76.30mg/kg while *Linum ustitatissimum* reduced blood glucose level to 84.20mg/dl and body weight 83.090mg/kg. From these results it can be concluded that both plants possesses anti-diabetic and anti-oxidant activity.

Keywords: Anti-diabetic, anti-oxidant, *Aerva javanica, Linum ustitatissimum.*

INTRODUCTION

The basic requirements of world population including food and medicine are fulfilled through native flora and fauna. It has been estimated that 60% of world population both in developing and developed countries use native plants for their basic health care and dietetic needs. Approximately 10% of the 250,000 species of higher plants are used globally for treating various health problems and are also known possesses antimicrobial properties (Bakht *et al.,* 2011 a, b, c, 2012; 2013 a,b,c; 2014 a,b,c; 2015; Nasir *et al*., 2015; Ullah *et al*., 2015; Zakir *et al*., 2015; Chaun *et al*., 2015; Bilal *et al*., 2016; Wajid *et al*., 2016 a, b; Amjad *et al*., 2016; Anwar *et al*., 2016; Shad *et al*., 2016). India with about 45,000 species in which 3000 to 6000 species are used therapeutically (Abiramasundar *et al*., 2011). Bangladesh has 5000 plant species in which more than 500 plants are customarily used as medicines. Likewise, 10% of 6000 species of Pakistani flora are important components in the cure system of local inhabitants.

Diabetes mellitus (DM) is the commonest endocrine disorder that affects vision, cause renal failure, atherosclerosis etc. According to International Diabetes Federation, approximately 285 million people are diabetic in the world whereas 7.1 million people in Pakistan suffer from diabetes (Ahmad *et al*., 2009). Pakistan has approximately 6000 species and about 200 to 700 species are claimed to possess anti-diabetic potential. The extracts and bioactive ingredients of many of Pakistani flora are used as anti-diabetic drugs in traditional way of healing.

Flax or Linseed **(***Linum ustitatissimum*) belongs to family Linaceae and locally known as Alsi. Five species of *Linum ustitatissimum* exists in Pakistan which are largely grown in Punjab and Sindh provinces. Both fiber and seed of flax are used for medicinal and commercial purposes. Flax fiber has the potential to treat heart related diseases (Hall *et al.,* 2011). It contains many physiologically essential compounds i.e. lignins, unsaturated fatty acids, flavonoids, saponins, tannins, retinol, Beta-carotene, vitamin B and some essential minerals like magnesium and manganese. Lignin is rich source of antioxidants and also acts as phytoestrogen. Omega-3-fatty acids have role in development of nervous system, maturation of retina and curing cardiovascular diseases. Further research has discovered that flax is active against breast and prostate cancers. Flax has been found to relieve swelling and oxidative tissue damage (Ullah *et al.,* 2009). It is also helpful in relieving bone disorder, intestinal problems, avert stone formation in kidney and anti-diabetic (Sudha *et al*., 2011; Mani *et al*., 2011; Ghule *et al*., 2012).

Aerva javanica belongs to family, Amaranthaceae locally known as "Sparai" whereas its English name is desert cotton. The plant is found in sandy, calcareous soils in semi-arid and arid regions of Africa and Asia and sub-continent. There are about 25 species of the genus *Aerva* found in Pakistan and India. The plant contains many important bio molecules, which comprise alkaloids, tannins, saponins, sulphates, flavonoids, lipids and carbohydrates. The plant is active against helmintis, used as anti-inflammatory and anti-oxidant agent (Vertichelvan *et al.,* 2002; Elayarani *et al*., 2011; Sethi and Sharma, 2011; Bhanot *et al.,* 2012; Ragavendran *et al*., 2012; Gajalakshmi *et al.,* 2012), helpful in diabetes (Khan *et al*. (2012), cough and infected lesions (Vetrichelvan and Jegadeesan, 2002). It is also used to treat urinary disorders (Waikar *et al*., 2007), respiratory complications, nasal hemorrhage and cracks (Kumar *et al*., 2007).

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MATERIALS AND METHODS

Plant collection

Alsi (*Linum ustitatissimum*) seeds were purchased from the local market of Peshawar KPK Pakistan. Similarly, plants of *Aerva javanica* were collected from the desert regions of Bannu district and suburbs of Khyber Pakhtunkhwa.

Sampling

The seeds of Alsi and aerial parts of the *Aerva javanica* were cleaned with distilled water. The species samples were dried in the shade at 24°C for one week till complete dryness. The dried plant material was grinded by tissue homogenizer to fine powder (Infinigen™ Tissue Mixer Mill, ACT Gene. The powdered materials were kept in plastic bags, sealed and stored at 4ºC in the refrigerator until used.

Preparation of crude extract

The powdered plant samples were macerated in four liters of methanol (Sigma-Aldrich) and kept at 24°C for one week. The mixture was stirred six times a day for thorough mixing and the solution was filtered (Whatman ™ Whatman UK). One litre of fresh methanol was added to the remaining residue and filtered again through Whatman filter paper and this process was repeated thrice. The filtered solution was evaporated with the help of a rotary evaporator (Rotavapor ^{R}-R 210/R215; BUCHIL Labortechnik AG). Methanol was separated at 45°C under vacuum pressure and a semi-solid extract was obtained (crude extract). The collected extract was mixed with known amount of water and partitioned with various solvents (Methanol, ethanol, DCM, ethyl acetate and hexane). Different fractions thus obtained were dried by rotary evaporator. The crude fraction was kept at 4°C in refrigerated until analyzed.

Determination of antioxidant activity by ABTS protocol

ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulphonate) analysis was carried out as described by Arnao *et al.* (2001). The stock solutions was prepared by mixing 7.4mM ABTS*+ and 2.6mM potassium per sulfate. The working solution was made by the addition of two stock solutions in equal quantities and then allowing them to react for 12h at room temperature in the dark. Dilution of the solution was done by mixing 1mL ABTS solution with 60mL methanol to get an absorbance of 1.170.02 units at 734nm. Fractions prepared from aerial parts of *Aerva javanica* and seeds of *Linum ustitatissimum* were reacted with 2850mL of the ABTS*+ for two hours in the dark and absorbance data recorded at 734nm. All the values obtained were compared with trolox (standard). Additional dilution was needed if the ABTS*+ value measured was over the linear range of the standard curve.

Estimation of antioxidant activity by DPPH protocol

Antioxidant activity by DPPH was measured as described by Lee *et al*. (2003) with certain modifications. The powdered materials of aerial parts of *Aerva javanica* and seeds of *Linum ustitatissimum* were extracted with methanol for 48 hrs and solvent was separated through vacuum evaporator. One ml of DPPH was mixed with ethanol and poured into about 2.5ml of each crude fraction. Antioxidant activity was measured by spectrophotometer at 517nm after 30 minutes of the reaction. All readings/data obtained was compared with trolox (standard). Radical scavenging activity was calculated using the following formula:

% radical scavenging activity = (control OD – sample OD/control OD) X 100.

IC_{50} represent the concentration of the sample required to scavenge 50% DPPH free radical.

Determination of antioxidant activity by FRAP protocol

In this protocol the antioxidant activity of the subject plants were measured by ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996). FRAP solution was made mixing 20mM ferric chloride hexa hydrate (BDH), 10mM of TPTZ (tripyridy1-s-triazine, Fluka Chemicals, Switzerland) and 300 mM acetate buffer (Riedel-de Haen, Germany). Plant material was mixed with 1.9ml of FRAP reagent. This mixture was systematically mixed and shaken for few minutes and reading was taken at 593 nm. The change in absorbance (D*A*593nm) between the final reading selected and the M1 reading was calculated for each sample and related to D*A*593nm of a FeII standard solution tested in parallel.

Anti-diabetic activity

Albino rats were used for the estimation of anti-diabetic potential of the subject plants. Rats were maintained under suitable temperature (22°C) and relative humidity (45-55%) for 12 hours and divided into seven groups. Group I consisted of normal healthy control rats, group II rats were chemically induced diabetes by Alloxan monohydrate (Sigma Aldrich, St. Louis, MO, USA) and not given crude extracts, Group III and Group IV were given crude extracts of *Aerva javanica* (250mg Kg^{-1} and 500mg Kg^{-1} body weight, respectively) (Viana *et al*., 2004). Similarly, Group V and VI were administered orally with crude extracts of *Linum ustitatissimum* (250 mg Kg^{-1} and 500mg/kg body weight, respectively). Group VII was given Glibenclamide (10mg Kg^{-1} body weight) as a control. Blood samples of all the tested groups were recorded at day 0, 5, 10 and 15 after crude extracts administration. Blood glucose level and body weight were measured and subsequently compared with Glibenclamid.

STATISTICAL ANALYSIS

The experiment was repeated in triplicate and MSTAT computer software was used for the analysis of the data. Standard deviation was calculated for each sample (Steel *et al.,* 1997).

Table 1: Antioxidant potential (IC_{50} (IC_{50} ±SD) of *Aerva javanica* using DPPH, ABTS and FRAP protocol.

SAMPLES	IC_{50} ±SD		
	DPPH	ABTS (TEAC)	*FRAP (mmol L^{-1})
Me.OH Extract	1.11±0.1951	0.613±0.1050	13.50±1.7312
DCM Extract	1.43±0.089	0.96±0.1276	9.47±0.8512
EtOAc Extract	1.416±0.150	0.86±0.06	11.56±1.8652
Hexane Extract	1.51±0.201	0.78±0.1417	16.07±1.1358
Trolox	1.05	1.09	-

Table 2: Antioxidant potential (IC_{50} ±SD) of *Linum ustitatissimum* using DPPH, ABTS and FRAP protocol.

SAMPLES	IC_{50} ±SD		
	DPPH	ABTS (TEAC)	*FRAP (mmol L^{-1})
Me.OH Extract	1.77±0.145	1.03±0.115	14.66±1.142
EtOAc Extract	1.326±0.150	0.56±0.06	10.37±1.8652
Hexane Extract	1.786±1.47	1.06±0.077	15.77±1.470
Trolox	1.05	1.09	-

SD represents Standard Deviation *Ferric reducing activity of plasma (expressed as mmole of $FeSO_4$ equivalent/liter of extract).

Table 3: Effect of crude fractions of *Aerva javanica* on blood glucose level (±SD, mg dl^{-1}) in induced diabetic and normal control rats

Groups and doses	Day Zero	Day 5	Day 10	Day 15
	Blood Glucose Level (±SD, mg dl^{-1})			
Normal Control	71.25±0.3650	73.02±0.07	72.17±0.2345	72.06±0.11015
Diabetic control	184.84±0.2059	186.01±0.1903	188.24±0.3843	191.93±0.4630
Aqu. MeOH (250ml)	191.03±0.0793	150.11±0.19924	109.13±0.1625	80.02±0.0721
Aqu. MeOH (500ml)	190.02±0.180093	147.01±0.0953	107.94±0.16623	77.08±0.4221
G.C 10	193.88±0.2302	155.14±0.2379	109.04±0.2389	75.13±0.3061

Table 4: Effect of crude fractions of *Aerva javanica* on body weight (g ±SD) in induced diabetic and normal control rats.

Groups and doses	Day Zero	Day 5	Day 10	Day 15
	Body Weight (g)			
Normal/Control	71.93±0.4266	73.24±0.335	72.96±0.3906	75.05±0.4194
Diabetic control	184.11±0.1556	185.14±0.1670	188.34±0.3704	191.11±0.3013
Aqu. MeOH (250ml)	183.09±0.1686	152.006±0.1101	108.37.±0.3622	80.97±0.2458
Aqu. MeOH (500ml)	189.12±0.2206	148.01±0.08082	106.03±0.06082	76.30±0.2836
G.C (10ml)	193.07±0.1357	155.03±0.06082	109.05±0.1342	75.11±0.2154

Table 5: Effect of crude fractions of *Linum ustitatissimum* on blood glucose (±SD, mg dl^{-1}) level induced diabetic and normal control rats.

Groups and doses	Day Zero	Day 5	Day 10	Day 15
	Blood Glucose Level (±SD, mg dl^{-1})			
Normal/Control	71.26±0.350	73.02±0.07	72.17±0.2345	72.06±0.11015
Diabetic control	184.84±0.2059	186.01±0.1903	188.24±0.3843	191.93±0.4630
Aqu. MeOH (250ml)	196.07±0.2003	156.80±0.1473	128.03.±0.1953	88.06±0.1184
Aqu. MeOH (500ml)	193.16±0.2946	147.961±0.4406	115.12±0.1715	84.20±0.2953
G.C (10ml)	193.88±0.2302	155.14±0.2379	109.04±0.2389	75.13±0.3061

SD represents Standard deviation

Table 6: Effect of crude fractions of *Linum ustitatissimum* on body weight (g ±SD) in induced diabetic and normal control rats

Groups and doses	Day Zero	Day 5	Day 10	Day 15
	Body Weight (g ±SD)			
Normal control	71.93±0.4266	73.24±0.3351	72.96±0.3906	75.06±0.4194
Diabetic control	184.11±0.1556	185.14±0.1670	188.34±0.3704	191.11±0.3013
Aqu. MeOH (250ml)	183.98±0.14106	156.11±0.2055	128.06.±0.0953	87.96±0.2722
Aqu. MeOH (500ml)	193.07±0.3874	147.961±0.1698	115.36±0.3704	83.09.±0.1352
G.C (10ml)	193.07±0.1357	155.03±0.06082	109.05±0.1342	75.11±0.2154

SD represents Standard Deviation

RESULTS

Antioxidant activity

The antioxidant activity of four different fractions of *Aerva javanica* using ABTS were determined and the average data of 3 replicates are presented in table 1. The data revealed that the antioxidant activity of DCM extract of *Aerva javanica* was maximum (IC_{50}: 0.96±0.1276) followed by ethyl acetate fraction (IC_{50}: 0.86±0.06) and hexane fraction (IC_{50}: 0.78±0.1417) when compared Trolox (table 1). Likewise, the methylated spirit fraction of *Linum ustitatissimum* had higher free radical scavenging activity (IC_{50}: 1.06±0.077) compared to methanolic fraction (1.03±0.115) (table 2). The crude fractions of the subject plants were also investigated for their antioxidant activity using DPPH protocol (table 1). It was observed that hexane fraction of *Aerva javanica* species had highest scavenging activity (IC_{50}: 1.51±0.201) followed by DCM fraction (IC_{50}: 1.43±0.089), however, DCM extract (IC_{50}: 1.43±0.089) and crude methanol fraction (IC_{50}: 1.11±0.1951) had the lowest scavenging activity. The data also suggested that methanol and hexane fractions of *Linum ustitatissimum* had good level of scavenging activity (IC_{50}: 1.786±1.47 and IC_{50}: 1.77±0.145 respectively) (table 2). Antioxidant activity was also measured by FRAP protocol. Our results suggested that all the crude fractions of both plants (*Aerva javanica* and *Linum ustitatissimum*) exhibited antioxidant activity in the range of 9.47±16.07mmol/L and 14.66±15.77mmol/L respectively. Hexane fraction of *Aerva javanica* measured highest activity (16.07±1.135 mmol/L) followed by aqueous methanol (13.50±1.731 mmol/L) and ethyl acetate (11.56±1.86mmol/L) fractions (table 1). Similarly, *Linum ustitatissimum* also possessed good level of antioxidant activity (table 2).

Anti-diabetic activity

To evaluate the anti-diabetic potential of the subject plants, both normal and induced diabetic rats were selected. Different extracts of both plants were used and their effect on blood glucose level and body weight was measured on day 5^{th}, 10^{th} and 15^{th} after treatment. Different extracts of both plants were used while glibenclamide was used as a standard. The data indicated that methanolic extracts of *Aerva javanica* (500ml) extract had maximum potential to decrease blood glucose level compared with other extracts. At day 15, this fraction reduced blood glucose level in diabetic rats from 190.02 mg/dl±0.1800 to 77.08mg/dl±0.4221 which was at par with glibenclamide treated diabetic rats (75.13mg/dl± 0.3061). *Aerva javanica* metanolic extract (250 ml) also decreased glucose level from 191.03mg/dl±0.0793 to 80.02mg/dl±0.0721 (table 3). *Linum usitatisumum* methanolic extracts (500ml) decreased glucose level from 193.16mg/dl±0.2946 to 84.20 mg/dl±0.2953 followed by methanolic extract of the same plant (250ml) and reduced glucose level from 196.07 mg/dl±0.2003 to 88.06 mg/dl±0.1184 (table 4). The effect of the tested plant extracts on body weight was also evaluated in the present investigation. Our results indicated that aqueous methanol (500ml) extract of *Aerva javanica* was most effective in controlling body weight of diabetic rats. It decreased body weight in diabetic rats to 76.30mg/dl±0.2836, which was at par with glibenclamide treated diabatic rates (75.11mg/dl±0.2154). Similarly, *Aerva javanica* methanolic extract (250ml) decreased body weight to 80.97mg/dl±0.2458 in diabetic rats (table 5). *Linum usitatisumum* fractions also significantly reduced body weight in diabetic rats. Aqueous methanol (500ml) of *Linum usitatisumum* significantly reduced body weight of diabetic rats to 83.09mg/dl±0.1352 at 15^{th} day of the treatment followed by aqueous methanol (250ml) of the same plant extract and decreased body weight to 87.96 mg/dl±0.2722 (t able 6).

DISCUSSION

Antioxidant compounds present in foods have been crucial in sustaining a healthy body by averting the risk of many fatal diseases including diabetes, cancer, scurvy, heart and metabolic abnormalities. Antioxidants protect the living body from the invasion of free radical oxidants through a mechanism known as “Free radical scavenging activity” which damage tissue structure and functions. Therefore, the search for potential free radical scavenging agent is imperative and indispensable**.** Different techniques and protocols have been employed by researchers to evaluate the antioxidant activity in different plants. The most important of these are ORAC (oxygen-Radical absorbance capacity), TRAP (Total Radical-Trapping antioxidant parameter), FRAP (Ferric Reducing ability of Plasma), DPPH (2, 2 diphemyl -1-Pcirylhydrazyl) and ABTs (2, 2 azinobis 3 ethylbenzathiazoline-6-Sulphonate) methods. However, in the present study DPPH, ABTS and FRAP methods were compared for the determination of antioxidant activity of subject plants.

The antioxidant activity of four different fractions of *Aerva javanica* using ABTS revealed that the free radical scavenging activity of DCM extract of *Aerva javanica* was highest followed by ethyl acetate fraction and hexane fraction using Trolox as a standard. Similarly, the hexane extract of *Linum ustitatissimum* had higher free radical scavenging activity compared to methanolic fraction. The possible reason of this moderate activity could be due to presence of the phytochemicals. The crude fractions of tested plants were also investigated for free radical scavenging activity by DPPH protocol. The data showed that hexane fraction of *Aerva javanica* species showed significant scavenging activity followed by methanol and DCM extract indicated the lowest scavenging activity. The data further suggested that methanol and hexane fractions of *Linum ustitatissimum* possessed good scavenging activity compared with other fractions. Free radical scavenging activity determined by FRAP revealed that all the crude fractions of both the tested plants (*Aerva javanica* and *Linum ustitatissimum*) exhibited different levels of antioxidant activity, however, hexane fraction of *Aerva javanica* measured highest activity followed by aqueous methanol fractions. From these results it can be concluded that both plants possesses considerable anti-oxidant activity as revealed measured by different estimation methods. Ahmed *et al*. (2006) and Goyal *et al*. (2011) revealed that *Aerva sp* contained flavanoids (kaempferol, quercetin, isorhamnetin, persinol, persinosides A and B) and exhibited high 2, 2-diphenyl-1-picrylhydrazyl antioxidant activity (IC_{50}= 110.74μg/mL) (Kumar *et al*., 2013). Hosseinian *et al*. (2007) and Chun *et al*. (2007) concluded that secoisolariciresinol (SECO 1) is the main lignan found in flaxseed (*Linum usitatissimum* L.) which contains a polymer called secoisolariciresinol diglucoside (SDG 2). SECO, SDG plays a very important role in many health issues including delay in the onset of type II diabetes. The health benefits of these compounds are partially due to their antioxidant properties. Our results are in agreement with Vertichelvan *et al.* (2002, Ullah *et al.* (2009), Sethi and Sharma (2011), Bhanot *et al.* (2012) Ragavendran *et al*. (2012), Gajalakshmi *et al.* (2012) and Muthukumaran *et al.* (2013).

For the determination of the anti-diabetic potential of *Aerva javanica* and *Linum usitatisumum*, normal and induced diabetic rats were selected. Different extracts of both plants were tested and their effect on blood glucose level and body weight was measured on day 5^{th}, 10^{th} and 15^{th} after treatment. The data showed that methanolic extracts of *Aerva javanica* had maximum potential to decrease blood glucose level compared with other extracts and at day 15 it reduced the blood glucose level in diabetic rats to a level at par with glibenclamide treated diabetic rats. Similarly, *Aerva javanica* and *Linum usitatisumum* metanolic extract also decreased glucose level when compared with glibenclamide treated diabetic rats. The current study also investigated response of body weight to different plant extracts. Our results revealed that aqueous methanol extract of *Aerva javanica* was most effective in controlling body weight of diabetic rates. It decreased body weight in diabetic rats, which was at par with glibenclamide treated diabatic rats. *Aerva javanica* and *Linum usitatisumum* methanolic extract also reduced body weight in diabetic rats. Aqueous methanol of *Linum usitatisumum* had significantly reduced body weight of diabetic rats at the 15^{th} day of the treatment followed by aqueous methanol of the same plant extract.

The alcoholic extract of *Aerva lanata* decreased the increased blood sugar level of alloxan-treated diabetic rats. *Aerva lanata* (400 mg/kg) treatment prevented a diabetic mice weight loss (Vetrichelvan and Jegadeesan 2002; Deshmukh *et al*., 2008; Goyal *et al*., 2011). Soluble fiber and other ingredients of flaxseed fractions may have an effect on insulin production, secretion and its mechanisms of action for maintaining plasma glucose homeostasis. It is reported that flaxseed decrease the postprandial blood glucose response in humans and lower incidence of type-1 diabetes (Cunnane *et al*., 1993; Jenkins *et al*., 1999; Prasad *et al*., 2000). Similar results are also reported by Broadhurst *et al*. (2000), Sudha *et al*. (2011) Mani *et al.* (2011) Ghule *et al.* (2012), Rajesh *et al.* (2012) and Khan *et al*. (2012) who revealed that both subject plants possesses promising anti-diabetic activities.

CONCLUSION

From these results it can be concluded that *Aerva javanica* and *Linum ustitatissimum* possesses significant antioxidant activity by employing DPPH, ABTS and FRAP. It was also observed that *Aerva javanica* had greater antioxidant potential than *Linum ustitatissimum.* Crude extracts of the subject plants contained anti-diabetic compounds to treat diabetic mellitus and regulating body weight.

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