EVALUATION OF THE ANTIOXIDANT AND ANTIDIABETIC PROPERTIES OF *PSAMMOGETON BITERNATUM* EDGEW. IN STREPTOZOTOCIN INDUCED DIABETIC MALE RATS

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ABSTRACT

The aim of present study was to screen various phytochemicals, antioxidant and antidiabetic investigation of ethanolic extract of whole plant of *Psammogeton biternatum* Edgew. which belongs to family Apiaceae. Phytochemical analysis showed the presence of phenols, flavonoids, alkaloid, terpenoids and tannins. Saponins were absent. The antioxidant activity was evaluated using different tests viz., Total phenolic content, total flavonoids content, FRAP, DPPH and β-carotene linoleic acid assay. It was observed that Total phenolic content showed maximum value i.e., 2.83±0.008 GAE mg/mL while Total flavonoid content was 2.50±0.01, and FRAP value were i.e., 1.92 ± 0.00 respectively. Antidiabetic potential of ethanolic extract of *P. biternatum Edgew.* (300mg/kg) were studied for 15 days in which following parameters were investigated i.e., Blood glucose, body weight, food consumption, water intake and organs weight. The STZ induced diabetic rats exhibited a heavy loss in weight and increase in blood glucose level. While oral intake of *P. biternatum Edgew.* (300mg/kg) extract showed maximum reduction in plasma glucose level from day 1 to 15 i.e., 314.67±0.88, 172.67±0.88 and 126.83±0.87 respectively. In diabetic rats administration of 300mg/kg extract have better sugar lowering effect than Metmorphine. Its antioxidant properties may reduce oxidative stress and hence prevent the diabetes. The present findings support the usage of *P. biternatum* for the treatment of diabetes after clinical trials.

Keywords: *Psammogeton biternatum*, TPC, Antioxidant, Streptozotocin, Antidiabetic, Phytochemical screening.

INTRODUCTION

According to a careful investigation, about 35000-70000 species of medicinal plants are used globally for curing different diseases (Akerele, 1992). The process of cellular oxidation is essential for all organisms, but it also produced free radicals which causes several diseases viz., diabetes and cardiovascular disorder etc. Plants may be used to prevent these radicals because they have various natural antioxidants i.e. phenol, alkaloids and flavonoids (Erlund, 2001; Ajaib *et al.*, 2017a).

Diabetes mellitus is described as loss of glucose due to disturbance in pancreas which directly influence on secretion of insulin (Scheen, 1999). Most common Symptoms of diabetes are polydipsia, polyuria, microvascular problems and kidney failure (Brownlee, *et al.*, 2001). It is overall estimated that the diabetic patients are between 40-59 years. In 2015, 415 million (8.8%) adults globally were affected due to diabetes (Timon *et al.*, 2014). The chief factor of diabetes are free radicals which produced during oxidation of macromolecules that destroy the cells defence mechanism. World ethnobotanical information reports that about 800-1200 medicinal plants used against diabetes (Oberley, 1988).

Psammogeton genus has 6 species, mostly distributed in sandy dunes of deserts in different regions of Iran, Afghanistan, Turkmenistan and Pakistan (Nasrabadi *et al.*, 2009). The selected plant *P. biternatum Edgew.* commonly known as "Sparki". It is an annual herb and about 15 to 20cm in length. The flowers are small and white or pink colour. Flowering period is March–April. This annual plant distributed in different regions of District Bhimber Azad Jammu and Kashmir (AJK). Medicinally it is very important plant as it is used in treatment of malaria, cough, typhoid and chest problems (Ikram *et al.*, 2015). *P. biternatum Edgew.* seeds are also ground and mixed in water and sugar and taken orally after delivery for postpartum infections (Bibii *et al.*, 2017).

MATERIALS AND METHODS

Plant material: *Psammogeton biternatum* Edgew was collected from different regions of District Bhimber Azad Jammu and Kashmir (AJK) for present study. Plant was identified with voucher specimen number MUST.BOT.5463 from MUST, AJK and plant sample was deposited to the Department of Botany of same University.
Maceration of plant material: The whole plant of *P. biternatum.* cleaned and dried under shade and macerated in ethanol solvent. The extract was dried on rotary evaporator to get final extract.
Phytochemicals analysis: The bioactive compounds were analyzed for plant extracts by using methodology of Riaz *et al.,* (2012).
Determination of Antioxidant Activity: For estimation of Antioxidant activity of whole ethanolic extract of *P. biternatum* Edgew. following parameters were used i.e., Total Phenolic Contents, Total Flavonoid Content, DPPH radical scavenging action, FRAP Assay and β-Carotene Linoleic Acid Emulsion System.
Total Phenolic Contents: For the determination of Total phenolic content the procedure of Ebrahimzadeh *et al.* (2010) were used.
Total Flavonoid Content: Total Flavonoid content were determined using procedure of Adedapo *et al.* (2009).
DPPH radical scavenging action: DPPH radical scavenging action were tested using (Wang *et al.*, 2011) assays.
FRAP Assay: For the determination of FRAP assay (Rehman, 2013) assys were used.
β-Carotene Linoleic Acid Emulsion System: For the determination β-Carotene Linoleic Acid Emulsion System procedure of (Mohdaly, 2010) were used.
Antidiabetic Activity: Investigation of Antidiabetic activity of *P. biternatum* Edgew. was performed by using (Gajdošík *et al*., (1999) assay. Rats were house in the Animal Research Laboratory, University of Health Sciences, Lahore. Animals fed on normal diet and water. All of protocols about animal treatment were approved by animal's ethical committee in the University.
Reagents: Streptozotocin, Metmorphine, Glucometer.
Experimental design: The animals were divided into four groups: **i)** Normal Control ii**)** Diabetic control iii**)** Diabetic Standard (Metmorphine/kg) **iv)** *P. biternatum* Edgew. extract (300 mg/kg).
Induction of experimental diabetes: After fasting 12h, diabetes was induced into rats by a single intra-peritoneal (i.p.) injection of STZ (60 mg/kg body weight) following base-line glucose estimations. This treatment schedule lasted 15 days during which the animals were weighed (daily), food consumption (daily), water intake (daily) and organs weight measured at the last day of treatment.

RESULTS

Phytochemicals analysis: A significant amount of different phytochemicals was present i.e. Alkaloids, flavonoids, cardiac glycoside and tannins were present in least amount. While terpenoids were present in highest concentration as compared to any other phytochemical. Saponins were absent.

Antioxidant activity: The antioxidant potential of *P. biternatum* Edgew. shows that Total phenolic contents were i.e., 2.83±0.008, Total flavonoids content were 2.50±0.01 and FRAP contents was i.e., 1.92 ±0.00 GAE/100g Dry weight). The % free radical scavenging activity of the sample was investigated by using DPPH solution and different concentration is employed (Fig: 1). Whole ethanolic extract showed a good result (34.61±002 at 250 µg/mL). β-carotene method is determined using different concentration of BHA, BHT with extract at different time intervals (Fig:2). Plant extract showed a significant results of β-carotene (0.234±0.54).

Antidiabetic Activity
Antidiabetic activity of plant showed excellent results in reduction of blood glucose level after 15 days' treatment (Fig: 3).

Effect of *P. biternatum* on weight gain of experimental rats: Effect of *P. biternatum* on diabetic rats showed increase in body weight from day 1 to 15. Normal group has the highest proportion weight gain than the diabetic. In the same way *P. biternatum* extract had higher weight gain than the Metmorphine treated group (Table 1).

Effect of *P. biternatum* on water intake of diabetic rats: The effect of *P. biternatum extract* on water consumption is shown in Table 2. The normal control rats represent the lowest intake of water throughout the experimental period as compared to the diabetic. The diabetic rats treated with Metmorphine and *P. biternatum* extract had an increase in water consumption in the 1st five days of the experiment and then reduction in water intake upto last day of treatment.

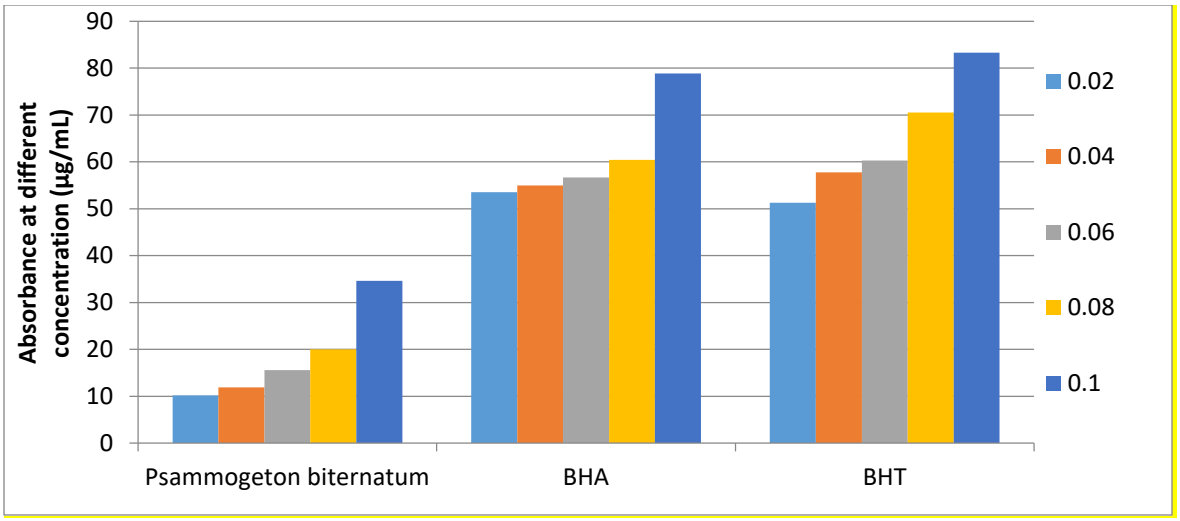

Fig.1. DPPH Scavenging activity of whole plant of *P. biternatum.*

In β-carotene assay absorbance value of antioxidant containing substance is decreased as BHT (Butylated hydroxytoulene) showed maximum decrease then BHA (Butylated hydroxyanisole) and then *P. biternatum.*

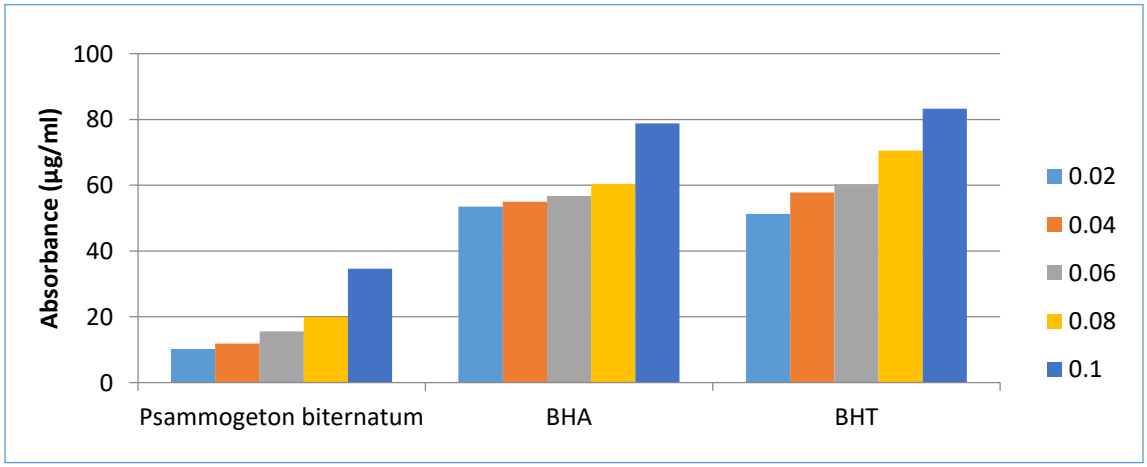

Fig. 2. β- Carotene Linoleic acid of whole extract of *P. biternatum.*

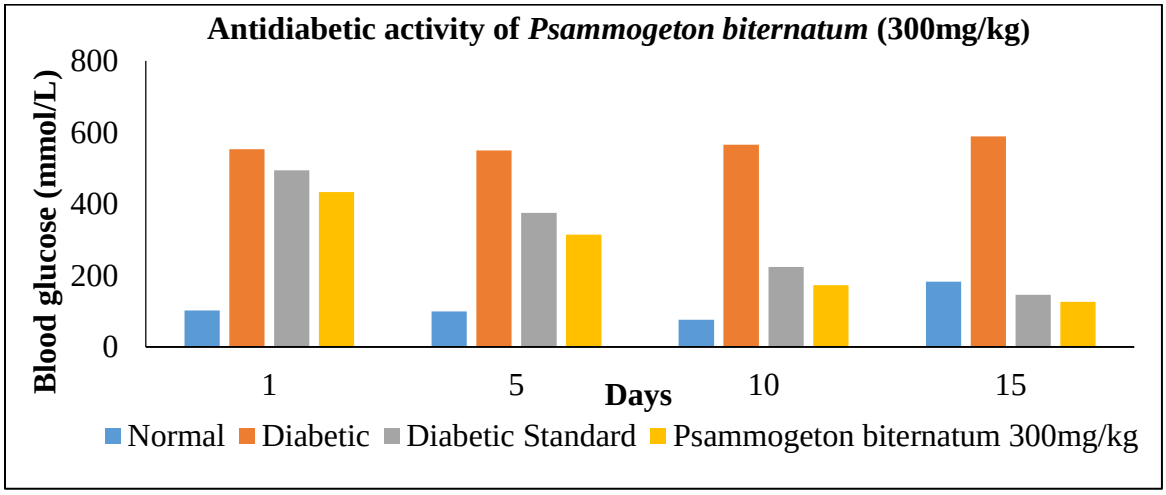

Fig. 3. Graphical representation of Glucose level in whole ethanolic extract of *P. biternatum* at 300mg/kg doses.

Table 1. Body weight (g) in different groups of Rats from Day 1-15.

Days	**Normal**	**Diabetic**	**Diabetic standard**	***P. biternatum***
1	274.33 ± 0.99	264.50 ± 0.89	256.33 ± 0.67	245.83 ± 0.60
5	277.00 ± 0.73	247.67 ± 0.49	259.50 ± 0.56	253.33 ± 0.88
10	277.00 ± 0.86	227.50 ± 0.62	266.33 ± 0.88	271.17 ± 0.70
15	274.83 ±0.48	207.33 ± 0.67	267.00 ± 0.73	271.67 ± 0.56

Table 2. Water intake (mL/l animal/1 day) in STZ treated male rats on day 1-15.

Days	Normal	Diabetic	Diabetic standard	*P. biternatum*
1	26 ± 0.56	120 ± 0.76	118 ± 0.60	120 ± 0.88
5	25 ± 0.48	133 ± 0.61	105 ± 0.97	109 ± 0.96
10	27 ± 0.91	124 ± 0.75	97 ± 088	76 ± 0.5
15	24 ± 0.80	109 ± 0.79	33 ± 0.43	28 ± 0.76

Diabetic rats showed an increase in food consumption. This increase was inhibited in experimental animals treated with plant extract *P. biternatum* at (300mg/kg) doses that is comparable to standard group (Table 3).

Table 3. Food Consumption (g/1 animal/1 day) in different groups (IV) of Rats on Day 1-15.

Days	**Normal**	**Diabetic**	**Diabetic standard**	***P. biternatum***
1	23 ± 0.47	28 ± 0.55	26 ± 0.65	25 ± 0.73
5	23 ± 0.67	35 ± 0.58	31 ± 0.33	30 ± 0.56
10	22 ± 0.76	35 ± 0.56	31 ± 0.33	26 ± 0.40
15	24 ± 0.99	35 ± 0.67	29 ± 0.60	25 ± 0.87

Table 4. Relative Organs weights (g/100 g of body weight) of in STZ treated male rats on day1- 15.

Organ	**Normal**	**Diabetic**	**Diabetic Standard**	***P. biternatum* (300 mg /kg)**
Heart	1.78±0.03	1.06±0.01	0.86±0.02	1.50±0.04
Lungs	3.27±0.01	2.06±0.03	2.39±0.01	2.30±0.13
Liver	12.67±0.16	9.43±0.35	12.40±0.47	10.74±0.30
Spleen	0.91±0.02	0.55±0.02	0.60±0.03	0.64±0.06
Kidneys	4.58±0.03	2.26±0.05	2.47±0.01	3.70±0.10
Pancreas	1.95±0.01	1.40±0.02	1.56±0.01	1.93±0.14
Testes	3.29±0.05	2.07±0.02	2.74±0.01	2.89±0.10

Effect of *P. biternatum* on organs weight of diabetic rats: The effect of *P. biternatum extract* on Different organs weight of all groups were measured at the last day of treatment (Table: 4). Diabetic rats have lowered their organs weight, meanwhile *P. biternatum* extract raise organs weight which is comparable to standard group.

DISCUSSION

The present investigation was carried out on phytochemicals assessment, antioxidant potential and *in vivo* antidiabetic potential of whole plant extract of *P. biternatum Edgew.* Phytochemical screening of the whole plant extract showed the presence of alkaloids, phenols, flavonoids, terpenoids and cardiac glycosides (Riaz *et al.,* 2012). In case of Alkaloids the results were compared with Ajaib *et al.* (2017b) which demonstrated that they play a significant role in living system. Phenols, flavonoids and FRAP contents were found in considerable amounts 2.83±0.008, 2.50±0.01, 1.92 ±0.00mg of GAE/100g Dry weight. DPPH and β-carotene also show good results i.e. (34.61±002), (0.234±0.54. The similar results noted by Ajaib *et al.,* (2013) during the estimation of antioxidant and antimicrobial activities of *Rivina humilis* L.

The antidiabetic study was designed to induce STZ (60 mg/kg b.w.) diabetogenic effect in albino rats. Induction of STZ damage pancreatic β-cells which ultimately cause under secretion of insulin (Szkudelski, 2001). The present study showed that oral administration of *P. biternatum* ethanolic extract improved the survival rate and reduced the elevated blood sugar level in STZ induced diabetic rats (Fig: 3). Metmorphine was used as standard drug which help in secretion of large amount of insulin from pancreas (Bedoya *et al.,* 1996). The STZ-induced rats initially showed a reduction in their body weight which may be due to the muscle squandering. But with the treatment with *P. biternatum* (300 mg/kg) improved body weight (Table:1). STZ Diabetic showed more consumption of food and water as compared to the normal group. However oral intake of *P. biternatum* (300mg/kg) extract have more pronounced effects as compared to all other groups (Table 2-3) similar results was obtained by Ajaib *et al.* (2016) during investigation of antidiabetic study of *Himalrandia tetrasperma* on Alloxan Induced Diabetic Mice. Organs weight of treatment group also indicate effectiveness of plant extract (Table 4).

The *in vitro* antioxidant and *in vivo* antidiabetic potential of *P. biternatum Edgew.*was evaluated. The results reveals good antioxidant activity and strong anti-diabetic potential comparable to the standard drug Metamorphine.

REFERENCES

Adedapo, A.A., F.O. Jimoh, S. Koduru, J.P. Masika and A.J. Afolayan (2009). Assessment of the medicinal potentials of the methanol extracts of the leaves and stems of *Buddleja saligna. BMC Compl. Altern.*,9: 9-21.

Ajaib, M., A. Zikrea, K.M. Khan, S. Perveen, S. Shah and A. Karim (2013). *Rivina humilis* L.: A Potential Antimicrobial and Antioxidant Source. *J. Chem. Soc. Pak.*, 35(5): 1384-1398.

Ajaib, M., M. Latif, S. H. Kamran, K. M. Khan, S. Perveen, S. Shah and A. Karim (2016). Comparative Anti-Diabetic Evaluation of Different Parts of *Himalrandia tetrasperma* in Alloxan Induced Diabetic in Mice. *J. Chem. Soc. Pak.*, 38(2): 313-317.

Ajaib, M., S.Q. Wahla, U.G Wahla,S. Perveen and S. Shah (2017a). Phytochemical Screening and Anthelmintic Activities of *Andrachne cordifolia. J. Chem. Soc. Pak.*, 39(3): 429-433.

Ajaib, M., Z. Ashraf and M.F.Siddiqui (2017b). *Cocculus laurifolius*: a rich antimicrobial, antioxidant and phytochemical source. *Pak. J. Bot.,* 49(1): 337-344.

Akerele, O (1992). Importance of medicinal plants: WHO's programme. In: Natural Resources and Human Health: plants of medicinal and nutritional value. *Elsevier, Amserdam, Netherlands.,* 63-77.

Bedoya, F.I., F. Solano and M. Lucas (1996). Nmonomethyl-arginine and nicotinamide prevent streptozotocin-induced double strand DNA break formation in pancreatic rat islets. *Experientia.,* 52: 344-347.

Bibii, T., A. Ahmad, A.I. Baloch, S. Muhammad and R. Manzoor (2017). Ethnomedicinal uses of plants for child birth and postpartum recovery in district pishin, Northern Balochistan, Pakistan. *Ijbpas.*, 6(9): 1730-1760.

Brownlee, M (2001). Biochemistry and molecular cell biology of diabetic complications. *Nature,* 14: 813–820.

Ebrahimzadeh, M.A., S.M. Nabavi, S.F. Nabavi, F. Bahramian and A.R Bekhradnia (2010). Antioxidant and free radical scavenging activity of *Hyssopus officinalis* L. var. *angustifolius, V. odorata, B. Hyrcana* and *C. speciosum. Pak. J. Pharm. Sci*., 23(1): 29-34.

Erlund, I., G. Alfthan and J. Maenpaa (2001). Tea and coronary heart disease: The flavonoid quercetin is more bioavailable from rutin in women than in men. *Arch. Intern. Med.,* 161: 1919-1920.

Gajdošík, A., M. Gajdošíková, J. Štefek, R. Navarová and Hozová (1999). Streptozotocin-Induced Experimental Diabetes in Male Wistar Rats. *Gen Physiol. Biophys.,* 18: 54-62.

Ikram, A., N.A. Zahra, Z.K. Shinwari and M. Qaiser (2015). Ethnomedicinal review of folklore medicinal plants belonging to family Apiaceae of Pakistan. *Pak. J. Bot.*, 47(3): 1007-1014.

Mohdaly, A (2010). Antioxidant properties of various solvent extracts of potato peel, sugar beet pulp and sesame cake. *Journal of the Science of Food and Agriculture*, 90 (2): 218-226.

Nasrabadi, R., M.B. Gholivand, H. Batooli and A. Vatanara (2009). Chemical Composition of the Essential Oil from Leaves and Flowering Aerial Parts of *Psammogeton canescens* (DC.) Vake from Iran. *J. Medicinal Plants*, 8(31): 82-86.

Oberley, L.W (1988). Free radicals and diabetes. *Free Radic. Biol. Med.*, 5: 113-124.

Periyar, S.S., P.M. Balu, P. Sathiyamoorthi and K. Murugesan (2009). Antihyperglycemic effect of mangiferin in streptozotocin induced diabetic rats. *J. Health Sci.*,55: 206-214.K

Rehman, J (2013). Phytochemical screening and evaluation of *in-vitro* antioxidant potential of *Monotheca buxifolia.E3 J. Biotechnol. Pharm. Res.*,4(4): 54-60.

Riaz, T., M.A. Abbasi, A. Rehman, T. Shahzadi, M. Ajaib and K.M. Khan (2012). Phytochemical screening, free radical scavenging, antioxidant activity and phenolic content of *Dodonaea viscosa*. *Jacq., J. Serb. Chem. Soc.*, 77 (4): 423–435.

Scheen, J.A (1997). Drug treatment of non-insulin dependent diabetes mellitus in the 1990s achievements and future development. *Drug.*, 54:355–68.

Szkudelski, T (2001). The mechanism of Alloxan and Streptozotocin Action in ß cells of the rat's pancreas. *Physiology. Res.*,50: 536-546.

Szkudelski, T (2001). The mechanism of Alloxan and Streptozotocin Action in ß cells of the rat's pancreas. *Physiolog. Res.*, 50: 536-546.

Timon, M.I., S.C. Collantes, S.A. Galindo and F.J. Gomez (2010). Type2 diabetes and cardiovascular disease. Have all risk factors the same strength? *World J. Diabetes*, 5 :444–70.

Wang, Z., Z. Pan, M.A. Haile and G.G. Atungulu (2011). Extract of phenolics from *pomegranate* peels. *The open food science journal*, 5: 17-25.

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