Antiurease and anti-oxidant activity of *Vaccinium macrocarpon* fruit

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Abstract: The objective of present study was to evaluate the antiurease and anti-oxidant activity of *Vaccinium macrocarpon* fruit. The parent extract was ethanolic extract while its sub fractions were prepared in n-hexane, chloroform and n-butanol. The method based on scavenging activity and reduction capability of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH). N-butanol fraction was the most effective antioxidant with 87.0±1.15 activity but the activity was less than ascorbic acid i.e. 93.74±0.12. Highly significant urease inhibition was shown by crude ethanolic extract (71.00±0.2a) with IC_{50} (392.66±2.1) followed by aqueous fraction (68.00±0.5e) with IC_{50} (159.83±2.8). The results of crude ethanolic extract and aqueous extracts were highly significant ($p<0.05$) than standard Thiourea. Present study showed that *Vaccinium macrocarpon* exhibits potent antiurease and antioxidant activities.

Keywords: *Vaccinium macrocarpon*, antiurease activity, anti-oxidant activity.

INTRODUCTION

In the human body, free radical reactions occur and the reactive species can react with biological active molecules that can lead towards the cellular injury and death. Oxidative stress causes imbalance of free radical formation and body anti-oxidant defense system, and there occurs over production of oxygen and nitrogen reactive species. For enhancement of food shelf life, the anti-oxidants are used in the food industries. There are potential health problems and toxicity risks by the use of synthetic anti-oxidants and researches have been conducted into identifying active constituents from natural sources that have powerful anti-oxidant activities and are safe than synthetic antioxidants. Vitamins C, A, E and carotenoids are anti-oxidants that are derived from food (Lobo *et al.*, 2010). Vegetables and fruits have abundant amount of phenolics that are beneficial for human health. The risk of cancer and cardiovascular disorders decreases in persons consuming vegetables and fruits. Excessive amount of phenolic contents is found in *Vaccinium macrocarpon* fruit (Sun *et al.*, 2002). *Vaccinium macrocarpon* products are consumed for health reasons mainly due to their potential effect on urinary tract infections (Vinson *et al.*, 2008). Urea is converted into ammonia and carbon dioxide through urease enzyme (Thakur and Ragavan, 2013). Naturally it is present in bacteria, fungi, algae and in many plants (Gouda and Abdel, 2002). Urease enzyme present in different bacteria is basic cause of various diseases such as ammonia encephalopathy, catheter encrustation, pyelonephritis, hepatic coma, urolithiasis and hepatic encephalopathy (Konieczna *et al.*, 2012). H-pylori also has urease enzyme and it is considered that bacteria causes disease mainly due to the presence of urease. It was postulated that urease present in bacteria hydrolyses urea into ammonia. This ammonia causes the neutralization of gastric pH because in acidic condition bacteria cannot survive so microbial urease is the basic cause of gastric adenocarcinoma, gastric lymphoma, gastritis, peptic and gastric ulcer (Lydia *et al.*, 2010).

MATERIALS AND METHODS

Equipments

Flasks, beakers, pipette and buckner funnel of Pyrex were purchased from Japan. Digital electronic balance of Precisa was acquired from Switzerland. Spray flasks of Witeg and glass sprayer of Precisa were purchased from Germany. Rotary evaporator of Velp Scientifica was acquired from Europe. Distillation plant of Thermo Scientifica was purchased from UK. Digital incubator of Memmert was obtained from Germany.

Materials

All solvents such as n-hexane (C6H14), methanol (CH3OH), ethanol (C2H5OH), chloroform (CHCl3) and n-butanol (C4H9OH) were purchased from Merck, Germany.

Plant material

Plant material for (*Vaccinium macrocarpon*) powder was provided by YAAX International, Inc. (USA) in the month of 7th September, 2015 and was confirmed by

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authenticated source, Department of Botany, Islamia University of Bahawalpur, Pakistan. Voucher specimen for plant material was also deposited. This study was carried out from 7th September, 2015 to 24th December, 2015.

Maceration

One kg of *Vaccinium macrocarpon* fruit dried powder was soaked in 4L of ethanol. Muslin cloth was used for filtration of material from solvent after 15 days. Later on, filter paper of whatsman # 1 was used for filtration. The material remained was then resoaked in ethanol for 15 days to obtain all the remaining constituents from plant material. The course of action of filtration was again repeated. The filtrate was 2.5 L.

Drying of filtrate

Both filtrates were mixed and rotary evaporator was used for drying. The weight of crude dried extract of *Vaccinium macrocarpon* fruit was 170 g after evaporation of solvent from filtrate.

Fractionation

n-butanol, chloroform and n-hexane soluble fractions of crude extract of *Vaccinium macrocarpon* fruit were made. For this intention, 170g of crude extract was suspended in 1L water. Separating funnel was used for separation of fractions. Suspension was shaken with n-hexane and two layers separated after allowing the solution to stand. N-hexane layer was collected. This procedure was repeated with the same aqueous fraction until the clear n-hexane layer was obtained. When clear n- hexane layer is obtained then in the same aqueous layer, chloroform and n-butanol was added respectively with same procedure to obtain chloroform and n-butanol soluble fractions. Remaining fraction was aqueous soluble fraction. Rotary evaporator was used for drying of all these fractions.

Antioxidant activity

Method used to investigate the antioxidant activity of *Vaccinium macrocarpon* fruit is given below:

$$\text{Inhibition (\%)} = 100 - \frac{(\text{Absorbanc e of test})}{\text{Absorbance of control}} \times 100$$

DPPH free radical scavenging assay

Antioxidant activity was determined by DPPH method (Ratshilivha *et al.*, 2014). DPPH concentration in methanol was 10uM.Total volume of assay was 100ul containing test solution (10ul) and DPPH solution (90ul). Mixture of contents was incubated at 37oC for half hour. The reduction in absorbance was determined by using micropipette reader at 517nm. Ascorbic acid was used as standard antioxidant. Triplicate was used for carrying out all experiments. IC_{50} were calculated by making serial dilution. The reduction in absorbance showed better radical scavenging potential which was calculated as under.

Antiurease assay

Antiurease assay was performed with slight modification. A total volume of 85ul in well of 96 well plate was taken (Xiao *et al.*, 2007). First 15ul of test compound, 15ul of buffer (1M, pH: 7), 15ul (0.25mg ml) of enzyme were incubated at 37˚C for 15 minutes. Then 40ul of substrate was added and reincubated at the same condition. After incubation, absorbance was measured at 630nm. This was taken as pre read. Then 45ul of phenol and 70ul of alkali reagent was added into the mixture and incubated for 50 minutes. After incubation, absorbance was measured at 630nm and taken as after read. Thiourea was taken as positive control and buffer was taken as control. For IC_{50} serial dilution was done. Result was measured by this formula:

$$(\%)\ \text{Inhibition} = \frac{(\text{Absorbanc e of test solution})}{\text{Absorbance of control}} \times 100$$

RESULTS

DPPH free radical scavenging assay

The results of *Vaccinium macrocarpon* extract and different fractions are presented in table 1.

STATISTICAL ANALYSIS

All the values are expressed as Mean ± SEM. The data were analyzed by one way ANOVA followed by LSD multiple comparison tests. A level of $P < 0.05$ was considered as statistically significant. A level of significance was noted and interpreted accordingly.

In vitro anti urease assay

The urease inhibition assays were performed on ethanolic extracts and fractions of *Vaccinium macrocarpon*. The results of antiurease activities of *Vaccinium macrocarpon* are presented in table 2.

DISCUSSION

Recently urease inhibitor drugs are gaining attention due to their activity to treat ulcer (Sanaz *et al.*, 2013). Urease activity is considered as a significant virulence determinant in the development of various pathological conditions, injurious for health of animal and human (Lateef *et al.*, 2012). Therefore, strategies are being made based on inhibition of urease for the management of infection caused by urease producing bacteria. The crude ethanolic extract and the fractions of *Vaccinium macrocarpon* investigated for urease inhibitory potential can be considered as a source of natural urease inhibitors. The current research suggests that *Vaccinium macrocarpon* is a potential source of urease inhibitors and antioxidants and the constituents may considered as a lead compound in the development of drug discovery and designing for the management of ulcer.

Table 1: Results of *Vaccinium macrocarpon* extract and different fractions

No.	Fraction used	Concentration (mg/ml)	DPPH value % inhibition	IC_{50} µg/ml
1	Crude ethanolic extract	5	86.66±0.8[a]	626.83±1.8
2	n-hexane fraction	5	10.9[b]	---
3	Chloroform fraction	5	21.80±0.6[c]	---
4	n-butanol fraction	5	87.0±1.15[d]	311.3±0.6
5	Aqueous fraction	5	85.96±2.4[e]	730.73±2.2
6	Ascorbic acid fraction	0.5 (mmol/ml)	93.74±0.12[f]	---

Values are mean (n=3) ± standard error. Superscript a, b, c, d, e and f showed that mean± standard error in the same column with different superscript are significantly different ($p < 0.05$)

Table 2: Results of urease inhibition activities of *Vaccinium macrocarpon*

No.	Fraction used	Concentration used ug/ml	% inhibition	IC_{50} µg/ml
1	Crude ethanolic extract	500	71.00±0.2a	392.66±2.1
2	n-hexane soluble fraction	500	42.40b	---
3	Chloroform soluble fraction	500	19.33±0.3c	---
4	n-butanol soluble fraction	500	61.00±0.5d	---
5	Aqueous fraction	500	68.00±0.5e	159.83±2.8
6	Thiourea	0.375mmol	99.29±1.18f	19±0.08umol

Values are mean (n=3) ± standard error. Superscript a, b, c, d, e and f showed that mean± standard error in the same column with different superscript are significantly different ($p < 0.05$)

CONCLUSION

In present study, most potent antioxidant (87.0±1.15) was n-butanol fraction but the antioxidant potential (93.74±0.12) of ascorbic acid was more than n-butanol. Highly significant urease inhibition was shown by crude ethanolic extract (71.00±0.2a) with IC_{50} (392.66±2.1) followed by aqueous fraction (68.00±0.5e) with IC_{50} (159.83±2.8). The results of crude ethanolic extract and aqueous extract were significant ($p<0.05$) than standard Thiourea. Antioxidant potential of plant is due to presence of phenolics. Plants extracts showed significant antiurease and antioxidant activity. Present study supports the claim of herbal practitioners on the therapeutic potential of *Vaccinium macrocarpon*.

REFERENCES

Gouda M and Abdel M (2002). Catalytic properties of the immobilized *Aspergillus tamari* xylanase.*Microbiol. Res.,* **157**:275-281.

Konieczna I, Zarnowiec P, Kwinkowski M, Kolesinska B, Fraczyk J, Kaminski Z and Kaca W (2012). Bacterial urease and its role in long-lasting human diseases. *Curr. Protein Pept. Sci.,* **13**:789-806.

Lateef M, Iqbal L, Fatima N, Siddiqui K, Afza N, Zia M and Ahmad M (2012). Evaluation of antioxidant and urease inhibition activities of roots of *Glycyrrhiza glabra. Pak. J. Pharm. Sci.,* **25**:99-102.

Lobo V, Patil A, Phatak A and Chandra N (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn. Rev.,* **4**(8):118–126.

Lydia E, Richard M and Keith T (2010). Helicobacter pylori and gastric cancer: Factors that modulate disease risk. *Clin.Microbiol. Rev.,* **23**:713–739.

Ratshilivha N, Awouafack M, Toit S and Eloff J (2014). The variation in antimicrobial and antioxidant activities of acetone leaf extracts of 12 *Moringa oleifera* (Moringaceae) trees enables the selection of trees with additional uses. *South Afric. J. Bot.,* **92**:59-64.

Sanaz G, Roya B, Sahand G, Farzaneh N, Zinat B, Behnam Y, Reza H, Shamsali R and Massoud A (2013). Urease inhibitory activities of β-boswellic acid derivatives. *Daru J. Pharm Sci.*, **21**:2-10.

Sun J, Chu Y, Wu X and Liu R (2002).Antioxidant and antiproliferative activities of common fruits. *J. Agri. Food Chem.,* **50**:7449-7454.

Thakur S and Ragavan V (2013). Biosensors in food processing. *J. Food Sci. Technol.,* **50**:625–641.

Vinson J, Bose P, Proch J, Kharrat H and Samman N (2008). Cranberries and cranberry products: powerful *in vitro, ex vivo*, and *in vivo* sources of antioxidants. *J. Agri. Food Chem.,* **56**(14): 5884-5891.

Xiao Z, Shi D, Li H, Zhang L, Xu C and Zhu H (2007). Polyphenols based on isoflavones as inhibitors of Helicobacter pylori urease. *Bioorg. Med. Chem.,***15**:3703-3710.