The Journal of Animal & Plant Sciences, 25(3 Supp. 2) 2015 Special Issue Page: 576-579
ISSN: 1018-7081

SOME PRELIMINARY STUDIES ON PHYTOCHEMICALS AND ANTIOXIDANT POTENTIAL OF *FAGOPYRUM ESCULENTUM* CULTIVATED IN CHITRAL, PAKISTAN

R. Abbasi[1], S. Janjua[2*], A. Rehman[2], K. William[2] and S. W. Khan[3]

[1]Institute of Natural and Management Sciences (INAM), Rawalpindi, Pakistan
[2]Bioresource Research Centre (BRC), Islamabad, Pakistan
[3]Department of Biological Sciences, Karakoram International University, Gilgit, Pakistan.
*Corresponding Author's Email: safiajanjua@hotmail.com

ABSTRACT

The current study was conducted to analyze the phytochemicals and antioxidant potential of *Fagopyrumesculentum* which was cultivated in region of Chitral, Pakistan. The phenolics content in the methanolic extract of *Fagopyrumesculentum* were analyzed by using Folin-Ciocalter reagent via spectrophotometer. To determine antioxidant activity, firstly DPPH and TLC were used and after confirmation of positive potential of Fagopyrum as antioxidant, DPPH assay and reducing power method were performed via spectrophotometer.As a result, *Fagopyrumesculentum* showed the strong antioxidant activity. The highest antioxidant activity was found at concentration 1 mg/mL (81.64%). The contents of flavonoids were estimated as 19.64 µg/250 µg of dry powder and percentage was 7.856 %. The content of phenolics was 0.80 µg/25.97 µg of dry powder and percentage was 3.08 %. Thus, our preliminary study on grains of *Fagopyrumesculentum* concluded that it has compounds which can have pharmacological and medicinal importance and it can be used as antioxidant drug.

Key words: Phytochemicals and antioxidant potential, *Fagopyrumesculentum*, Chitral, Pakistan.

INTRODUCTION

Fagopyrumesculentum (Buckwheat) is known as a crop of secondary importance in various countries and is also used for various purposes due to which it is also known as multipurpose crop. Flowers of *Fagopyrumesculentum* are used for extraction of rutin while its leaves are eaten as vegetable. It has been cultivated at approximately 500 to 2500 m in altitude(Ohnishi, 1988).It is also known as important food crop in Himalayan region of Pakistan. Buckwheat contains carbohydrates, fats, lipids, proteins, and other essential amino acids. It also has rutin in it which has great medicinal value. In the buckwheat seeds, starch is abundantly found i.e.59% to 79%.Globulin is the abundantly found protein in buckwheat and is almost one half of the total protein. Palmitic, oleic, linoleic, stearic and linolenic acid are the abundantly found fatty acids in Buckwheat (Ohnishi, 1993).The pure buckwheat is dark in color and it has strong flavor. Buckwheat germinates quite rapidly and it also produces a canopy which shades the soil. It is also reported to be used for the control of weeds (Ohnishi, 1995). *Fagopyrumesculentum* is well known for its medicinal values. The present study was conducted by keeping in mind that different environmental and ecological factors can widely affect the secondary metabolites present in plant (Fujii*et al*. 2003 and Gilani *et al*., 2003). Therefore, the variety of *Fagopyrumesculentum* cultivated in Himalayan regioni.e. Chitral, Pakistan was tested for its preliminary tests for phytochemicals and antioxidant potential.

MATERIALS AND METHODS

The seeds of *Fagopyrumesculentum*were collected from Chitral and they were identified by taxonomist. These seeds were then washed, properly dried and grounded so that it formed fine powder and used for extract preparation. For extract preparation, methanol was selected as solvent. The Crude methanolic extract was then diluted and concentrations of 1000 ppm, 100 ppm, 10 ppm, 1 ppm and 0.1 ppm were made. The serial dilutions wereprepared from these dilutions.

The qualitative and quantitative tests were performed forthe determination of the phytochemical content present in CME seed of *Fagopyrumesculentum*. Screening was done for the following phytochemicals; saponins, tannins, steroids, alkaloids, phlobatannins, carbohydrates, anthraquinones and glycosides and methodology described by Sofowora, (1993), Harborne, (1998) and Kokate, (2008) was followed.

For quantification of flavonoids, protocol described by Ordenoz*et al*. (2006) was followed. The reaction mixture for this assay contains 250 µL of stock solution (5000 µg/mL), 1975 µL of methanol, 100µL of 10% $AlCl_3$, 100µL potassium acetate (1M) and 2575 µL of distilled water. The samples were subjected to spectrophotometer for the analysis of flavonoids.

To determine the quantity of phenolic acids protocol by Wolfe *et al*. (2003) was followed; 6% Na_2CO_3 and Folin-Ciocalteu reagent were prepared. The total phenolic content of CME was determined by the Folin-Ciocalteu method in which 2.5 mL of fresh Folin reagent and 0.5 mL of different concentrations of the extract were mixed thoroughly and then 2 mL of Na_2CO_3 (7.5%) was added in this solution. It was left for 90 minutes at 30 °C.

For determination of antioxidant activity, DPPH assay and reducing power assay were performed. Test sample of 100 mg/mL in methanol was prepared. In wells of 96 well plate, 5 µL of test sample was added. 95 µL of DPPH solution was added in each well. It was then incubated at 37 ^oC for 1 hour. By using micro plate reader (Thermo electron Fluoroskan Ascent), absorbance was measured at 515 nm. For determination of reducing power, the methodology described by Oyaizu (1986) was followed.2.5 mL of the extract was mixed with 2.5 mL of phosphate buffer (pH 6.6) and 1% potassium ferric cyanide. The mixture was then incubated for 20 min at 50 ^oC.Then after cooling it was with 2.5 mL of 10 % Tricarboxylic acid(w/v).Then it was centrifuged at 3000 rpm for 10 minutes. The upper aqueous layer was then mixed with 5 mL of deionized water and 1 mL of 0.1 % $FeCl_3$. It was then allowed to stand for 10 min. after 10 min, the absorbance of solution was measured at 700 nm.

RESULTS AND DISCUSSION

Different species of buckwheat are being used as medicinal plants by various local communities to cure diseases for a long period (Gilani *et al.,* 2009; Mohy-ud-Din *et al.,* 2010; Shinwari, 2010). According to Fujii *et al.* (2003) and Gilani *et al.* (2003), plants which are grown in different habitats tends to have different biological potential. Methanol is one of the well known solvent and it is commonly used for extraction purposes in plants as it has tendency to solubilize maximum secondary metabolites (Tiwari *et al.*2011 and Das *et al.,* 2010). Thus, methanolic extracts were synthesized and its yield is mentioned in Table 1. Qualitative tests results indicated the presence of flavonoids, anthraquinones, carbohydrates, amino acids, alkaloids, phlobatannins and tannins while saponins and steroids were absent in CME (Table 2).The *F. esculentum* which is variety of Chitral, Pakistan is also rich in phytochemicals like other species of genus *Fagopyrum* (Liu *et al.* 2008) and also like other *F. Esculentum* variety found in different regions (Quettier- Deleu *et al.* 2000). Thus, it is beneficial for health.

Table 1:The percentage yield of methanolic extract of *Fagopyrumesculentum*.

Replication	Sample Weight (g)	Extract Weight (g)	Yield (%)
Average	5	1.497±0.015	9.8±0.58

Table 2: Qualitative Phytochemical analysis of *Fagopyrum esculentum*

Serial No.	Phytochemicals	Results
1	Alkaloids	+
2	Amino acids	+
3	Anthraquinones	+
4	Carbohydrates	+
5	Flavonoids	+
6	Phlobatannins	+
7	Saponins	-
8	Steroids	-
9	Tannins	+

(+) sign indicates the presence of compounds whereas (-) Sign indicates the absence of compound

The FCR method was used for determination of phenolic compounds in CME of *F. esculentum* (Table 3). This method is based on principle that tyrosine which has hydroxyl phenol group, it reduces the phospho-molybdate which is present in the FCR and due to this reaction it turns blue. It can be measured colorimetrically at 765 nm (Singleton and Rossi, 1965).

Table 3: The total flavonoids and phenolics present in grains of *F. esculentum* variety of Chitral, Pakistan.

	Phytochemical	Quantity (µg/mL)	Percentage (%)
1	Flavonoids	19.64	7.856
2	Phenolics	0.80	3.08

To evaluate the antioxidant activity of plant samples, many assays are used but among all these assays DPPH assay is a well-known and considered as avalid assay for estimation of free radical scavenging potential of plant extracts or different compounds (Choi *et al*. 2002; Katsube *et al*. 2004; Wong *et al*.2005 and Kidare *et al*. 2011). The concentration of extract is directly proportional to the absorbance which indicates the reducing power of the extract (Figure 1). Reducing ability of CME of *Fagopyrumesculentum* is shown in Figure 2.

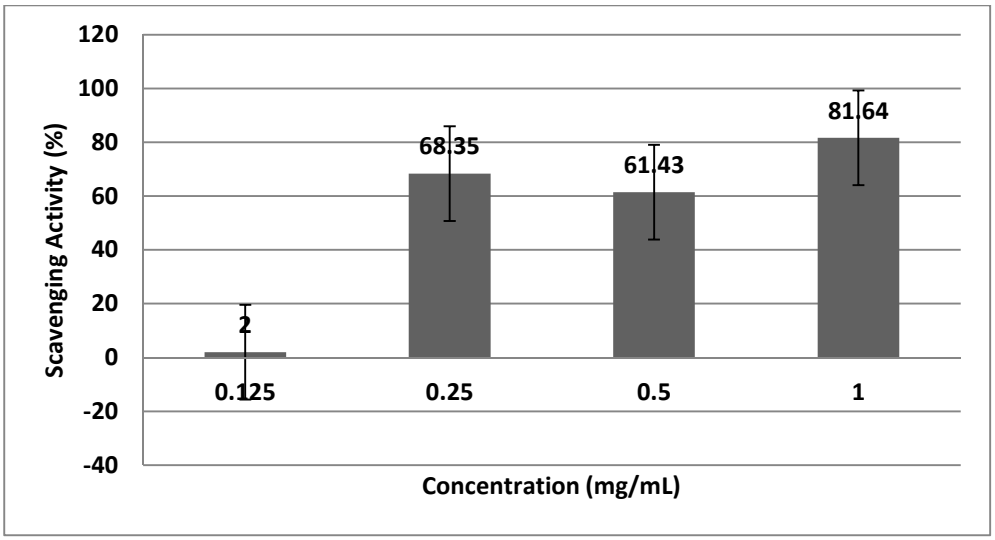

Figure 1: The percent scavenging activity of different dilutions of CME of *Fagopyrumesculentum*. Concentration of 1 mg/mL showed highest results while concentration of 0.125 mg/mL showed least results i.e. only 2%. These values are means of three replications.

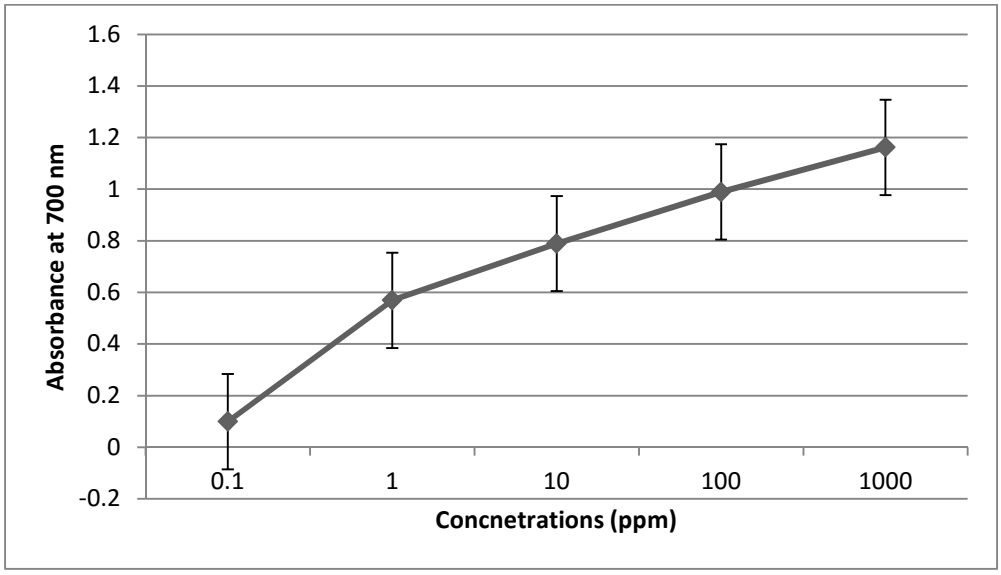

Figure 2: Reducing ability of CME of *Fagopyrumesculentum*.

Conclusion: The current study concluded that *Fagopyrumesculentum* which was collected from Chitral, Pakistan is rich source of phytochemicals. Results showed the presence of alkaloids, amino acids, anthraquinones, carbohydrates, flavonoids, phlobatannins and tannins in the grains of *Fagopyrumesculentum*. Its CME contains 3% phenolics and 85% flavonoids. Results of DPPH assay and reducing power assay showed that *Fagopyrumesculentum* CME (1000 mg/mL) has about 80% scavenging activity. Therefore, it is concluded from this preliminary study that *Fagopyrumesculentum* can be used for isolation of important compounds with medicinal and pharmacological importance.However, its nutraceutical and local use as food emphasizes the need for further isolation of biologically active compounds.

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