
**ISSN Print:** 2394-7500
**ISSN Online:** 2394-5869
**Impact Factor:** 8.4
IJAR 2019; 5(6): 479-484
www.allresearchjournal.com
Received: 10-04-2019
Accepted: 19-05-2019

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# Screening of buckwheat and wheat flour extracts for potential antioxidant and anti-proliferative activities

**Saiyma Sheikh and Dr. Yasir Ali Arfat**

**Abstract**
The purpose of the study was to evaluate the total phenolic content, anti-oxidant activities, and anti-proliferative activity of buckwheat and wheat using different solvents. Both the samples of each extract were evaluated for DPPH radical scavenging activity, reducing power, lipid per-oxidation, $H_2O_2$ scavenging activity, hydroxyl radical scavenging activity. The results showed that both the samples with all the solvents exhibited appreciable amounts of total phenolic compounds and anti-oxidant activity but methanol proved to be best extract in all the tests except for DPPH radical scavenging activity which was found to be higher in ethanol. Buckwheat was found to be a more potent anti-oxidant and anti-proliferative source with higher total phenolic content (70.62 GAEμ/ml) when compared to wheat (60.4mg GAEμ/ml). The anti-proliferative activity of buckwheat and wheat was tested in C-6 cells. Both, buckwheat and wheat inhibited the growth of cancer cells but buckwheat (49.58%) proved to be more potent anti-proliferative source when compared to wheat (35.59%).

**Keywords:** Buckwheat flour, wheat flour, antioxidant activity, antiproliferative activity

## Introduction
Buckwheat (*Fagopyrum esculentum*) is highly nutritious pseudocereal known as dietary source of protein with flavourable amino acid compostion (Eggum *et al*., 1981) [11], vitamins, starch dietary fiber, essential minerals and trace elements (Bonafaccia *et al*., 2003) [5]. Buckwheat is thought to have originated in central and western china from a wild Asian spices Fagroprum cymosum, belonging to the Polygonaceae family. In comparison to most frequently used cereals, buckwheat has been reported to possess higher antioxidant activity, due to high content of rutin, tocopherols and phenolic acids, and can thus be stored for a long time without apparent chemical changes (Dietrych-szostak & Olsezek, 1999; Sakac *et al*., 2011) [10]. Antioxidant-rich diets have been associated with a lower incidence of cardiovascular disease, cancers, and age-related degenerative processes (Kaliora & Dedoussis, 2007) [19]. The primary antioxidants in buckwheat are rutin, quercetin, hyperin, and catechins (Morishita, Yamaguchi, & Degi, 2007; Chlopika, 2008) [9] Buckwheat contains more rutin than most plants. Rutin is a flavonol glycoside plant metabolite with antioxidative, anti-inflammatory and anticarcinogenic effects, and can also reduce the fragility of blood vessels related to hemorrhagic disease and hypertension in humans. (Oomah & Mazza, 1996; Ikeda, 2002; Li and Zhang, 2001) [24, 17, 20]. Rutin and isovitexin are the only reported flavonoids of buckwheat seed. Buckwheat hulls contain rutin, orientin, vitexin, quercetin, isovitexin and isoorientin (Dietrych-szostak & Olsezek, 1999) [10]. The total flavonoid concentrations of buckwheat seed and hull are 18.8 mg/100 g and 74 mg/100 g, respectively. Flavonoids isolated from buckwheat hulls showed radical scavenging activity when analyzed in the purified form (Watanabe, 1998) [32] (Watanabe, Ohshita, & Tsushida, 1997). Variation in antioxidant activity of buckwheat was mainly due to the cultivars and environment effects (Oomah & Mazza, 1996) [24]. Whole buckwheat contains 2–5 times more phenolic compounds than oats or barley, while buckwheat bran and hull have 2–7 times higher antioxidant activity than barley, triticale, and oats (Holasova *et al*., 2002; Zdunczyk *et al*., 2006). The increasing attention for buckwheat cultivation and utilisation of buckwheat products is due to rising number of data focused on its functional characteristics, which can provide many health benefits based on buckwheat products consummation, first of all during prevention and healing chronic diseases (Li and Zhang, 2001) [20].

Based on the fact that antioxidative components from buckwheat flour significantly contribute to its functionality, the aim of this work was to investigate antioxidative properties of the commercially accessible buckwheat flours in comparison to the wheat flour the most frequently used wheat products for bakery industry, by measuring DPPH radical scavenging activity, reducing power, total phenolics content, lipid peroxidation, $H_2O_2$, hydroxyl radical scavenging activity and anti proliferative activity.

## Materials and Methods

### Procurement of Material

Buckwheat (*F. esculentum*) was procured from Sheri-Kashmir University of Agricultural Sciences and Technology (SKUAST), Kashmir. Whole buckwheat was ground to pass a 1 mm screen and stored at 4 °C before experiment and wheat flour was procured from local market.

### Extraction

0.9g of the buckwheat and wheat flour each were weighed and put into a 50 ml bottle. 30ml of, 96% ethanol, methanol, and dd water were added to each bottle, respectively. The solution was subjected to stirring for 2 hrs on magnetic stirrer, after proper mixing of solution, the solution was placed in tubes for centrifugation at 3500 rpm for 10 min, and the supernatant and the sediment were separated. The residue was extracted a second time described as the first extraction. The first and second extraction solutions were combined and evaporated in rotary evaporator to form powder which is stored as stock sample at 4ºc for further use.

### Determination of antioxidant activity by DPPH method

The antioxidant activity of the buckwheat and wheat extracts was measured in terms of hydrogen-donating or radical-scavenging ability, using the DPPH method (Brand-willliams *et al.*, 1995; Chen Wang, Rosen, & Ho, 1999; Baba *et al.*, 2014) with slight modifications. Different concentrations of the extracts of buckwheat or wheat (100µl) were added to1.0ml of 0.01% of methanolic solution of DPPH. The reaction mixtures were shaken vigorously and incubated in the dark for 30 min. The absorbance of the solution was measured at 517nm. The % inhibition was calculated against a control and compared to BHT as standard.

The relative inhibition of antioxidants against DPPH was calculated according to the following equation:

$$\% \text{ Inhibition} = A_{\text{control } 517} - A_{\text{sample}517} / A_{\text{ control } 517} \times 100$$

Where $A_{\text{control } 517}$ is the absorbance of the control and $A_{\text{sample}517}$ is the absorbance of extract or α-tocopherol.

### Reducing power

The reducing power was determined according to the method of (Oyaizu 1986) with slight modifications. Different concentrations of extract of the buckwheat or wheat (100µl) was mixed with 0.02M sodium phosphate buffer ph 6.6 (2.5ml) and 1% (w/v) of aqueous potassium ferric cyanide (2.5ml). The mixture was incubated at 50ºc for 20 min. 10% (w/v) trichloroacetic acid (2.5ml) was added to the mixture, which was then centrifuged at 3000 g for 10 min. the supernatant 2.5mlwas diluted with de ionised water 2.5ml and 0.1% (w/v) ferric chloride 0.5ml was added. The absorbance was measured at 700nm against a blank and compared to α- tocopherol as standard. A higher absorbance indicates a higher reducing power.

Reduction % = [1-(1-AC/AS)] x100
Ac = absorbance of standard
As = absorbance of sample

### Total phenolic content

Phenolic content was determined by the Folin–Ciocalteau colourimetric method as described previously with minor modifications (Waterhouse, 2001; Yu & Zhou, 2004; Jan *et al.*, 2015) [33, 37, 18]. The varying concentration of the supernatant 200l was added to 2.5ml of freshly prepared folins reagent (1;10v/v) with water the mixture was allowed to equilibrate for 4 mins and then mixed with 2ml of 75g/litre sodium carbonate solution after incubation at room temperature for 2hrs. Absorbance of samples was measured at 765 nm after 2 h using gallic acid as a standard. Results were expressed as mg of gallic acid equivalents g-1 d.m.

### Lipid peroxidation

Lipid peroxidation was performed according to the method of Wright *et al.* [19] with minor modifications. Different concentration of buck wheat or wheat was mixed with 1ml of linoleic acid 0.1g in 100ml of pure ethanol,0.2ml of $H_2O_2$ 30mM, 0.2ml of ascorbic acid 100mM and 0.2ml of ferric nitrate 20mM. this was followed by incubation at 37ºc in water bath for 1hr the reaction was stopped by the addition of 1.0ml trichloroacetic acid (TCA),(10%w/v), followed with with 0.1ml of TBA (thiobarbutric acid, 1%w/v) and all tubes were placed in a boiling water bath for 20 mins. The tubes were then centrifuged at 5000 rpm for 10mins the amount of malonaldehyde formed in each sample was assessed by measuring the optical density of supernatant at 535nm against a reagent blank.

### Hydroxyl radical scavenging activity

The OH scavenging ability of buckwheat or wheat was examined by following the procedure previously described (Cheng *et al.*, 2003) [8], with some modifications. Briefly, the reaction mixtures contained 25mM of calf thymus 2-deoxyribose (1ml), 10Mm ferric chloride (200µl), 100mM Ascorbic acid (20µl), 2.8mM $KH_2PO_4$ and various concentrations of extracts of buck wheat or wheat. The mixture was vortexed and incubated at 37 ºC for 1hr then 1ml of 1%TBA and 1ml of 3%TCA were added and heated in water bath at $100^0$C for 20min. The extent of oxidation was estimated from the absorbance of the solution at 532 nm. The hydroxyl radical-scavenging activity of the buck wheat or wheat extract was reported as the percentage of inhibition of deoxyribose degradation and was calculated according to the following equation:

$$\% \text{ Inhibition} = A_{\text{control } 532} - A_{\text{sample } 532} / A_{\text{ control } 532} \times 100$$

Where $A_{\text{ control } 532}$ is the malondiaaldehyde produced by Fenton reaction treated alone, is the absorbance of the control and $A_{\text{ sample}532}$ is the malondiaaldehyde produced in presence of extract or a-tocopherol.

### $H_2O_2$ scavenging activity

The ability of buckwheat or wheat extracts to scavenge hydrogen peroxide was evaluated according to the method

Rush *et al*. with minor modifications. A solution of $H_2O_2$ (2mM)was prepared in phosphate buffer (pH7.5).Various concentrations of buck wheat or wheat extracts were added to$H_2O_2$ solution (0.6ml).Absorbance of $H_2O_2$ at 523nm was determined after 5min against blank solution containing phosphate buffer without $H_2O_2$.BHT was taken as standard. The scavenging activity of buck wheat or wheat extracts on $H_2O_2$ was expressed as.

% inhibition = [(A0- A1)/A0] x 100

Where A0 is the absorbance of control and A1 is the absorbance of presence of buck wheat extracts and known standards.

**Antiproliferation effects of buckwheat extract on C6 human cancer cells**

Cell proliferation inhibitions were investigated according to (Mosmann, 1983) with minor modifications. C6 human cancer cell was used for the MTT assay. Cells were plated in 96 well plate at 5000-7000 cell density per well. Cell were grown overnight in100µl's of 10% FBS. After 24 hours cells were replenished with fresh media and extracts of buckwheat or wheat were added to the cells. Different concentrations of the aqueous extracts (10, 25, 50, 75 & 100mg/ml) of buckwheat or wheat (100µl) were added to wells in triplicates. Cells were incubated with the extract for 24 hours. After 24hours 20µl's of MTT dye (5mg/ml) were added to each well and further incubated for 3 hours. Before read-out, precipitates formed were dissolved in 150µl's of DMSO using shaker for 15minutes. All the steps performed after MTT addition were performed in dark. Absorbance was measured at 590nm.

**Statistical Analysis**

Experiments were performed in triplicates. The data was analyzed using one way analysis of variance (ANOVA) and Duncan test by SPSS.

**Results and Discussion**

**Total phenolics**

Phenolic compounds are the main constituents of plants and contribute to their antioxidant activity. The powerful antioxidant activity of phenolics is due to hydroxyl groups. The extracts of both buckwheat and wheat were screened for their potential anti oxidant activity. In-vitro antioxidant assay indicated that buckwheat possesses a potent antioxidant activity than wheat. The TPC of wheat and buck wheat differed greatly ranging from 60.4 GAEµ/ml in wheat and 70.62GAEµ/ml in buckwheat as shown in Fig (1). Total phenolic content of both the samples was found to be affected by extraction solvents and were in the following order:

methanol>ethanol>aqueous. Our results are in agreement with Przybylski, Lee & Eskin (1998) [26] in which methanol was found as a more potent extraction solvent when compared to ethanol and distilled water.

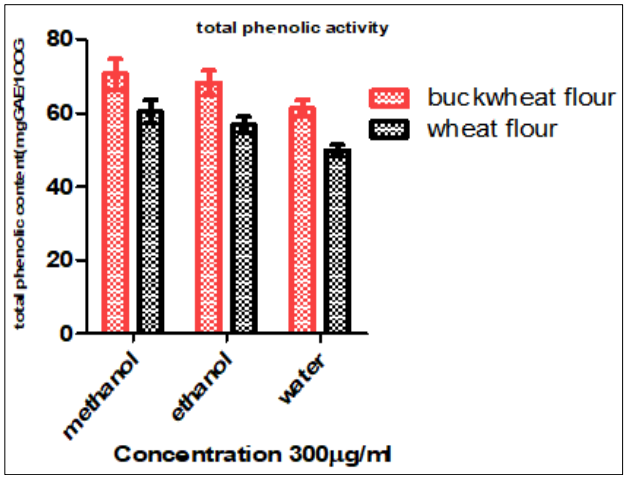


**Fig 1:** Showing total phenolic content of buckwheat and wheat extracts using different solvents at different concentrations

**DPPH**

DPPH (1, 1, Diphenyl-2-picrylhydrazyl) is a stable free radical and is widely used to evaluate antioxidant activity of phenolic compounds from fruits, vegetables and cereals. Scavenging of DPPH radical is based on measurement of reducing ability of antioxidants towards DPPH (Huang *et al*., 2005; Prior, Wu & Schaich, 2005) [16, 35]. In this study, the antioxidant activity of wheat and buckwheat was evaluated using DPPH radical scavenging activity. Our results indicated in Fig (2) that DPPH radical scavenging ability of buckwheat was higher than wheat in ethanolic solvents which could be because of the higher phenolic content present in buckwheat as compared to wheat. Same results were reported by Sedeji *et al*. in which DPPH scavenging activity of buckwheat was found higher than wheat flour. Our results showed significant effect of solvents on the DPPH scavenging activity of extracts and the highest activity was found in ethanol followed by methanol and distilled water.

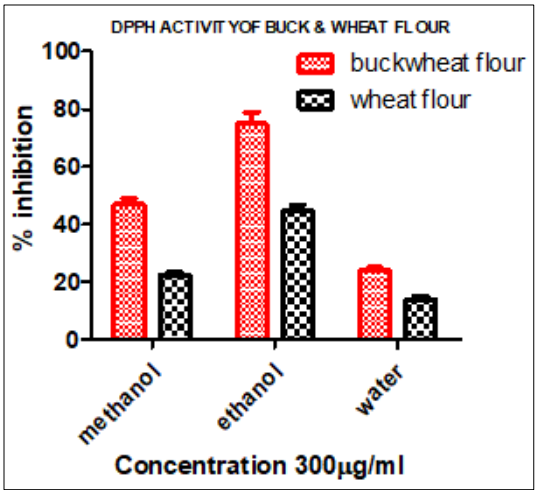


**Fig 2:** Showing DPPH radical scavenging activity of buckwheat and wheat extracts using different solvents at different concentrations

**Lipid peroxidation**

Lipid peroxidation results in oxidative modification of biological molecules particularly polyunsaturated fatty acids. Initiation of lipid peroxidation by ferric nitrite/ ascorbic acid / water takes place either through ferryl-perferryl or through OH radical through fentons reaction. Ferric nitrite/ ascorbic acid / water induce lipid peroxidation and the damages are examined by the formation of MDA (malonyl dialdehyde) the product of lipid peroxidation which forms a pink coloured complex with TBA (thiobarbituric acid) that absorbs at 535 nm. The results obtained for buckwheat at a concentration of 300µg/ml in methanol, ethanol and aqueous, are 61.02, 45.44, and 38.93%. For wheat, in same solvents and at same concentration the % age inhibition recorded is 46.65, 35.70 and 28.55% which is less as compared to buck wheat Fig (3). Our findings are in inconsistent with Zelinsky and Kozlowsko (2000) [38] who reported that among several grains wheat, rye, barley and buckwheat the methanol extract prepared from buck wheat exihibited the strongest protective effect against lipid peroxidation. This fact can be attributed to the isoflavanoid rutin present in the seeds of buckwheat which makes major contribution to the antioxidant activity in buckwheat (Dietrych-Szostak and Oleszek, 1999) [10].

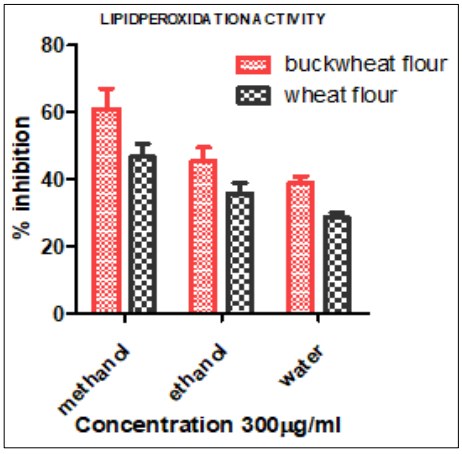


**Fig 3:** Showing Lipid peroxidation activity of buckwheat and wheat extracts using different solvents at different concentrations

**Hydroxyl radical scavenging activity**

The activities of extract on hydroxyl radical are shown in Fig (4). The hydroxyl radical is an extremely reactive free radical formed in biological system and has been implicated as highly damaging species in free radical pathology capable of damaging almost every molecule found in living cells (Hochestein and Atallah, 1988). This radical has the capacity to join nucleotides in DNA and causes strand breakage which contributes to carcinogenesis, mutagenesis and cytotoxicity. Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity (Babu *et al*., 2001) [4]. The hydroxyl radical scavenging activity of buck wheat extracts in different solvents was found in the same order as in other tests i.e, methanol> ethanol>aqueous. The results obtained for buck wheat extract in methanol, ethanol and aqueous were 54.79%, 43.68 and 39.96%, respectively. While the results obtained for wheat extracts in methanol ethanol and aqueous were 49.87, 32.32 and 30.16% respectively at concentration of 300µg/ml which are less than standard tocopherol (60.45%) at same concentration. Zhou *et al*. in 2012 found a strong correlation between TPC and OH scavenging activity. Also it is known that phenolics posses relevant radical scavenging activity which would support their putative role in radical scavenging properties of Buckwheat honey (Henriques *et al*., 2001) [15].

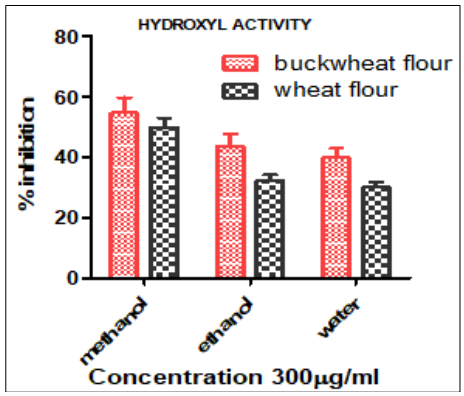


**Fig 4:** Showing hydroxyl radical scavenging activity of buckwheat and wheat extracts using different solvents at different concentrations

**Hydrogen peroxide scavenging activity**

Hydrogen peroxide is non reactive and its high concentration is toxic to living cells, and changed to free radical called hydroxyl radical. Hydrogen peroxide can cross cell membranes rapidly once inside the cell $H_2O_2$ can probably react with $Fe^{2+}$ and possibly with $Cu^{2+}$ ions to form hydroxyl radical and this may be the origin of many toxic effects (Halliwell *et al*., 1993) [13]. The Hydrogen peroxide radical scavenging potency of extracts of buckwheat and wheat in different solvents can be ranked as methanol (47.07% and 29.32)> ethanol (32.08 and 16.5%)>aqueous (21.63 and 12.53%) at concentration of 300µg/ml respectively as shown in Fig (5). Our results indicate that extracts of buck wheat show more effective results than wheat it is due to fact that buckwheat has more rutin and quercetin content in buck wheat species than common wheat (Fujita *et al*., 2005) [12]. It may be due to the higher hydrogen capability of methanol than ethanol and water.

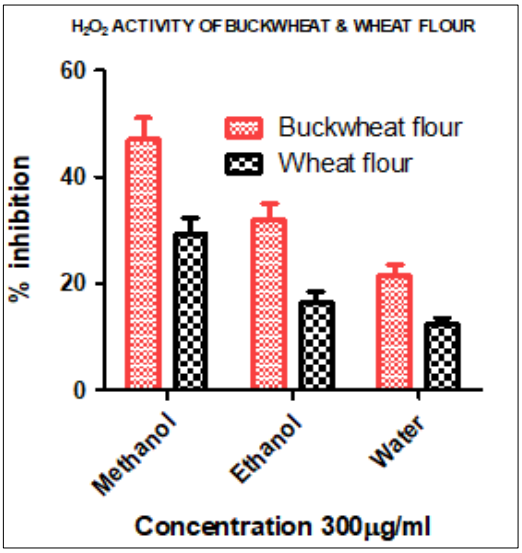


**Fig 5:** Showing Hydrogen peroxide scavenging activity of buckwheat and wheat extracts using different solvents at different concentrations

**Reducing power**

It has been reported that the reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Shimida, Fujikawa, Yahara & Nakamura, 1992) [31]. The reducing ability of buckwheat extracts in different solvents *viz*, methanol, ethanol, & aqueous is shown in fig (6). The % age inhibition of buck wheat in different solvents *viz*, methanol, ethanol & aqueous at concentration of 300mg/ml were 44.31, 36.59 23.63% respectively. While results obtained in wheat extracts in methanol, ethanol, & aqueous at same concentration were 37.30, 26.15, 19.80%. The result shows higher difference in AOA comparing buckwheat & wheat extracts, better antioxidant activity was found in buckwheat than in wheat, Strong anti oxidant activity of buckwheat extracts might be attributed to the presence of polyphenols, especially rutin, as main antioxidative component in buckwheat (Dietrych-Szostak & Oleszek, 1999) [10]. Rutin possesses all structural features which has been demonstrated to increase antioxidant activity of flavonoids and their O- glycosides (Afanas *et al*., 1989) [1]. Wheat, as other cereal, has been known to contain hydroxycinnamic acid derivatives, which demonstrated antioxidant activity (Andreasen *et al*., 2001). Ferulic acid was reported to be predominant phenolics acid in wheat (Zhou *et al*., 2004) [37] However contains lower antioxidant capacity than rutin, According to structural characteristics of these components (Cook and Samman, 1996). This fact could explain the higher AOA of ethanolic extract of buckwheat in comparison to wheat. In addition, Liyana & Shahidi (2007) found that wheat flour possessed lower ferulic acid among milling fractions of wheat, so this was reflected in its lower antioxidant activity.

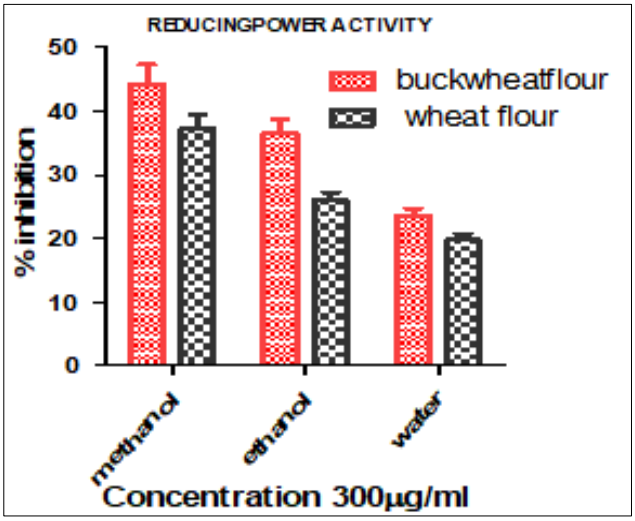


**Fig 6:** Showing Reducing power of buckwheat and wheat extracts using different solvents at different concentrations

**Anti-proliferative effects of buckwheat than wheat extract on C6 human cancer cells:**

In order to study the anti proliferative activity of wheat and Buckwheat, human C6 cancer cells were used. Fig (7) shows the antiproliferative activity shown by the extracts are concentration dependent with increase in concentration of antiproliferative activity of both extracts of wheat and buck wheat increases. The aqueous extract of the buckwheat significanltly reduced the growth rate of C6 cancer cells at concentration of 100µg/ml after 24h treatment. Buckwheat and wheat at initial concentration of 25 µg/ml had lesser antiproliferative activity than at slightly higher concentration of 100 µg/ml on C6 cancer cells. However, available information document that common and tartary buck wheat extracts induce apoptosis (Ren *et al*., 2003) [37] and have antimutagenic action (Brindozova *et al*., 2009) [7] they have effective growth inhibition of various cancer cells (Zheng *et al*., 2012) [36].

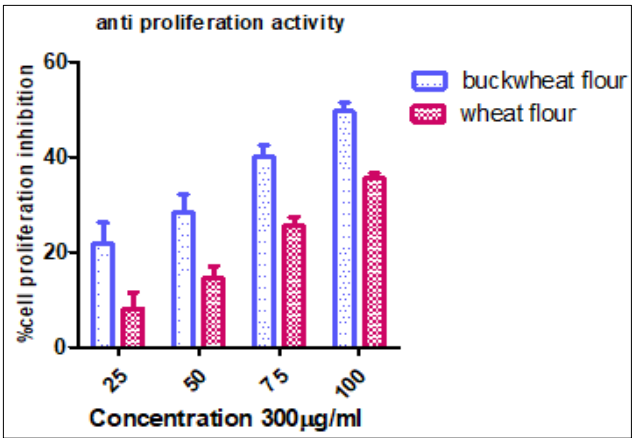


**Fig 7:** Showing Antiproliferative activity of buckwheat and wheat extracts on C6 human cancer cells at different concentrations

**Conclusion**

In the present study, it was found that buckwheat flour extract possessed potent antioxidant and antiproliferative properties as evaluated by different assay techniques. Due to presence of many prophylactic compounds such as polyphenols and flavonoids, concentrated in the buckwheat grain. Our results conclude that buckwheat can be used as natural antioxidant source in order to replace the synthetic ones. However, further characterization of chemical composition, structure of secondary metabolites, and mechanism of antiproliferative activity is required so that it can be effectively and safely used as anticancer and antioxidant source.

**References**

1. Afanas'ev IB, Dorozhko AI, Brodskii AV, Koostyuk VA, Potapovitch AI. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. Biochemical Pharmacology. 1989;38:1763-1769.
2. Andeasen MF, Landbo AK, Chiistensen LP, Hansen A, Meyer AS. Antioxidant effects of phenolic rye (*Secale cereal* L.) extracts monomeric hydroxycinnamates, and ferulic acid dehydrodimers on human low-density lipoproteins. Journal of Agricultural and Food Chemistry. 2001;49(8):4090-4096
3. Baba N, Rashid I, Shah A, Ahmad M, Gani A, Masoodi FA. Effect of microwave roasting on antioxidant and anti-cancerous activities of barley flour. J Saudi Soc Agric Sci. 2014;27:143-154.
4. Babu BH, Shylesh BS, Padikkala J. Antioxidant and hepato protective effect of Alanthus icicifocus. Fitoterapia. 2001;72:272-277.
5. Bonafaccia G, Marocchini M, Kreft I. Composition and technological properties of the flour and bran from common and tartary buckwheat. Food Chemistry. 2003;80:9-15.

6. Brand-Williams WE, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidative activity. LWT Food Science and Technology. 1995;28:25-30.
7. Brindozova, *et al*. Evaluation of mutagenicity and antimutagenicity of extracts from oat buckwheat and wheat bran in salmonella/microsome assay. J of food composition and analysis. 2009;22(1):87-90.
8. Cheng, *et al*. 2, 2-Diphenyl-1-1picrylhydrazyl radical-scavenging active components from polygonum multiflorum Thunb. Journal of agricultural and Food Chemistry. 1999;47:2226-2228.
9. Chlopicka J. Buckwheat as functional food. Bromat. Chem. Toksykol. 2008;41(3):249-252.
10. Dietrych-szostak D, Oleszek W. Effect of processing on the flavonoid content in buckwheat *fagopyrum esculentum* Moench grain. Journal of agricultural and food chemistry, 1999, 4384-4387.
11. Eggum BO, Kreft I, Javornik B. Chemical composition and protein quality of buck wheat (*Fagopyrum esculentum* Moench). Plant Foods Hum Nutr. 1981;30:175-179.
12. Fujita K, *et al*. The proceeding of international symposium the buckwheat and dietary culture in china, 2005. (Xhichang, china)
13. Halliwell B, Gutteridge JM, Aruoma OI. The deoxyribose method a simple test tube assay for determination of rate constants for reactions of hydroxyl radicals. Anal Biochem. 1987, 2005:165:215-219.
14. Halosava M, Fiedlerova V, Smcrinova H, Orsak M, Lachman L, Vavreinova S. Buckwheat-the source of antioxidant activity in functional foods. Food Research International. 2002;35:207-211.
15. Henriques A, Jacksin S, Cooper RA, Burton N. Free radical production and quenching in honeys with wound healing potential. Journal of antimicrobial chemotherapy. 2006;58:773-777.
16. Huang, *et al*. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry. 2005;53:1841-1856.
17. Ikeda K. Buckwheat: composition, chemistry and processing. Adv Food Nutr Res. 2002;44:395-434.
18. Jan U, Gani A, Ahmad M, Shah U, Baba WN, Masoodi FA, *et al*. Characterization of cookies made from wheat flour blended with buckwheat flour and effect on oxidation properties. J Food Sci Technol. 2015;52(10):6334-6344.
19. Kaliora AC, Dedousis CV. Natural antioxidant compound in risk factor for CVD. Pharmucol Res. 2007;56(2):99-109.
20. Li SQ. Zhang QH. Advance in the development of functional foods from buckwheat. Critical Reviews in food science and nutrition. 2001;41(6):451-464.
21. Liyana-pathirana C, Shahidi F. Importance of insoluble-bound phenolic to antioxidant properties of wheat. Journal of Agricultural and Food Chemistry. 2006;54:1256-1264.
22. Mosmann, *et al*. Rapid colorimeter assay for cellular growth and survival: application to proliferation and cytotoxity assays. Journal of immunological Research. 1983;65:55-63.
23. Mosmann, *et al*. Rapid colorimeter assay for cellular growth and survival: application to proferation and cytotoxity assays. Journal of immunological Research. 1983;65:55-63.
24. Oomah BD, Mazza G. Flavonoids and antioxidative activities in buckwheat. Journal of Agricultural and Food Chemistry. 1996;44:1746-1750.
25. Oyaizu M. Studies on products of browning reaction. Antioxidant activities of products of browning reaction prepared from glucoamine, Japanese Journal of nutrition. 1986;44:307-315.
26. Przybylski R, Lee YC, Eskin NAM. Antioxidant and radical scavenging activities of buckwheat seed components. Journal of the American Oil Chemists Society. 1998;75:1595-1601.
27. Ren, *et al*. Moleclar basis of fas and cytochrome c pathways of apoptosis induced by tartary buckwheat flavonoid in HL-60 cells. In methods and findings in experimental and clinical pharmacology. 2003;25(6):431-436.
28. Rush, *et al*. The Reaction Between Ferrous Polyaminocarboxylate Complexes And hydrogen peroxide investigation of the reaction intermediates by stopped flow spectrophotometry, J Inorg Biochem. 1987;29(3):199-215.
29. Sakac M, Torbica A, Sedej I, Hadnadev M. Influence of bread making on antioxidant capacity of gluten free breads based on rice and buckwheat flours. Food Res Int. 2011;44:2806-2813.
30. Sedej I, Sakac M, Mandic A, Misan A, Pestoric M, Simurina O. Quality assessment of gluten-free crackers based on buckwheat flour. LWT Food Sci Technol. 2011b;44:694-699.
31. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autooxidation of soyabean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry. 1992;40:945-948.
32. Watanabe M. Catechins as antioxidants from buckwheat (*Fagopyrum esculentum* Moench) groats. Journal of agricultural and Food Chemistry. 1998;46(3):839-845.
33. Waterhouse AL. Determination of total phenolics. In R. E. Wrolstad (Ed), Current protocols in Food Analytical Chemistry. New York: John Wiley and Sons, 2001, 11.1.1-11.1.8.
34. Wright JR, Colby HD, Miles PR. Cytosolic factors which affect microsomal Lipid peroxidation in lung and liver. Arch Biochem Biophys. 1971;206:296-304.
35. Wu HC, Pan BS, Chang CL, Shiau CY. Low-molecular-weight peptides as related to antioxidant properties of chicken essence. Journal of Food and Drug Analysis. 2005;13:176-183.
36. Zheng C, HU C, MA X, Peng C, Zhang H, QIN L. Cytotoxic phenylpropanoid glycosides from *Fagopyrum tataricum* (L.) Gaertn. In Food chemistry. 2012;132(1):433-438.
37. Zhou K, Yu L. Effect of extraction solvent on wheat bran antioxidant activity estimation. LWT-Food Science and Technology. 2004;37:717-721.
38. Zielinski H, Kozlowska H. Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. Journal of Agricultural and Food chemistry. 2000;48:2008-2016.