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## A comparative study of buckwheat and wheat cookies and effect of baking on antioxidant and anti-proliferative activities

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### Abstract

Total phenolic content, anti-oxidant activities, and anti-proliferative activity of buckwheat cookies were compared with wheat cookies using different solvents. During baking process buckwheat cookies showed greater percentage increase in antioxidant and antiproliferative activity than in wheat cookies. Samples of each extract were evaluated for DPPH radical scavenging activity, reducing power, lipid per-oxidation, H<sub>2</sub>O<sub>2</sub> 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 cookies was found to be a more potent anti-oxidant and anti-proliferative source with higher total phenolic content (66.16 GAE/100 g) when compared to wheat cookies (54.50 mg GAE/100 g). The anti-proliferative activity of buckwheat and wheat cookies was tested in C-6 cells. Both, buckwheat and wheat cookies inhibited the growth of cancer cells but buckwheat cookies (18.34%) proved to be more potent anti-proliferative source when compared to wheat (6.49%).

**Keywords:** Buckwheat and wheat, antioxidant and anti-proliferative, plant use, herbalist

### 1. Introduction

Buckwheat (*Fagopyrum esculentum*) is highly nutritious underutilized pseudocereals found in hilly regions of India and is known for its unique nutrient composition that includes lysine rich protein, dietary fibre, mineral and trace elements, antioxidant rich vitamins and bioactive compounds such as rutin, quercetin and other flavonoids. Buckwheat is thought to have originated in central and western china, 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, 28]. Rutin has strong anti-oxidant anti-inflammatory, anti-hypertensive properties which are beneficial in cardiovascular diseases thus its demand is increasing in the industries, pharmaceutical industries and cosmetic. The primary antioxidants in buckwheat are rutin, quercetin, hyperin, and catechins (Morishita, Yamaguchi, & Degi, 2007; Chlopika, 2008) [32, 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) [23, 17, 19]. 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) [33]. Variation in antioxidant activity of buckwheat was mainly due to the cultivars and environment effects (Oomah & Mazza, 1996) [23]. 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 (Holasoava *et al.*, 2002; Zdunczyk *et al.*, 2006) [14, 15]. 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

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buckwheat products consumption, first of all during prevention and healing chronic diseases (Li and Zhang, 2001) [19]. Although buckwheat is a rich source of nutrients in general and antioxidants in particular, the effect of baking on the antioxidant activity of buckwheat is very rarely reported. Since buckwheat flour is gluten free, thus replacing wheat flour with buckwheat flour will definitely dilute the wheat gluten proteins and thereby help in preventing celiac disorders, and hence act as a good replacement for wheat flour in cookie making. Several researchers have incorporated various natural components (like whey protein, mango peel powder, green tea powder, etc.) in wheat flour, to improve functional & nutraceutical properties of products prepared from it. However, limited studies have been reported on cookie making behavior of buckwheat flour.

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 cookies in comparison to the wheat cookies the most frequently used wheat products for bakery industry and to study its effect on the retention of antioxidants properties during baking, by measuring DPPH radical scavenging activity, reducing power, total phenolics content, lipid peroxidation, H<sub>2</sub>O<sub>2</sub>, hydroxyl radical scavenging activity and anti-proliferative activity.

## Materials and Methods

### Procurement of Material

Buckwheat (*F. esculentum*) was procured from market. 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.

### Cookies, preparation and evaluation

The cookies were prepared according to formula described by Tyagi *et al.* (2007) [32]. The cookies' formula based on flour weight was: 100 g flour, 53 g sugar, 26.5 g shortening, 1.1 g sodium bicarbonate, 0.89 g sodium chloride and 12 cm<sup>3</sup> water. Prepared dough was then sheeted to a thickness of 6mm with a rolling pin. The cookies were cut round in shape with a cookie die of diameter 5.5 cm and transferred to a tray lined with aluminum foil and were baked at 180 °C for 15 min in an electric oven. The baked cookies were cooled to room temperature and packed in airtight containers for further analysis.

### Extraction

0.9 g of the buckwheat and wheat cookies each were weighed and put into a 50 ml bottle. 30 ml 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 4 °C for further use.

### Determination of antioxidant activity by DPPH method

Scavenging activity of the extracts was determined

according to the method described by Baba *et al.*, (2014) [5] with some slight modifications. The absorbance at 517 nm was measured after each sample solution had incubated in dark for 30 minutes. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Percentage inhibition was calculated by using the formula  

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

Where

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

### Reducing power

The reducing power was determined according to the method of (Oyaizu 1986) [24] with slight modifications. Different concentrations of extract of the buckwheat or wheat cookies (100  $\mu$ l) was mixed with 0.02M sodium phosphate buffer pH 6.6 (2.5 ml) and 1% (w/v) of aqueous potassium ferric cyanide (2.5 ml). The mixture was incubated at 50 °C for 20 min. 10% (w/v) trichloroacetic acid (2.5 ml) was added to the mixture, which was then centrifuged at 3000 g for 10 min. the supernatant 2.5 ml was diluted with de ionised water 2.5 ml and 0.1% (w/v) ferric chloride 0.5 ml was added. The absorbance was measured at 700nm against a blank and compared to  $\alpha$ -tocopherol as standard. A higher absorbance indicates a higher reducing power.

Reduction % =  $[1 - (1 - AC/AS)] \times 100$

Ac = absorbance of standard

As = absorbance of sample

### Total phenolic content

Total phenolic content (TPC) was determined by Folin Ciocalteu spectrophotometric method Shah *et al.*, (2014), with some modifications. The results were expressed as Gallii acid equivalents (nmoles/ $\mu$ l) of sample.

### 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 cookies was mixed with 1 ml of linoleic acid 0.1 g in 100 ml of pure ethanol, 0.2 ml of H<sub>2</sub>O<sub>2</sub> 30 mM, 0.2 ml of ascorbic acid 100 mM and 0.2 ml of ferric nitrate 20 mM. this was followed by incubation at 37 °C in water bath for 1hr the reaction was stopped by the addition of 1.0 ml trichloroacetic acid (TCA), (10% w/v), followed with 0.1 ml of TBA (thiobarbitric 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), with some modifications. Briefly, the reaction mixtures contained 25 mM of calf thymus 2-deoxyribose (1 ml), 10mM ferric chloride (200  $\mu$ l), 100mM Ascorbic acid (20  $\mu$ l), 2.8 mM KH<sub>2</sub>PO<sub>4</sub> and various concentrations of extracts of buck wheat or wheat. The mixture was vortexed and incubated at 37 °C for 1hr then 1 ml of 1% TBA and 1 ml of 3% TCA were added and heated

in water bath at 100 °C for 20 min. 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:

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

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

### H<sub>2</sub>O<sub>2</sub> scavenging activity

The ability of buckwheat or wheat cookies extracts to scavenge hydrogen peroxide was evaluated according to the method *Rush et al.* with minor modifications. A solution of H<sub>2</sub>O<sub>2</sub> (2mM) was prepared in phosphate buffer (pH7.5). Various concentrations of buck wheat or wheat extracts were added to H<sub>2</sub>O<sub>2</sub> solution (0.6 ml). Absorbance of H<sub>2</sub>O<sub>2</sub> at 523nm was determined after 5min against blank solution containing phosphate buffer without H<sub>2</sub>O<sub>2</sub>. BHT was taken as standard. The scavenging activity of buck wheat or wheat extracts on H<sub>2</sub>O<sub>2</sub> was expressed as.

$$\% \text{ inhibition} = \left[ \frac{A_0 - A_1}{A_0} \right] \times 100$$

Where A<sub>0</sub> is the absorbance of control and A<sub>1</sub> 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 in 100 $\mu$ l'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 (100 $\mu$ l) were added to wells in triplicates. Cells were incubated with the extract for 24 hours. After 24hours 20 $\mu$ l'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 150 $\mu$ l's of DMSO using shaker for 15minutes. All the steps performed after MTT addition were performed in dark. Absorbance was measured at 590 nm.

### Statistical analysis

Experiments were performed in triplicates. The data was analyzed using one way analysis of variance (ANOVA) and Duncan test by SPSS (version 16.1).

## 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 cookies 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 cookies differed greatly ranging from 54.50 GAE $\mu$ /100g in wheat cookies and 66.16 GAE $\mu$ /100g in buckwheat cookies 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) in which methanol was found as a more potent extraction solvent when compared to ethanol and distilled water.

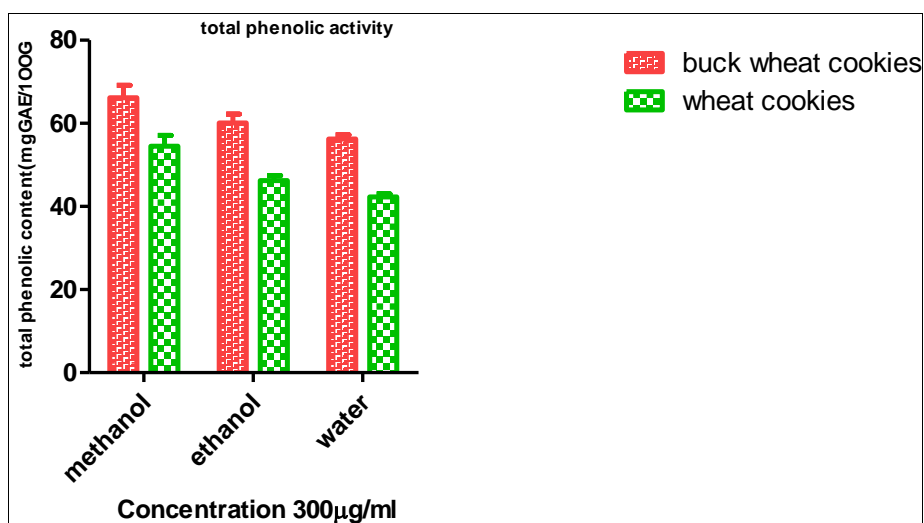


Fig 1: Showing total phenolic content of buckwheat and wheat cookies extracts using different solvents at different concentrations

### 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*) [16]. In this study, the antioxidant activity of wheat

and buckwheat cookies was evaluated using DPPH radical scavenging activity. Our results indicated Fig (2) that DPPH radical scavenging ability of buckwheat cookies was higher than wheat cookies in ethanolic solvents which could be because of the higher phenolic content present in buckwheat as compared to wheat. Same results were reported by Seedeve *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.

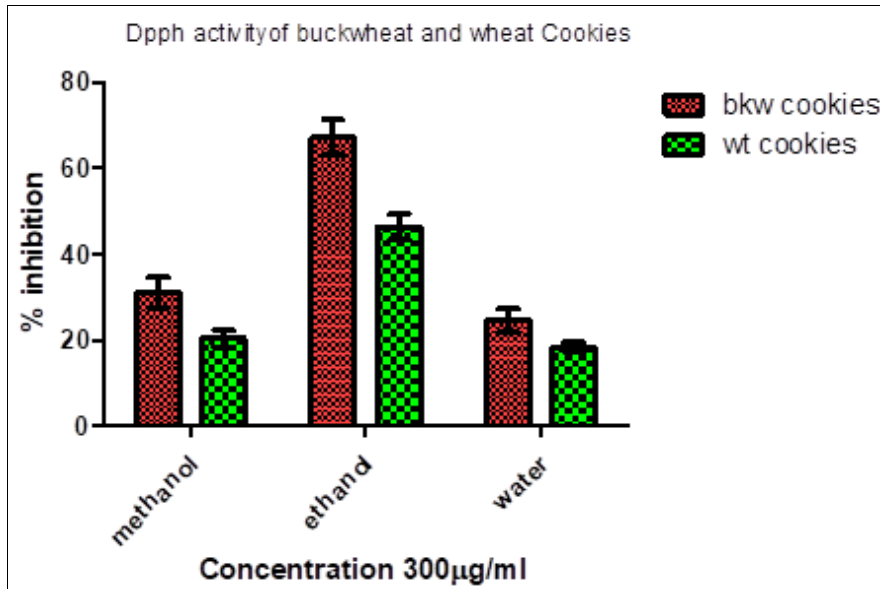


Fig 2: Showing DPPH radical scavenging activity of buckwheat and wheat cookies extracts using different solvents at different concentrations

**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 cookies at a concentration of 300

µg/ ml in methanol, ethanol and aqueous, are 64.20, 47.70, and 32.33%. For wheat cookies, in same solvents and at same concentration the %age inhibition recorded is 53.36, 30.24 and 20.20% which is less as compared to buck wheat cookies Fig (3). Our findings are in inconsistent with who reported that among several grains wheat, rye, barley and buckwheat the methanol extract prepared from buck wheat exhibited the strongest protective effect against lipid peroxidation. This fact can be attributed to the is of flavanoid rutin present in the seeds of buckwheat which makes major contribution to the antioxidant activity in buckwheat (Dietrych-Szostak and Oleszek, 1999) [10].

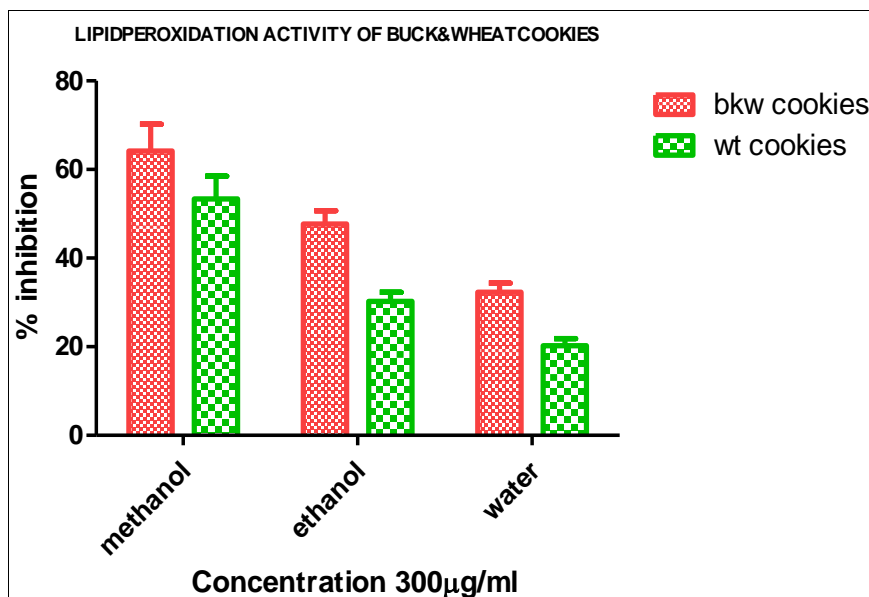


Fig 3: Showing lipid peroxidation activity of buckwheat and wheat cookies extracts using different solvents at different concentrations

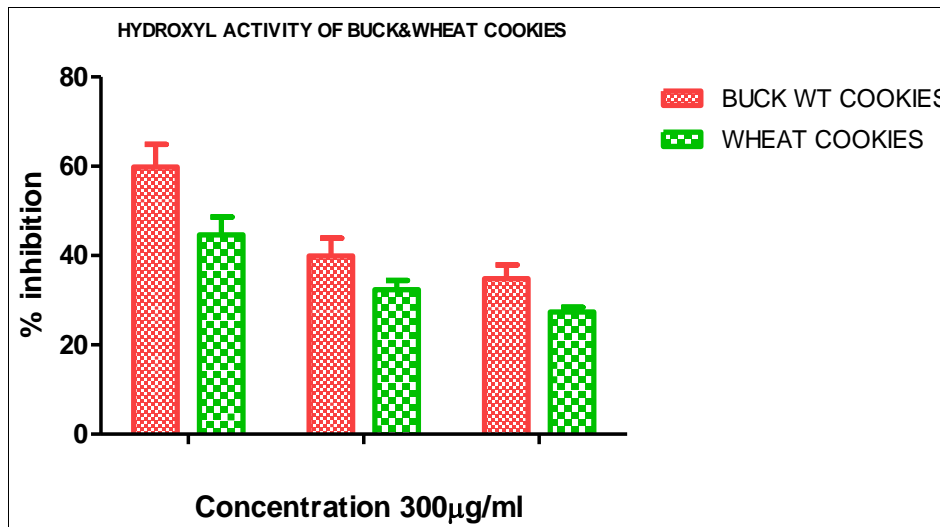
**Hydroxyl radical scavenging activity**

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. 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) [6]. The hydroxyl radical scavenging activity of buck wheat cookies 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 cookies extract in methanol, ethanol and aqueous were 59.83%, 39.87 and 34.85 %, respectively. While the results obtained for wheat cookies

extracts in methanol ethanol and aqueous were 44.63, 32.39 and 27.40% respectively at concentration of 300 µg/ml which are less than standard tocopherol (60.45 %) at same concentration. found a strong correlation between TPC and OH scavenging activity. Also it is known that phenolics possess relevant radical scavenging activity which would support their putative role in radical scavenging properties of Buckwheat honey (Henriques *et al.*, 2006) [15].

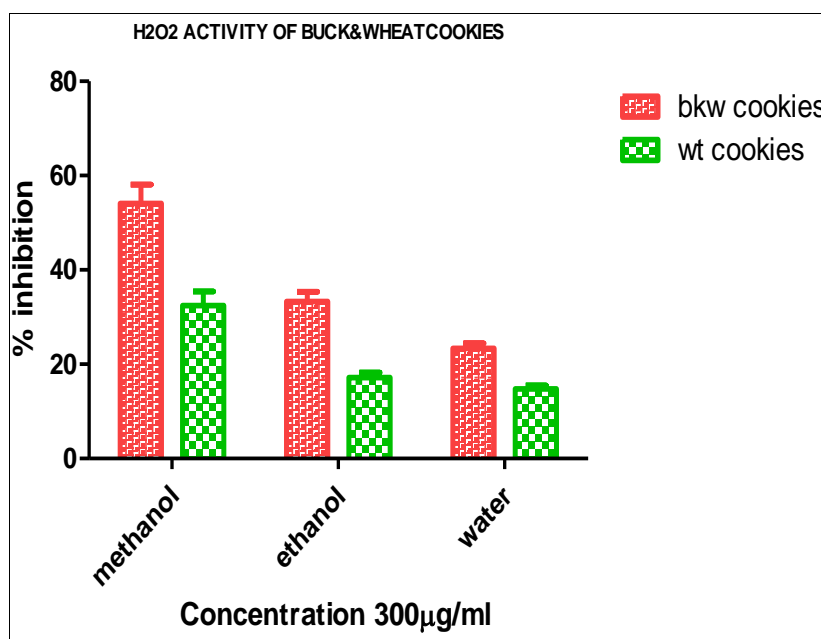


**Fig 4:** Showing hydroxyl radical scavenging activity of buckwheat and wheat cookies extracts using different solvents at different concentrations

**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<sub>2</sub>O<sub>2</sub> can probably react with Fe<sup>2+</sup> and possibly with Cu<sup>2+</sup> 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 cookies in different solvents can be ranked as methanol (54.08 % and 32.40)> ethanol (33.33 and 17.15%) >aqueous (23.40 and 114.77%) at concentration of 300 µg/ml respectively as shown in Fig (5). Our results indicate that extracts of buck wheat cookies 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) [11]. 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 cookies 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 cookies 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 51.34, 41.42, 39.43% respectively. While results obtained in wheat cookies extracts in methanol, ethanol, & aqueous at same concentration were 45.65, 36.77, 31.65%. The result shows higher difference in AOA comparing buckwheat & wheat cookies extracts, better antioxidant activity was found in buckwheat than in wheat, Strong antioxidant activity of buckwheat cookies 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) [11]. Wheat, as other cereal, has been known to contain hydroxycinnamic acid derivatives, which demonstrated antioxidant activity. Ferulic acid was reported to be predominant phenolics acid in wheat 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) [20] found that wheat flour possessed lower ferulic acid among milling fractions of wheat, so this was reflected in its lower antioxidant activity.

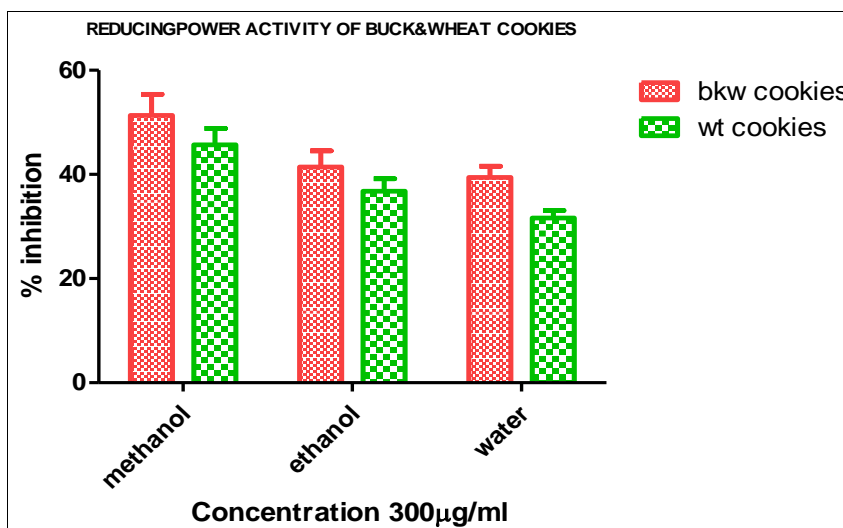


Fig 6: Showing Reducing power of buckwheat and wheat cookies extracts using different solvents at different concentrations

**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 cookies, 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 significantly reduced the growth rate of C6 cancer cells at

concentration of 100 µg/ ml after 24h treatment. Buckwheat and wheat at initial concentration of 25 µg/ ml had lesser antiproliferative activity than at slightly higher concentration of 75 µg/ ml on C6 cancer cells. However, available information document that common and tartary buck wheat extracts induce apoptosis (Ren *et al.*, 2003) [26] and have antimutagenic action (Brindozova *et al.*, 2009) [9] they have effective growth inhibition of various cancer cells (Zheng *et al.*, 2012) [36].

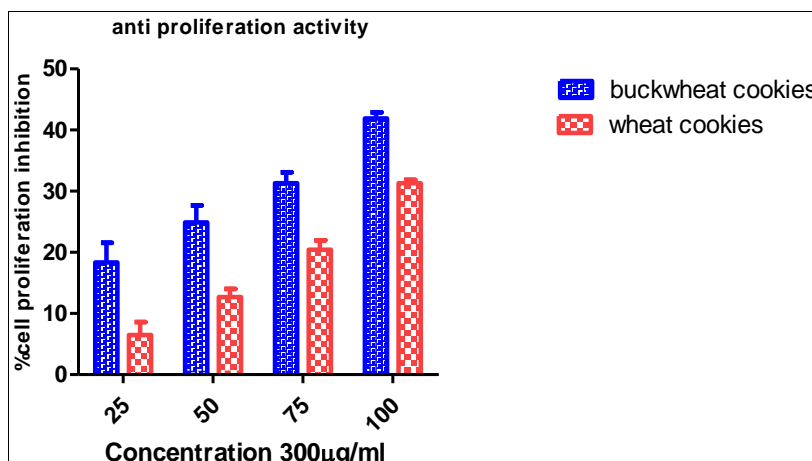


Fig 7: Showing Antiproliferative activity of buckwheat and wheat cookies extracts on C6 human cancer cells at different concentrations

## Conclusion

In the present study, it was found that buckwheat cookies extract possessed potent antioxidant and antiproliferative properties as evaluated by different assay techniques. Although baking decreased total phenolic content but an increase in overall antioxidant activity was observed, Buckwheat cookies showed greater increase in antioxidant activity than wheat cookies can be attributed to greater generation of Melanoides in the former which is supported by higher NEB values of buckwheat cookies than wheat cookies. Therefore 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.

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