



A Comparative Study on Proximate and Antioxidant Activity of *Brassica oleracea* (Kale) and *Spinacea oleracea* (Spinach) Leaves

Ayushi Agarwal¹, Nishtha Raj² and Neelam Chaturvedi^{3*}

^{1,2}.Research Scholar, ³ Associate Professor, Department of Food Science and Nutrition, Banasthali University, Dist- Tonk, Rajasthan. 304022,

*Corresponding author: neelam295chaturvedi@rediffmail.com

Abstract

Green leafy vegetables have excellent content of vitamins as well as minerals and also contain an enormous variety of bioactive components which provide health benefits beyond basic nutrition. The purpose of the study is to evaluate the proximate composition by standard AOAC method, antioxidant properties and DPPH free radicals scavenging activity of aqueous extract of *Brassica oleracea* and *Spinacea oleracea*. The study revealed that crude fiber, protein, iron and calcium content in *Brassica oleracea* was significantly increased by 41%, 41.6%, 8.7% and 37.23% respectively, when compared to *Spinacea oleracea* at $p \leq 0.05$ level. In aqueous extract of *S.oleracea* recorded the higher content of total phenols and total flavonoids i.e 41.2 ± 0.18 mg GAE/g and 20.40 ± 1.23 mg QE/g respectively as compared with *B.oleracea*. Although both leaves extract showed good amount of antioxidant components whereas free radical scavenging activity was higher *B.oleracea* leaves (IC_{50} -26 μ g/ml) while *S.oleracea* had (IC_{50} - 48 μ g/ml) when compared to standard ascorbic acid. The study may conclude that *Brassica oleracea* leaves are as good as *Spinacea oleracea* leaves which could be incorporated in food formulation as therapeutic agent apart from its nutritional essence which could be explored to provide affordable remedy to masses.

Keywords: *Brassica oleracea*, *Spinacea oleracea*, DPPH (2, 2-diphenyl-1-picrylhydrazyl), Total Phenols Content, Total Flavonoids Content.

Introduction

Green leafy vegetables play an important role in supporting the normal functioning of the different body systems and preventing diseases (Emebu et al., 2011). It provides high amounts of micro-minerals which participate in vital functions of nutrient metabolism and retard various degenerative diseases (Duma et al., 2014). Phytochemicals found in vegetables are strong antioxidants which reduce the risk of chronic disease by protecting against free-radical damage, by modifying metabolic activation and detoxification of carcinogens, or even influencing processes that alter the course of tumor. Vegetables

contain low amounts of fat and calories (Yadav et al., 2013). They are rich sources of carotene, ascorbic acid, riboflavin, folic acid and minerals like calcium, iron and phosphorus. In addition, they contain antinutrients which reduce their bioavailability. They also act as buffering agents for acid substance obtained during the digestion process (Gupta et al., 2008).

Kale (*Brassica oleracea*) is a leafy green vegetable belonging to the cabbage family Brassicaceae that contains a large amount of health-

promoting phytochemicals (Adams, 2013). It is the species of plant that includes many common foods as cultivars including cabbage, broccoli, cauliflower, kale, Brussels sprouts, collard greens, Savoy, kohlrabi and Chinese kale (Lewis et al., 2015). It is one of the most nutritious vegetables particularly rich in dietary fiber, protein, minerals and antioxidant compounds (Acikgoz, 2011). Kale has therefore gained increased attention as a source of beneficial effects (Nishi *et al*, 2011). Kale is among a small number of foods that contain measurable and negligible amounts of oxalates (0.02 mg/100 g), naturally-occurring substances found in plants, animals and human beings (Nazzaro et al., 2014).

Spinach (*Spinacea oleracea*) belongs to the chenopodiaceae family and it is dense in vitamins and minerals, low in calories and fiber content which help to prevent constipation and promote a healthy digestive tract (Ware, 2015). Spinach is available all year round but is in season during the spring (Lewin, 2015) and have possible health benefits include improving blood glucose control in diabetics, lowering the risk of cancer, lowering blood pressure, improving bone health, prevention of bone mineral density, lowering the risk of developing asthma and it also contains a unique and beneficial mixture of phytonutrients, as well as anti-oxidants and inflammatory benefits, flavonoids and carotenoids (Khare, 2007). Spinach and other green vegetables contain chlorophyll which has shown to be effective at blocking the carcinogenic effects of heterocyclic amines which are generated when grilling foods at a high temperature and helpful for the cancer patients.

The major aim of the present study was to compare the *Brassica oleracea* (Kale) and *Spinacea oleracea* (Spinach) on nutritional components and antioxidant property

Materials and Methods

Sample collection and preparation of plant material

Fresh leaves of *Bassica oleracea* (Kale) and *Spinacea oleracea* (Spinach) were used in the study and were collected from local market of Kashipur (Uttarakhand). The plant samples were identified by horticulturist of KVK of Banasthali University. The plant material was washed, dried at 50 °C for 18 hours, powdered and stored in airtight containers at 5 °C for further analysis. Aqueous extracts were prepared by soaking 100g each of the dry leaves powdered in 1 litre of distilled water

at room temperature for 48 h. The extracts were filtered first through a Whatmann filter paper No. 42 (125 mm). The extracts were concentrated using a rotary evaporator with the hot water bath set at 40 °C. The percentage yield of extracts ranged from 5 - 20% (w/w) (Aqil et al., 2006).

Determination of Proximate composition:

Proximate determination which involved protein, fats, carbohydrates, dietary fiber, moisture, ash and energy were carried out according to AOAC methods (2009). Ash content was determined by weight difference after sample mineralization at 600 °C for 6 h whereas moisture content was determined by drying in an oven at 85 °C to constant weight. Crude protein was determined indirectly from the analysis of total nitrogen (crude protein = amount of nitrogen × 6.25) using Kjeldhal method by Kel Plus analyzer (Pelican, Model: KES-061). Crude fat was determined through Socs Plus system (Pelican, Model: SCS-6) by using petroleum ether. Crude fiber content of seeds was determined by digesting dry sample with 1.25% H₂SO₄, followed by 1.25% NaOH solution in Fibra Plus Fiber analyzer (Pelican, Model: FES-4). Vitamin C was obtained by titrimetric method by using 2-6 dichlorophenol indophenols dye. Carbohydrate content was estimated by subtracting the values of protein, moisture, ash, fiber and fat content from 100. Energy value (Kcal/100g) = 4 X % carbohydrates + 9 X % crude fat + 4 X % crude protein. Mineral elements estimations indicate the amount of inorganic elements present in the sample. The determination was carried out using standard procedures. The mineral element determined were Iron (Fe) by Wong's method and Calcium (Ca) by titration against standard potassium permanganate solution (KMnO₄).

Determination of Total Phenol Content:

Total phenols were determined by Folin Ciocalteu Reagent. A dilute extract of each seed (0.5 ml of 1:10 g/ml) or Gallic acid (standard phenol compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and 4 ml of aqueous sodium carbonate (1 M). The mixtures were kept at dark ambient condition for 15 min and the total phenols were determined by spectrophotometer at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/L solutions of Gallic acid in aqueous extract. Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound (Blois et al., 2009).

Determination of Total Flavonoids Content:

Aluminum chloride colorimetric method was used for flavonoids determination. Each seed extracts (0.5 ml of 1:10 g/ ml) in aqueous were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a UV-Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 mg/g in aqueous extract (Olsen et al., 2009).

DPPH radical scavenging activity:

The ability of the aqueous extracts to scavenge free radicals was determined against a very stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) determined Spectrometric method. Aliquots of the

sample extract at different concentrations 0.02, 0.05, 0.1, 0.15, 0.25 mg/ml were added to 1 ml aqueous solutions of DPPH. Each mixture was vortexed vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm and activity was expressed as percentage. DPPH scavenging relative to control using the following equation:

DPPH scavenging activity (%) = (Absorbance of control – Absorbance of sample)/ Absorbance of control × 100, IC₅₀ Value was also determined by graph (Hemalatha et al., 2010).

Statistical Analysis:

The results obtained were expressed as mean ±SD and analysis determinations and also statistically analyzed to ascertain its significance. The significance was estimated at (p≤0.05 level).

Results and Discussion

Table1- Proximate analysis of *Bassica oleracea* and *Spinacea oleracea* leaves on dry weight basis.

Proximate composition	<i>Spinacea oleracea</i>	<i>Brassica Oleracea</i>	Increase/decrease Difference	
Moisture (g/100g)	6.7±0.07	9.08±0.6*	(26.2%)	↑
Ash(g/100g)	6.61±0.4	4.34±0.7*	(52.9%)	↑
Fat(g/100g)	3.6±0.3	1.62±0.3*	(55%)	↓
Crude Fiber(g/100g)	4.6±0.4	7.8±0.42*	(41%)	↑
Protein(g/100g)	2.99±0.4	5.12±0.43*	(41.6%)	↑
Carbohydrate(g/100g)	75.5±0.07	72.04±0.56*	(4.58%)	↓
Energy(kcal/100g)	334.04±0.8	304.88±0.6 ^{ns}	(7.8%)	↓
Iron(mg/100g)	12.32±0.2	13.5±0.4*	(8.7%)	↑
Calcium(mg/100g)	77.42±0.04	123.40±0.4*	(37.23%)	↑
Vitamin-C(mg/100g)	25.9±0.4	24.5±0.4 ^{ns}	(5.7%)	↓

(n=3), Values are expressed as means± SEM.* significant at P < 0.05, ns- non significant when compared with *Spinacea oleracea* leaves powder.

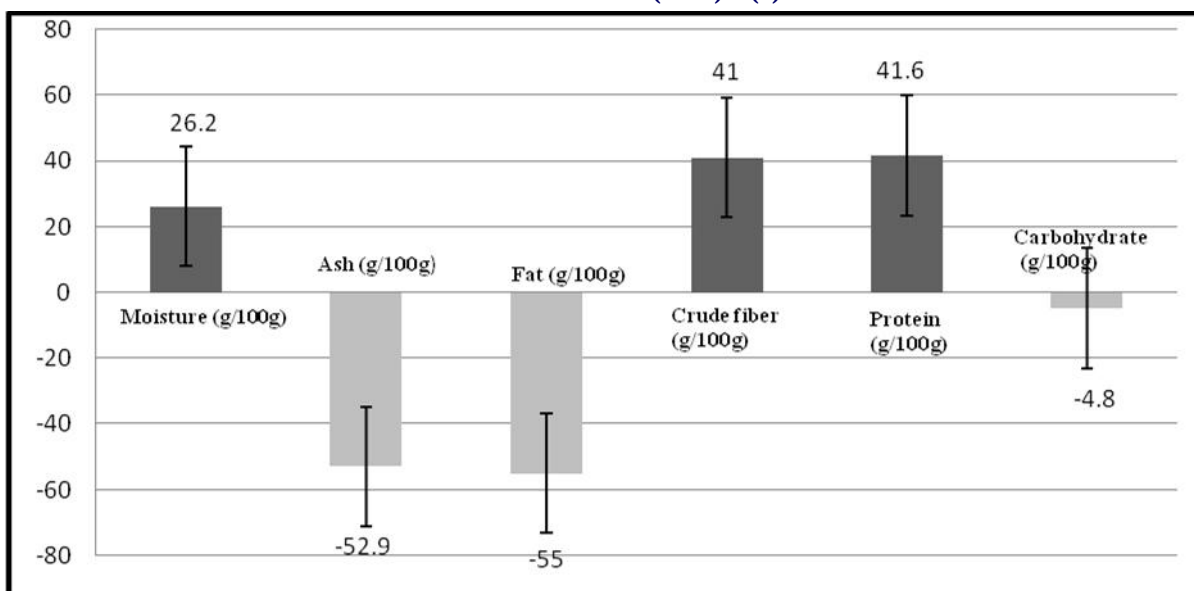


Figure 1: Percent difference of *Brassica oleracea* over *Spinacea oleracea* leaves powder with Moisture, Ash, Protein, Fat, Crude Fiber, Carbohydrate.

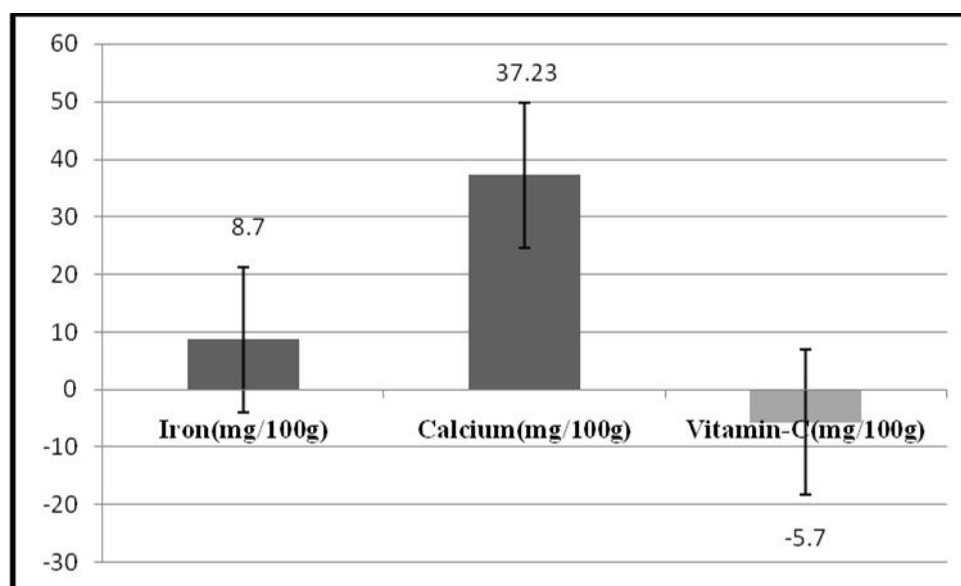


Figure 2- Percent difference of *Brassica oleracea* over *Spinacea oleracea* leaves powder with Iron, Calcium and Vitamin C.

The proximate composition of *Brassica oleracea* (Kale) and *Spinacea oleracea* (Spinach) is shown in Table 1 and figure 1. The fat and carbohydrate content of *B. oleracea* were 1.62 ± 0.3 and 69.48 ± 0.56 (g/100g) respectively. The data depicted that the values of *B. oleracea* were significantly decreased by 55% and 4.58% at $P \leq 0.05$ level when compared to *Spinacea oleracea*. However, the fat content of Kale as observed in this study was lower. According to Baloch et al., 2015, the fat content in *Brassica oleracea* was also found to be 2.16g/100g which was lower value than other vegetables such as cauliflower

and Broccoli. The crude fiber and crude protein content were found to be high in *Brassica oleracea* 7.8 ± 0.42 and 5.12 ± 0.43 (g/100g) respectively when compared to *S. oleracea*. The values were significantly increased by 41% and 41.6% at $P \leq 0.05$. The present results are in fair agreement with Leibman, 2007 that crude fiber content in *brassica oleracea* was 8.05 ± 0.56 g/100g. Korus et al., 2009, who reported the protein content of *Brassica oleracea* was 11.6g/100g which is higher value than present data.

The iron and calcium content were found to be high in *B. oleracea* 13.5 ± 0.4 and 123.40 ± 0.4 (mg/100g) respectively when compared to *S. oleracea*. The data depict that the value were significantly increased by

8.7% and 37.13% at ($P \leq 0.05$). The similar data were also obtained by Emebu et al., 2011 and Lewis et al., 2015 that the iron and calcium content were 8.94 and 41.05 (mg/100g) respectively in *Brassica oleracea*.

Table 2- Antioxidant potential of aqueous of *Bassica oleracea* and *Spinacea oleracea* leaves

Antioxidants	<i>B.oleracea</i>	<i>S.oleracea</i>
Total Phenols (mg GAE/g)	$35.64 \pm 0.56^*$	41.2 ± 0.18
Total Flavonoids (mg QE/g)	$13.98 \pm 0.23^*$	20.40 ± 1.23

n=3, Values are expressed as means $\pm$ SEM.* significant at $P < 0.05$, ns- non significant when compared with *S. oleracea* leaves powder

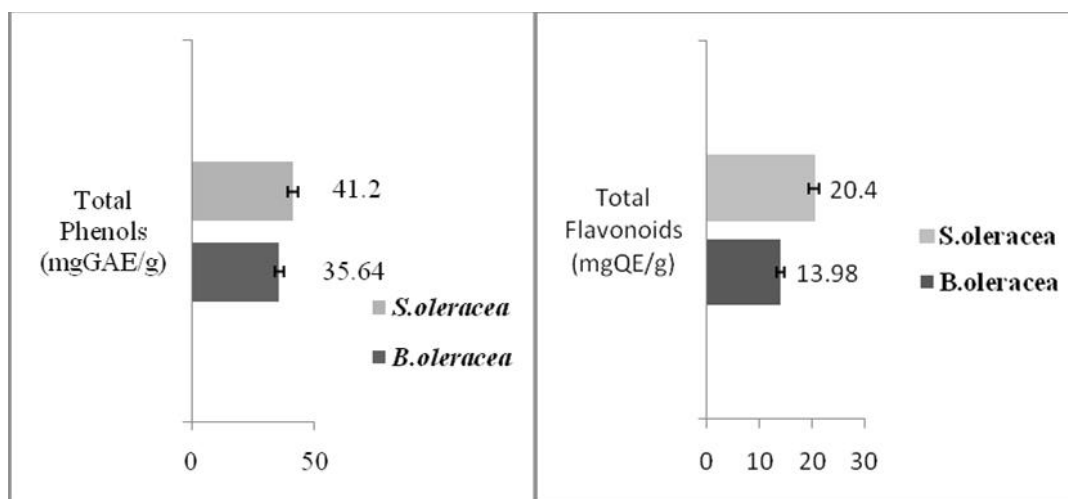


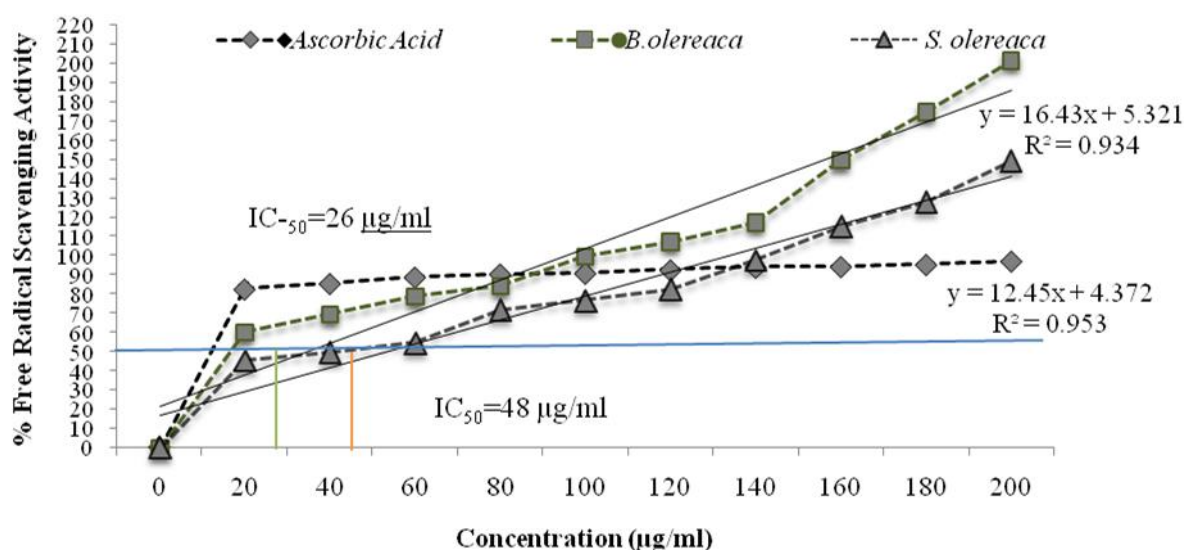
Figure 3- Total Phenols and Flavonoids content of aqueous leaves extract of *Brassica oleracea* and *Spinacea oleracea*

The content of total phenols in aqueous extracts expressed as mg Gallic acid equivalent (GAE) per gram of extract. The total phenol content of *B. oleracea* and *S. oleracea* were 35.64 ± 0.56 and 41.2 ± 0.18 (mg GAE/100g) respectively. The results depicted that values of *B. oleracea* was significantly decreased by 18% at $p \geq 0.05$ when compared to *S. oleracea* leaves extract. The similar data was also illustrated by Kural et al., 2011 that *B. oleracea* leaves aqueous extract had 34.3 ± 1.6 (mg GAE/100g) that may have protective effect on the oxidation of lipoprotein even at low concentration. Total flavonoids

content was determined by using Aluminum chloride method and amount of chloride was expressed as mg quercetin equivalent (QE) per gram of extract (QE/g). Correspondingly, total flavonoids content was found to be low in *B. oleracea* (13.98 ± 0.23 mg QE/g) when compared to *S. oleracea* (20.40 ± 1.23 mg QE/g). The data revealed that the value of *B. oleracea* was significantly decreased by 9% at $p \geq 0.05$ level. According to Lakshmi et al., 2016 that the flavonoids content was 17.44 to 4.37 (mgQE/g) in *B. oleracea* leaves which is closer value to our data.

Table 3- The DPPH free radical scavenging activity of *Brassica oleracea* and *Spinacea oleracea* aqueous leaves extracts

Concentration (µg/ml)	Ascorbic Acid	<i>B oleracea</i> aqueous extract	<i>S oleracea</i> aqueous extract
20	82.4	60.2	45.4
40	85.3	69.5	49.6
60	88.7	78.7	54.5
80	90.3	84.3	71.7
100	90.7	99.6	76.6
120	92.4	107.3	82.2
140	93.8	117.4	97.5
160	94.2	149.6	115.2
180	95.2	174.8	127.7
200	97.03	201.7	149.4
IC ₅₀ Values (µg/ml)		18.00	48.00

**Figure 4- % free radicals scavenging activity of *Brassica oleracea* and *Spinacea oleracea* aqueous extract.**

The % free radical scavenging activity of the aqueous extracts were evaluated by using the parameter IC₅₀ which means the concentration of antioxidant required for 50% scavenging of DPPH radicals in the particular time period (Malencic et al., 2000). *In vitro* activities of both leaves extracts were measured with the standard antioxidant (ascorbic acid). Here, the DPPH free radicals scavenging activities of both leaves extracts are shown in (Table 3 and figure 4). The antioxidants activity of both leaves with IC₅₀ (Inhibitory concentration) values ranged from (20-

210µg/ml) from which, *B.oleracea* leaves exhibited high scavenging activity at IC₅₀ (26µg/ml) when compared with *S.oleracea* (48 µg/ml).It was noticed that leave extract of *B.oleracea* showed strong hydrogen donating abilities to act as an effective antioxidant. This result was also in line with the study of Woolley, 2015 that maximum DPPH scavenging activities were found in aqueous of *B.oleracea* leaves extract with IC₅₀ values ranging from 0.72 mg/ml to 2.17 mg/ml respectively.

Conclusion

From the results of this study, showed that *Brassica oleracea*, though a lesser known vegetable, has enormous nutritional potentials and can favorably be used as a substitute for most of the commonly used vegetables. It has been recognized as a good source of vegetable fiber and protein content and showed lower value for total phenols, flavonoids content but higher free radical scavenging activity as compared to *spinacea oleracea* aqueous extract. Therefore, both leafy vegetables possess strong anti antioxidative potential to manage against metabolic disorders such as diabetes and cardiovascular diseases.

Conflict of interest statement

We declared that we have no conflict of interest.

Acknowledgments

Authors are thankful to Prof. Aditya Shastri (Vice Chancellor) of Banasthali University for providing all the required lab facilities in Food Science and Nutrition department that helped us for the successful completion of the project work.

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DOI: 10.22192/ijarbs.2017.04.04.004	

How to cite this article:

Ayushi Agarwal, Nishtha Raj and Neelam Chaturvedi. (2017). A Comparative Study on Proximate and Antioxidant Activity of *Brassica oleracea* (Kale) and *Spinacea oleracea* (Spinach) Leaves. Int. J. Adv. Res. Biol. Sci. 4(4): 22-29.

DOI: <http://dx.doi.org/10.22192/ijarbs.2017.04.04.004>