



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(1): 2159-2162
Received: 06-11-2017
Accepted: 07-12-2017

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Phyto-nutritional and mineral composition of Indian Horse Chestnut (*Aesculus indica*) seeds

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Abstract

Primarily, Indian Horse Chestnut Seed Extract (HCSE) was used only pharmacologically for human purposes. It has an anti-allergic, anti-inflammatory, anti-microbial, anti-cancer, antiviral and anti-diarrheal activity. But now days, it gain nutritional importance rapidly. The seeds varying nutritionally in raw fresh flour and processed flour (*tatvakhar*) form after processing. The estimated total sugars (% glucose) of fresh and processed flour were 20.23 and 13.47, respectively. Starch (%) was 27.83 and 66.47 content in fresh and processed flour correspondingly. The mineral contents of fresh flour *viz.* calcium (8.20), phosphorus (19.00), potassium (81.00), copper (0.60), manganese (0.50), iron (8.50) and zinc (705.90) (mg/100g) were determined. Phytochemicals present in the seeds, such as total phenols, tannins and flavonoids values were followed as 785.25, 747.43 and 47.00 (mg GAE/g).

Keywords: Fresh flour, mineral, phytochemicals, processed flour, starch, sugar

Introduction

Indian Horse Chestnut is generally known as *bankhor* in India, is botanically known as *Aesculus indica* and belongs to *Hippocastanaceae* family. The tree is distributed in the hilly areas or temperate region from Kashmir to Nepal, at an altitude from 900 to 3,600m. Zhang *et al.* (2010) ^[1] reported *Aesculus hippocastanum* species was used for chronic venous insufficiency, hemorrhoids, and postoperative edema. *Aesculus* seed extracts possessed miscellaneous activities, including anti-inflammatory, antitumor, antiviral, antifungal, antiangiogenic (vascular protection), anti-obesity, antioxidative, and antigenotoxic properties. But in India, the seeds are eaten by wild animals and cattles. The seeds are lethal, if consumed without processing or raw, due to the presence of anti-nutrients like saponin and tannin. So the seeds must be decorticoid, crushed and soaked for overnight in water for 10-12 days with repeatedly changing water, to remove its poisonous substance and then dried under sunlight. Locally, this dried seed is grounded to make flour, is called as *Tatvakhar* or processed flour. Occasionally, it is mixed with wheat flour to make chapatti by the tribal community of Himachal Pradesh and extraction of saponin/aescin content (0.08 g/100g) in processed flour was reported by Sood *et al.* (2015) ^[2]. The fat/oil, protein and mineral content of Indian Horsechestnut seeds determined by Majeed *et al.* (2010) ^[3] were 2.02 per cent, crude protein (7.18%), and minerals *i.e.* potassium (0.79%), phosphorus (0.18%), sulphur (0.07%), calcium (0.08%), iron (159 ppm), copper (41.2 ppm), zinc (25.6 ppm) and manganese (6.95 ppm). The oil was used for rheumatism. On dry basis, Uppal (1952) ^[4] investigated starch 25(%) content from Indian horse chestnut. Singh *et al.* (2004) ^[5] analyzed the reducing sugars (as dextrose), 0.83; sucrose, 9.07; starch, 38.3; water sol, 36.4 and aescin obtained from *Aesculus indica* seeds has an effective antiviral against in controlling the growth of cucumber mosaic virus (cmv) that affected plants like vegetable plants, fruit plants, seeds, leaves, ornamental plants and other plants like cucumber, beans, spinach, crucifers, peppers, melons etc. Rafiq *et al.* (2015) ^[6] isolated the starch content from dehydrated Horse Chestnut slices at different temperature. The sample dried at 50°C had highest yield of starch. The isolated starch yield was 28(%), whereas ash and protein were 0.29%, and 0.31%, respectively and no lipid. Parmar and Kaushal (1982) ^[7] determined the moisture content about 50.5 per cent; total sugars (5.58 per cent), whereas the reducing and non-reducing sugars are 4.59 and 0.94 per cent respectively. Protein and mineral values were 0.388 and 1.934 per cent, in that order and the minerals elements *viz.* Phosphorus, 0.124; potassium, 0.733; calcium, 0.0495; magnesium, 0.042 and iron, 0.0084 percent. Kapusta *et al.* (2007) ^[8] extracted flavonoids from Horse chestnut seeds which were higher and significant amount. The waste of the seeds got in Industry could be used to made quercetine and kaempferol glycosides for cosmetic, nutraceutical, and food supplement. Kaur *et al.* (2011) ^[9] evaluated the phyto-chemicals of *Aesculus indica* in leaves in qualitative and quantitative way.

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In qualitative terms, there were presence of saponin, tannin, flavonoid, amino acids, steroids, terpenoids, and carbohydrate test in aqueous and ethanolic extract. And the yield of total saponin and tannin were 13.4 and 5 percent, respectively.

It has important nutritional value; hence an effort was done to determine the nutritional, phyto-chemical and mineral content because the seeds are wasted every year in the forest and ate by the wild animals. Thus, an effort was attempted to find out the nutritional characteristics such as total sugars starch and phyto-chemicals of the fresh seed and processed flour (*tatwakhar*) which is not available readily, and minerals contents in fresh seed samples. People used to consume processed flour traditionally in various forms without knowing its effect on us.

Materials & Method

Indian Horse Chestnut (*Aesculus indica*) seeds were obtained from *Brot* village of Mandi, District and brought to the laboratory of the Department of Food Science, Nutrition and Technology, College of Home Science, CSK HPKV, Palampur. The seeds were cleaned manually, washed to remove any adhering dirt, dust and foreign particles. Then ground into a fine powder with the help of stainless steel grinder and stored in airtight plastic containers so as to prevent changes till further analysis.

Sugars

The sugar content in seeds was estimated by the standard method of AOAC, (1990) ^[10].

Preparation of extract

Transferred 5.675 g finely ground powder of Indian Horse Chestnut seeds in 100 ml Erlenmeyer flask. The flasks were kept tilted so that whole of the flour is at one side, then added 5 ml alcohol to wet the flour followed by 50 ml acetate buffer solution. Shook the flasks and immediately added 2 ml sodium tungstate solution and again mixed thoroughly. The contents were filtered through the Whatman filter paper No. 4 discarding first 8-10 drops.

Starch

Starch in seeds of Horse Chestnut was determined by the method of Clegg (1956) ^[11].

Extraction

Transferred 500 mg sample in a round bottom flask followed by 25 ml ethanol (80 per cent) was added to powdered sample of Indian Horse Chestnut. The flask was connected to condenser and kept on heating mantle for 30 minutes with occasional stirring. The extract was cooled, centrifuged at 8000 rpm for 15 minutes and the supernatant was discarded. The above procedure was repeated twice each time extracting the residue in 25 ml of 80 per cent ethanol. This sugar free pellet was used for estimation of starch.

Minerals

Digestion

One gram of finely ground sample (seeds) was taken in 150 ml conical flask. To this 25 ml of diacid mixture (HNO₃: HClO₄ in 5:1 v/v) was added and kept overnight. Digestion was done on the next day by heating till clear white precipitates settle down at the bottom. The crystals were dissolved by diluting in double distilled water. The contents were filtered through Whatman filter paper No. 42. The filtrate was made to 25 ml volume with double distilled water

and used for determination of potassium, copper, zinc, manganese, and iron by using atomic absorption spectrophotometer, Model 3100, Perkin Elmer. Calcium was determined with the help of flame photometer, Mediflame, 127.

Phosphorus

Phosphorus was determined Colorimetrically by the method of Chen *et al.* (1956) ^[12].

Tannins

Tannins were determined by the method developed by Makkar *et al.* (1993) ^[13]. For the estimation of total tannins, total and simple phenol needed to be estimated in seeds.

Extraction

Weighed 0.20 g of finely ground samples and transferred in to a beaker and 10 ml of 70 per cent acetone was added. The beaker was placed in a water bath adjusted at 37°C for 2 hours. Frequent shaking was given for better extraction. After expiry of this period, extract was centrifuged for 20 minutes at 3000 rpm. The supernatant was collected in a test tube and was used further for the estimation of total and simple phenols.

Procedure

Took 0.10 ml of aliquot extract (as obtained above) and volume was made 1.00 ml with distilled water, 2.50 ml of 20 per cent sodium carbonate solution was added followed by 0.50 ml of folin ciocalteau reagent. Contents were left for 40 minutes for colour development (purplish blue). Absorbance was read at 725 nm after 40 minutes against a suitable blank and calculations were done for total phenols using standard curve which was prepared using tannic acid (0.1 mg/ml).

For the estimation of simple phenols 1 ml of filtrate was taken to which 1 ml of PVP was added and was kept for 15 minutes in ice-cold water. After this, 0.2 ml of aliquot was taken in a test-tube to which 2.5 ml of 20 per cent sodium carbonate was added followed by folin ciocalteau reagent. Incubation of 30-40 minutes was given for colour development and the absorption was read at 725 nm against a suitable blank. Further calculation was done for simple phenols using standard curve which was prepared using tannic acid (0.1mg/ml).

Calculations

Total tannins= [Total Phenols- Simple phenols]

Flavonoid

The flavonoid content in the sample of various Indian Horse Chestnut seeds was determined by the method given by Boham and Kocipia (1994) ^[14].

Results and Discussion

Nutritional characteristics of fresh seed flour and processed flour (*tatwakhar*) of Indian horse chestnut

Sugar is an important source of energy; especially our brain relies on it. It provides sweetness in the food stuff. Perusal of data from the Table 1 showed that the total sugars confined in fresh and processed flour of Indian Horse Chestnut revealed significant differences. Fresh flour had 20.23(%) total sugars, whereas in processed flour, the values for the same constituents obtained at lower ebb 13.47(%). The higher percentage of total sugar in fresh flour is because of the crude form. While processing the sugar content might get dissolved and leached out during frequent change of water. The lower

value of total sugar in processed flour is justified. It can be clearly seen that statistically significant difference was found in the percentage of reducing sugar in fresh and processed flour of Indian Horse Chestnut. The values were obtained as 13.62(%) and 9.52(%) in fresh and processed flour. The lower value of reducing sugar might be due to dissolving and leaching of sugar during processing of flour. The values for this parameter were found to be 6.72 per cent and 3.91 per cent for the fresh and processed flour respectively. Statistically both samples differed significantly at 5% level of significance. Singh *et al.* (2004) [5] reported the nearby values.

Table 1: Nutritional Characteristics of Fresh Seed Flour & Processed Flour (*Tatwakhar*) of Indian Horse Chestnut

Parameters	Fresh flour	Processed flour	CD (P≤0.05)
Total sugars (% glucose)	20.23 ^a	13.47 ^b	0.20
Reducing sugars (% glucose)	13.62 ^a	9.52 ^b	0.28
Non-reducing sugars (% sucrose)	6.72 ^a	3.91 ^b	0.12
Starch (%)	27.83 ^b	66.47 ^a	2.23

Each value represents mean of three replicates. In the same column, significant differences according to CRD are indicated by different letters. Same letter represent that their values are at par.

Mineral composition

The mineral elements *viz.* calcium, phosphorus, potassium, copper, manganese, iron and zinc in fresh Indian horse chestnut seeds were analyzed and the values are presented in Table 2. As is evident from the data, the value of zinc element was found to be high (705.90 mg/ 100 g) among all elements. This shows that the sample is good source as zinc element. The potassium content in the fresh flour was 81.00 mg/100 g. The amount of calcium and phosphorus present in the flour was 8.20 and 19.00 mg/ 100g respectively. The same sample contained manganese (0.50 mg/100 g) and copper (0.60 mg/100 g) which were comparatively low. Iron being the important constituent in the blood. The same content was analyzed as 8.50 mg/100g in the fresh seed flour of the *Aesculus indica*. Majeed *et al.* (2010)[3] reported the mineral content of Indian Horse Chestnut seeds *i.e.* potassium (0.79%), phosphorus (0.18%), sulphur (0.07%), calcium (0.08%), iron (159 ppm), copper (41.20 ppm), zinc (25.6 ppm) and manganese (6.95 ppm). Slight variations might be due to different soil conditions.

Table 2: Mineral Composition of Fresh Seed Flour

Elements	(mg/100g)
Calcium	8.20
Phosphorus	19.00
Manganese	0.50
Potassium	81.00
Zinc	705.90
Iron	8.50
Copper	0.60

Phyto-chemical estimation of fresh seed of Indian horse chestnut flour (*Aesculus indica*)

Tannins are naturally occurring as plant polyphenols and widely distributed in the plant kingdom. Their main characteristic is that they bind and precipitate proteins. They are responsible for the astringent taste. It acts as a defense mechanism in plants against pathogens, herbivores and hostile environmental conditions. Tannins induce a negative response when consumed. Tannins may reduce intake by decreasing palatability and by negatively affecting digestion. Tannins

Starch is considered as very important nutritional constituent for our body. The starch content was significantly low 27.83(%) in the fresh sample, but on the other hand, maximum starch content was found in the processed flour *i.e.* 66.47(%). The variation is due to the loss of starch during processing to make it palatable due to which the starch content of processed flour is reduced. Uppal (1952) [4] reported the 25(%) starch in fresh flour which is pretty close to the calculated result. Whereas, no pertinent information/literature is available related to total sugars and starch content of processed flour.

have traditionally been considered anti-nutritional but it is now known that their beneficial or anti nutritional properties depend upon their chemical structure and dosage. The total tannin per cent was determined by the total and simple phenol. It was found from the Table 3 that the seed contains total phenol of 785.25 mg per 100 g, whereas the simple phenol was very less in amount *i.e.* 37.82 mg/g. So for calculating tannins from the total phenols and simple phenols, it came out to be 747.43 mg/100 g. These contents might be high due to the presence of saponin, alkaloids and other phenolic compound which are further responsible for the toxic effect. After scanning literature thoroughly no related references could be find out. Flavonoids (or bioflavonoids) are secondary metabolites and the most common group of polyphenol compounds in the human diet. The daily intake of flavonoids with normal food, especially fruit and vegetables, is 1-2 g. it can be used to treat many important common diseases, stimulate some hormones and neurotransmitters, and to scavenge free radicals. It have an anti-allergic, anti-inflammatory, anti-microbial, anti-cancer, and anti-diarrheal activities. The flavonoid content in the Horse Chestnut was found to be 47.00 mg / 100g in Table 3. However, Kapusta *et al.* (2007) [8] reported escalated values for the total concentration of flavonoids in Horse Chestnut seeds (*Aesculus hippocastanum*) as 0.88 per cent on dry matter basis. This could be due to the difference in the method used for extraction or the difference in species.

Table 3: Phyto-chemical Estimation of Fresh Seed of Indian Horse Chestnut Flour (*Aesculus indica*)

Parameter	(mg GAE/g)
Total Phenol	785.25
Simple Phenol	37.82
Tannins	747.43
Flavonoids	47.00

Conclusion

The total sugars (% glucose) 20.23 per cent of fresh flour were more than processed flour and starch content of processed flour was highest 66.47 per cent. In fresh seed flour, zinc (705.90 mg/100g) was higher than the other minerals followed by potassium (81.00 mg/100g). Zinc deficiency is associated with a person more susceptible to disease and illness. So, it might be the good food source. The determined values of phyto-chemicals were 785.25, 747.43 and 47.00 (mg GAE/g), respectively of total phenols, tannins

and flavonoids. There are no anti-nutritional factors present in the seed after processing i.e. in processed flour. Hence the processed flour (*tatwakhar*) could be used as food and nutritional supplement for human beings and might be the superior food substitute for food and nutritional security.

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