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Proximate analysis and its comparison after extraction of crude Saponin/Aescin different treated flour of Indian horse chestnut (*Aesculus indica*)

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Abstract
Indian Horse Chestnut (*Aesculus indica*) seeds were processed by the different treatments for the extraction or removal of crude Saponin/Aescin for its edible quality. The seeds were treated in various way *viz.* Percolator/ cold extraction (T_1), Roasted (T_2), Soxhlet (T_3), Microwave cooking whole seed (MW), Microwave cooking crush seed (MC), Pressure cooking(PW), 10 minute whole seed boiling (B_1W), 20 minute whole seed boiling (B_2W), 30 minute whole seed boiling (B_3W), 10 minute crush seed boiling (B_1C), 20 minute crush seed boiling (B_2C), and 30 minute crush seed boiling (B_3C). After the extraction of crude Saponin/Aescin from the seeds, the flour was analyzed for its proximate composition and compared with fresh sample and processed flour or *tatwakhar*. Apart from the highest value in fresh sample, the moisture content (5.45 per cent) in T_2 sample was on the uppermost. The protein percent (6.13) was found highest in B_1W treatment looking after fresh one. Most of the fat percent were ranges from 1.30-1.66 which is almost similar in different treatment whereas the fresh sample has highest value. Ash content (3.42 %) was found highest in the treatment (MW) subsequent to fresh sample. The total carbohydrate percentage was found highest in (T3) sample i.e. 89.09. There was no fibre present in the seeds of *Aesculus indica*. That's why it was not included in the calculation of proximate analysis.

Keywords: Carbohydrate, crude Saponin/Aescin, proximate analysis, treatments

Introduction
Indian Horse chestnut scientifically called as *Aesculus indica* fit in the family of *Hippocastanaceae* which is very large and rounded tree found in the area like Himachal Pradesh and Kashmir belt of Northern India. It has large leaves and flowers make it right for as large sized bonsai. Its leaves, seeds and other parts of the tree are used as fodder in some parts of Northern India and wild animals. Oil of the seeds is traditionally used in the treatment of skin diseases, rheumatism and headaches. The fruits/seeds are given to horses suffering from colic disorder. The nuts are used in piles and obstinate constipation too [1]. The seed colour was shiny dark chocolate to brown and smooth ovoid shape. The length of the fruit was ranged from 3.98 ± 0.35 cm and the diameter about 3.60 ± 0.34 cm. More importantly, no gluten content present in the processed flour. It might be vital for the patients experienced from celiac disease [2]. *Aesculus indica* seeds are utilized as an astringent, nutritious, whereas the roots are used in leucorrhoea management [3]. The plant contains a mixture of saponins, chemical constituent i.e. aescin. It also contains flavonoids, glycosides, tannins and phenolic substances. It is usually known for its medicinal properties. The seeds have pharmacologically value as anti-oxidant, anti-viral, immune-modulatory, anti-inflammatory properties [5]. The composition of the oil *viz.* arachidic acid, 5. 67; myristic, 1.3; palmatic, 8.77; oleic, 57.49; linoleic, 16.91 and linolenic, 7.92 per cent [5]. In order to determine its 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 was estimated [6]. Indian horse chestnut seeds are a good source of starch [8]. And the toxic component present i.e. saponin is bitter in taste makes unsuitable for human consumption [7]. The studies were conducted to remove its toxicity by different pre-treatments together with conventional techniques. Seeds were dehulled and crushed/grated into a mass and prepared for treatments. The defined pretreatments were soaking, blanching, cooking, pressure cooking and standardized separately [9].

In some parts of the Himachal Pradesh, people used to dried and ground the flour, called as *tatwakhar*. After processing, the flour is not toxic and used to make some products like halwa, or *falahar* during fast [4, 10].

Materials and Methods

The complete matured seeds of Indian Horse Chestnut *Aesculus indica* was acquired from *brot* village and remove all dirt and bruises manually through under water. Then dried it and peel off the outer covering. After that the treatments were given to the seeds.

Experimental Layout

The experiment was scientifically planned and laid out according to CRD design. In order to reduce the determinate type of error, for each type of parameter, observations were repeated thrice times.

Statistical analysis

The data collected on all the parameters *viz.,* proximate analysis was analyzed statistically. Analysis of variance by completely randomized design (CRD) in which the mean and critical difference (CD) value was determined and their statistical significance was ascertained.

Proximate analysis

The different treated seeds of *Aesculus indica* were analyzed for the Moisture, Ash, Protein Fat and Carbohydrate (NFE) by using standard methods of (AOAC, 1990) [11]. The crude protein was calculated by multiplying nitrogen per cent with 6.25 factor. No fibre was present in any treatments of the seeds as well as in fresh and processed flour. It is not involved in the calculations.

Statistical analysis

The data collected on all the parameters *viz.,* proximate analysis and its comparison to the fresh and processed flour (*tatwakhar*) were analyzed statistically. Analysis of variance by completely randomized design (CRD) in which the mean and critical difference (CD) value was determined and their statistical significance was determined.

Results and Discussion

Proximate composition of flour after the extraction of crude Saponin/Aescin from the Indian Horse Chestnut by using different methods of extraction

Table 1 represents the following parameters of proximate composition in Saponin/Aescin free flour. The moisture content of the flour after the extraction of crude Saponin/Aescin on Indian Horse Chestnut flour. By using different methods of extraction and treatments, the moisture content was found high in all treatments except T_0. The treatment T_2 attained the value (5.45 per cent) T_2 and lowest (0.79 per cent) was found in B_2C then following treatments like in T_1, MC, B_1W and B_1C, there range varied from 2.55-3.50 per cent, statistically they are at par and hence no effect of treatment was observed on the moisture content of the different processed flour. After that, following treatments like T_3, MC, PW, B_3W and B_1C was ranged from 2.03 to 2.29 per cent. The value for B_3C was calculated as 1.83 (%) was just above from the lowest value moisture content from different flour. All the values were compared with the fresh flour analysis of proximate composition. The variation in the results might be due to the use of different processing methods. There was no pertinent literature is available with regard to this component. In fresh sample the protein content was found higher than the other treatments. The treatment B_1W possesses 6.13 per cent protein followed by B_2W as 5.73 (%) Protein content in microwave cooking (MW and MC), PW, B_3C, B_1C and B_2C were varied from 5.17 to 5.48 per cent. Mostly, the low protein content was found in Soxhlet, roasted and cold extraction method which ranged from 4.45 to 4.55 per cent in 100 g of flour. The fat content in T_3, T_1, MC, B_1W, B_2W, B_3W, B_2C and B_3C ranged from 1.10 -1.99 per cent. And the low fat content was found mainly in MW (0.85 per cent). B_1C; PW and T_2 shared the 0.90 to 1.00 per cent fat. The ash content was found highest in the fresh flour up to 4.00 per cent whereas, the ash content was decreased gradually in different methods of extraction. In different treatments *viz.* B_2C; B_1W and B_2W, PW, MW and T_3 the values ranged from 2.90 to 3.42 per cent followed by T_2, T_1, MC, B_3W and B_1C where values ranged from 2.67-2.78 per cent. The lowest (2.32 per cent) protein was found in B_3C. The highest value of carbohydrate was obtained by the T_3 method with the value 89.09 per cent followed by the T_1 i.e. 88.72 per cent. The treatment MW obtained 88.53 per cent carbohydrates. The treatments, PW, B_3W, B_1C, B_2C and B_3C shared the calculated values as 88.52, 88.53, 88.83, 89.00 and 88.82 per cent, respectively by the flour after extraction of crude saponin. They lied in the same range and statistically there was no effect found by the treatments on the total carbohydrate content. In T_2 86.27 per cent was calculated, MC shared 87.25 per cent carbohydrates. Whereas, B_1W and B_2W possessed carbohydrates to the tune of 86.17 and 86.94 per cent, respectively. The lowest carbohydrate content 76.59 (%) was found in the T_0. In nutshell, the moisture, protein and ash contents were found comparatively high in T_0 and differed statistically significantly whereas, very less effect was shown by different method of extraction on fat content. On the other hand, the carbohydrate content was high. The losses for all these nutrients might be due to hydrolysis and leaching out in /water.

Table 1: Proximate composition of flour after extraction of crude saponin/Aescin

	Treatment	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Total Carbohydrate (%)
1	Soxhlet(T_3)	2.36^{de}	4.55^{g}	1.10^{bc}	2.90^{cd}	89.09^{a}
2	Roasted(T_2)	5.45^{b}	4.50^{g}	1.00^{c}	2.78^{d}	86.27^{ab}
3	Percolator/ cold extraction(T_1)	2.76^{cde}	4.45^{g}	1.30^{abc}	2.77^{d}	88.72^{a}
4	Microwave cooking whole seed(MW)	2.03^{de}	5.17^{e}	0.85^{c}	3.42^{abcd}	88.53^{a}
5	Microwave cooking crush seed(MC)	3.50^{cde}	5.21^{e}	1.31^{abc}	2.73^{d}	87.25^{b}
6	Pressure cooking(PW)	2.23^{de}	5.25^{e}	1.00^{c}	3.00^{cd}	88.52^{a}
7	10 minute whole seed boiling(B_1W)	2.88^{cde}	6.13^{b}	1.55^{abc}	3.27^{bcd}	86.17^{b}
8	20 minute whole seed boiling(B_2W)	2.55^{cde}	5.73^{c}	1.58^{abc}	3.20^{bcd}	86.94^{b}
9	30 minute whole seed boiling(B_3W)	2.29^{de}	4.83^{f}	1.58^{abc}	2.77^{d}	88.53^{a}
10	10 minute crush seed boiling(B_1C)	2.29^{de}	5.31^{de}	0.90^{c}	2.67^{d}	88.83^{a}
11	20 minute crush seed boiling(B_2C)	0.79^{f}	5.48^{d}	1.66^{abc}	3.07^{bc}	89.00^{a}
12	30 minute crush seed boiling (B_3C)	1.83^{e}	5.40^{de}	1.63^{abc}	2.32^{e}	88.82^{a}
13	Fresh sample(T_0)	9.93^{a}	7.48^{a}	1.99^{ab}	4.00^{a}	76.59^{c}
	CD ($P \leq 0.05$)	0.96	0.14	0.46	0.40	1.08

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

Proximate composition of flour after extraction of crude Saponin/Aescin compared with processed flour

Table 2 depicted the moisture, ash, protein, fat and carbohydrate content in the processed flour. The moisture content (13.83%) in the T_4 was high in comparison to other treatments. Though the statistical values for moisture content were same just like when the values were compared with T_0. The protein content was also found maximum in T_4 (7.26%) and the Table 4.11 is depicted the statistical significant difference of protein content of the T_4 was just like when the values were compared with fresh flour. The highest fat content was found in B_2C and B3C with the values 1.66 and 1.63 per cent, respectively. The treatments viz.B_1W, B_2W, and B3W possessed 1.55, 1.58 and 1.58 per cent respectively. The treatment MC contained 1.31 per cent fat followed by T_1 with the value 1.30 per cent. In T_3, the fat content was decreased to 1.10 per cent whereas; PW and T_2 shared 1.00 per cent fat. The treatments MW and B_1C calculated the fat per cent as 0.85 and 0.90 respectively. On the other hand, the treatment T_4 had bagged the lowest fat content among all the treatments. The highest ash content (3.42 %) was found in MW while 3.27, 3.20, and 3.07 per cent ash was obtained by the treatments B_1W, B_2W and B_2C, respectively. In PW (3.00 per cent) and in T_3, 2.90 per cent ash was analyzed. In B_3W; B_1C and MC had the ash per cent 2.77, 2.67 and 2.73, respectively. Nearly same values (2.78 and 2.77 per cent) were shared by T_2 and T_1 samples. Further B_3C had 2.32 per cent ash content. On the contrary, T_4 had the lowest ash content. The highest carbohydrate content was found in T_3 (89.09 per cent) followed by T_2 86.27(%),T_1 88.72 (%),MW 88.53 (%), PW 88.52 (%), B_3W 88.53 (%), B_1C 88.83 (%), B_2C 89.00 (%) and B_3C 88.82 (%). There was not much effect of treatments on the carbohydrate content found. The treatments B1W; B_2W; MC possessed carbohydrates 86.17 (%), 86.94 (%), and 87.25 (%) which was calculated by NFE. Overall effect on the nutrients like moisture and protein contents was similar when compared with processed flour. But ash content was found high in MW; the fat content was found high in B_2C; likewise the T_3 shared the highest carbohydrate content when processed flour compared with the other treatments. Hence, it showed that the nutrients were lost during the processing of flour in processed flour in comparison to other methods of extraction.

Table 2: Proximate composition of flour after extraction of crude Saponin/Aescin compared with processed flour

	Treatment	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Total Carbohydrate (%)
1	Soxhlet (T_3)	2.36^{de}	4.55^{g}	1.10^{bc}	2.90^{cd}	89.09^{a}
2	Roasted(T_2)	5.45^{b}	4.50^{g}	1.00^{c}	2.78^{d}	86.27^{ab}
3	Percolator/ cold extraction(T_1)	2.76^{cde}	4.45^{g}	1.30^{abc}	2.77^{d}	88.72^{a}
4	Microwave cooking whole seed(MW)	2.03^{de}	5.17^{e}	0.85^{c}	3.42^{abcd}	88.53^{a}
5	Microwave cooking crush seed(MC)	3.50^{cde}	5.21^{e}	1.31^{abc}	2.73^{d}	87.25^{b}
6	Pressure cooking(PW)	2.23^{de}	5.25^{e}	1.00^{c}	3.00^{cd}	88.52^{a}
7	10 minute whole seed boiling(B_1W)	2.88^{cde}	6.13^{b}	1.55^{abc}	3.27^{bcd}	86.17^{b}
8	20 minute whole seed boiling(B_2W)	2.55^{cde}	5.73^{c}	1.58^{abc}	3.20^{bcd}	86.94^{b}
9	30 minute whole seed boiling(B_3W)	2.29^{de}	4.83^{f}	1.58^{abc}	2.77^{d}	88.53^{a}
10	10 minute crush seed boiling(B_1C)	2.29^{de}	5.31^{de}	0.90^{c}	2.67^{d}	88.83^{a}
11	20 minute crush seed boiling(B_2C)	0.79^{f}	5.48^{d}	1.66^{abc}	3.07^{bc}	89.00^{a}
12	30 minute crush seed boiling (B_3C)	1.83^{e}	5.40^{de}	1.63^{abc}	2.32^{e}	88.82^{a}
13	*Tatwakhar* (processed flour) (T_4)	13.83^{a}	7.26^{a}	0.41^{d}	0.83^{f}	77.66^{c}
	CD ($P \leq 0.05$)	0.96	0.14	0.46	0.40	1.08

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

Conclusion

It can be concluded that different treated samples were compared to the fresh sample and the processed flour. There was almost no difference found in fat extraction of all treated samples when compared with fresh sample whereas significant more difference was found when compared with processed flour. The difference in all treatments when compared with both flours they shared same critical difference. Moisture content was found more in processed sample than the other treated flour. Mineral content was more in the treatment of microwave cooking whole seeds among both flours compared. The critical difference between the fresh sample and processed flour sample shared the same values. it is all due to the nutrients lost during the processing in processed flour in comparison to other methods of extraction.

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