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Soaking and germination effect on bioactive components of fenugreek seeds (*Trigonella foenum* graecum L.)

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Abstract

The research was aimed at observing the changes in bioactive component (polyphenol, tannin, ascorbic acid and antioxidant activity) in fenugreek seed during soaking and germination. Soaking was carried out for 12 hours at the ratio of 1:5 (seed:water) in dark condition. Germination was carried out for 72 hours at 25°C and 90% RH. The raw, soaked and germinated seeds were dried and ground to powder to pass through 100 µm mesh size screens. The ascorbic acid and phenol content increased significantly during soaking and germination whereas tannin content decreased significantly. The scavenging activity of fenugreek seed (10.4%) increased significantly during soaking (13.6%) and germination (55.5%) in comparison to gallic acid (89.3%).

Introduction

Fenugreek (*Trigonella foenum-graecum*-known as 'Methi' in Nepali and Hindi) belongs to the family leguminosae and is an important medicinal spice used in Nepal and various other Asian, African and European countries from ancient times (Leela and Shafeekh, 2008; Agrawal *et al*., 2015). Its seeds, leaves and tender shoots show anti-carcinogenic activity, hypocholoesterolemic activity, hypoglycemia activity, anti-fertility effect, antiulcer effect, lowers blood pressure and are helpful in digestive disorders such as flatulence, diarrhea, dyspepsia, chronic cough as well as serves as appetizer and reduce allergies (Singhal *et al.*, 1982; Sowmya and Rajyalakshmi, 1999; Basch *et al*., 2003; Bin-Hafeez *et al.*, 2003; Srinivasan, 2006; Aggarwal and Shishodia, 2006; Singh and Garg, 2006; Faeste *et al.*, 2009). Besides health effect, there are many food application of fenugreek such as emulsifier, prebiotic effect, thickening agent (Lee, 2006; Hefnawy and Ramadan, 2011).

Fenugreek has been consumed as spices and various preparations (Mathur and Choudhry, 2009). For various preparations, fenugreek is soaked, germinated or boiled in all parts of the world (Alarcon-Aguilara *et al*., 1998; Mathur and Choudhry, 2009). In Nepal also Mee Kwa (fenugreek soup), newari cusine was used to serve after lunch or dinner.

Fenugreeks are rich in dietary fiber and also contain protein like globuline, histidine, albumin and lecithin (Raju *et al*., 2001, Mathur and Choudhry, 2009). The protein content was found up to 43.8 g/100 g endosperm (Naidu *et al*., 2011). Fenugreeks are also rich in vitamins like pyridoxine, niacin and choline (Sharma, 1986). The ascorbic acid was found up to 12 mg/100 g (Leela and Shafeekh, 2008). Minerals like calcium, iron and zinc were also reported to be high in curry prepared from fenugreek compared to curry prepared from potato (Jani *et al*., 2009). Phenolics and flavonoids are found in fenugreek which contributes to the antioxidant properties, which have been reported to have various beneficial effects (Balch, 2003; Dixit *et al*., 2005). Balch (2003) reported that germinated fenugreek seeds have a more beneficial effect on human health.

The major objective of this study was to determine the effect of soaking and germination process on bioactive component of fenugreek seeds (T*rigonella foenum* graecum).

Materials and Methods

Soaking

Fenugreek seeds collected from Asan, Kathmandu, Nepal were first cleaned and then sterilized with 70% ethanol for 2.5 minutes, followed

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by 2.5% sodium hypochlorite solution for 15 minutes (Huang *et al*., 2003). Ethanol and sodium hypochlorite were removed with four rinses of sterile water. After disinfection, seeds were soaked in distilled water at room temperature for 12 hours in dark condition. A seed to water ratio of 1:5(w/v) was used. Then, water was drained and the seeds were dried at 55±5°C until moisture content goes below 14%.

Germination

The soaked seeds were germinated in sterile petri-plates lined with wet whatman no. 1 filter paper for 72 hours at 25°C at 90% RH. The seeds were rinsed in distilled water and dried at 55±5°C till moisture content goes below 14%. The dried samples of raw, soaked and germinated seeds were ground to fine powder in an electric grinder to pass through 100 µm mesh size screens.

Chemicals and Instrument

2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich company, Germany. Phenol reagent was purchased from Finar limited, India. Gallic acid was purchased from LOBA Chemie, India. Methanol was purchased from Fisher Scientific, India. Sodium carbonate was purchased from Merck limited, India. All chemicals used were of analytical grade. The spectrophotometer used was of model GENESYSTM 10S Vis Spectrophotometer from Thermo ScientificTM, Germany.

Analysis of fenugreek seeds powder

The ascorbic acid of dry, soaked and germinated fenugreek seeds powder was determined as a method described by AOAC (2005). For polyphenol and tannin, extraction process was carried out with modification as described by Kostic *et al.*, (2013). 5 g of fenugreek seeds powder were mixed with 80% methanol (30 ml) and this was kept under continuous shaking for 20 minutes and then was filtered through Whatman no. 1 filter paper. The residue was again submitted to two more extraction cycle for 20 minutes each totalizing 60 minutes of extraction time. The filtrate was combined in a volumetric flask, and the volume was made up to 100 ml. The extracts of fenugreek seeds powder were stored in the refrigerator until analysis of total polyphenol, tannin and antioxidant activity. Total polyphenol content was estimated by Folin–Ciocalteau Colorimetry as per Adom and Liu (2002); whereas tannin content was estimated by Folin–Ciocalteau Colorimetry as per Afify *et al.* (2012). For antioxidant activity, different concentrations of extract (25, 50, 100, 200, 400, 800, 1600 and 3200) µg/ml were prepared from stock solution of fenugreek seeds powder (50000 µg/ml). Total Antioxidant activity was estimated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) colorimetry method (Brand-Williams *et al*., 1995). Then the % inhibitions were plotted against respective concentrations used and from the graph, IC_{50} was calculated by using linear regression line. Data obtained were analyzed by a statistical program known as Genstat release 7.22, Discovery edition, 2004, developed by VSN International Ltd. Sample means were compared by LSD method at 5% level of significance ($p< 0.05$).

Results and Discussion

Ascorbic acid content, total phenolic content and tannin content

The ascorbic acid content, total phenolic content and tannin content of dormant, imbibed and germinated seeds powder were determined and shown in Table 1. Ascorbic acid of raw fenugreek seeds powder was found to be 5.6 mg% which was lower than 7.07±1.27 mg% ascorbic acid in fenugreek, reported by Dinesh *et al*. (2015) whereas greater than 3 mg% ascorbic acid in fenugreek seed, as reported by Manas (2014). Leela and Shafeekh (2008) reported ascorbic acid 12 mg/100 g in raw seed. The ascorbic acid of fenugreek seed increased significantly during soaking and germination. El-Shimi *et al*. (1984) reported an increase in ascorbic acid during germination whereas decrease in ascorbic acid during soaking. The data suggests that ascorbic acid content increases on germination which is in accordance with the findings of Shah *et al*. (2011). They reported that ascorbic acid increases with increase in germination time for mung bean. This difference might be due to different seed species, germination procedure or degree of hydration prior to prolonged germination (Finney, 1982). Increase in ascorbic acid in soaked fenugreek may be due to increased activity of L-galactono-Y-lactone dehydrogenase in imbibed bean (Siendones *et al*. 1999; Moriyam and Oba, 2008)

There was a significant increase in total phenol content of germinated fenugreek seeds but the tannin content gradually decreased as the seeds developed into sprouts (532.08 to 210.08µg GAE/g). The values of total phenol content obtained for the dormant seeds is quite similar to the one reported by Bukhari *et al.* (2008). The observed reduction in tannin content after germination may be due to the result of the formation of a hydrophobic association of tannins with seed proteins and enzymes. In addition, loss of tannins also might be due to the leaching of tannins into the water (Shimelis and Rakshit, 2007).

Table 1. Ascorbic acid, Total phenol content and tannin content of dry, soaked and germinated fenugreek seeds

	Ascorbic acid content (mg/100 g)	Total phenol content (mg of GAE/g)	Tannin content (µg of GAE/g)
Dry seed	5.36±0.4[a]	5.68 ±0.22[a]	532.08±2.62[a]
Soaked seed	7.84±0.75[b]	9.43 ±0.45[b]	321.16±0.92[b]
Germinated seed	18.09±0.6[c]	14.50 ±0.16[c]	210.08±0.89[c]

*The values in the table are the mean of triplicates with (±). standard deviation

**Values in the same row bearing different superscript are significantly different at 5% level of significance

Lopez-Amoros *et al.* (2006) indicate that germination modifies the quantitative and qualitative phenolic compounds of legumes and the changes depend on the type of legume and the germination conditions. These changes influence the functional properties of the legumes as the consequence of variation in antioxidant activity. It is expected that total phenolics and tannin content changed differently in different legumes (Khandelwal *et al*., 2010). Khandelwal *et al*., (2010) reported that total phenolics and tannin content was reduced significantly in germinated green gram compared to Bengal gram, red gram and lentil. Loss of total phenolics and tannin contents could be as high as 96% in germinated kidney bean as shown by Shimelis and Rakshit (2007). However, Duenas *et al*. (2009) found that germination increased total phenolics content in lupin seeds after 9 days. Similarly, Chai (2011) reported changes in phenolic content during germination of peanut.

Free radical scavenging activity

The radical (DPPH) scavanging activity % at a different concentration of fenugreek seeds powder (Dry, soaked and germinated) extract and IC_{50} are shown in Figure 1, Figure 2 and Figure 3 respectively. Figure 1 shows the free radical scavenging activity of different amounts of extracts of fenugreek seeds powder (Dry, soaked and germinated). Radical scavenging activity increased with increase in the amount of extract. Among the extracts, the methanolic extract of germinated fenugreek showed the highest DPPH radical scavenging activity followed by imbibed and dormant stage. The evaluation of the antioxidant activity of the extracts indicated that the activity was concentration-dependent and increased when higher concentrations of the extracts were applied.

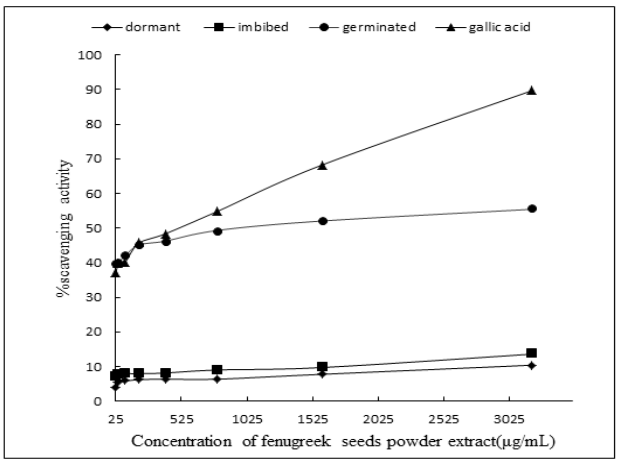

Figure 1. Free radical scavenging activity of fenugreek seeds at different concentration

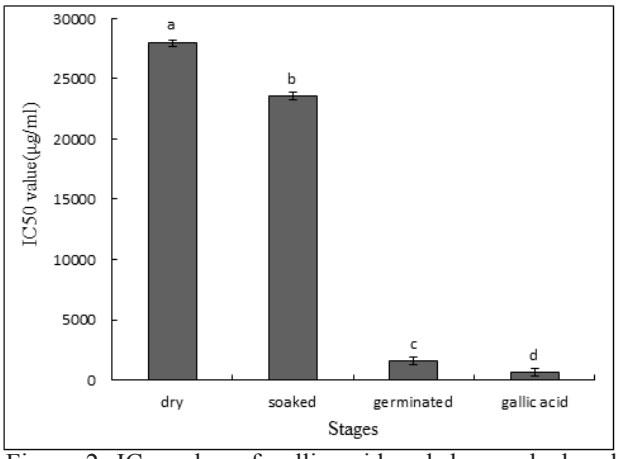

Figure 2. IC_{50} value of gallic acid and dry, soaked and germinated fenugreek seeds

*The values in the table are the mean of triplicates with (±). standard deviation

**Values in the same row bearing different superscript are significantly different at 5% level of significance

The IC_{50} value for dry, soaked, germinated (fenugreek seeds) and standard GA was found to be 27975 µg/mL, 23615.67 µg/mL 1582.12 µg/mL and 614.84 µg/mL, respectively (Figure 2). At 200 mg concentration, extracts of husk, fenugreek seeds, and endosperm exhibited 72%, 64%, and 56% antioxidant activity respectively by free-radical scavenging method (Naidu *et al*., 2011). The inhibitory concentration (IC_{50}) of fenugreek leaves for DPPH scavenging activity was found to be 0.7mg/mL^{-1}(Premnath, 2011).

The extracts of dry, soaked and germinated fenugreek seeds powder exhibited free radical scavenging activities of 10.4%, 13.6% and 55.45% (Figure 3) respectively in comparison with the Gallic acid (control) which exhibited free radical scavenging activity of 89.83%. Bukhari *et al.* (2008) revealed antioxidant activity for methanolic extract of raw seed was less than 20%. Extraction processes

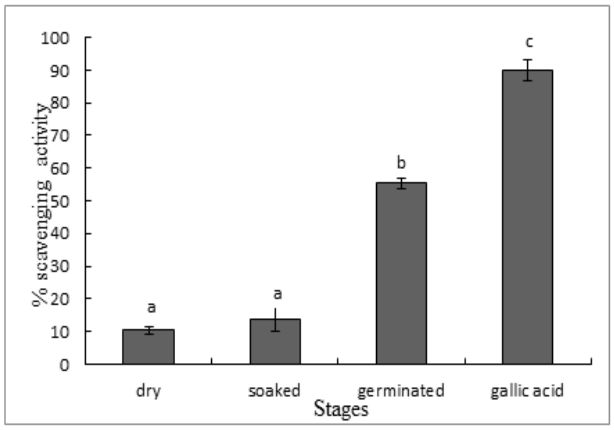

Figure 3. Radical scavenging activity (%) of gallic acid and dry, soaked and germinated fenugreek seeds at a concentration of 3200 µg/mL

*The values in the table are the mean of triplicates with (±). standard deviation

**Values in the same row bearing different superscript are significantly different at 5% level of significance

influence the value of antioxidant activity (Dixit *et al*., 2005). Lopez-Amoros *et al*. (2006) revealed that quantitative and qualitative change of phenolic compounds during germination influence the antioxidant property.

Legumes contain other bioactive compounds besides phenolic such as vitamins and carotenoids at different concentrations that might also behave as an antioxidant (Prodanov *et al*., 1998; Atienza *et al*., 1998). These compounds might also exert synergetic activities among themselves and with phenolic compounds, which could be the main reason for the observed differences in the antioxidant activities.

Conclusion

Soaking and germination of fenugreek seeds resulted in significant changes in bioactive components and anti-oxidant activity. From the above results, it may be concluded that germinated fenugreek possess more health potential compared to non-germinated fenugreek seeds.

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