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Phytochemical analysis of Giloy stem powder and sensory analysis of Giloy (*Tinospora cordifolia*) stem powder incorporated sweet balls

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Abstract
Giloy is popularly known as *Tinospora cordifolia*. Therefore, the aims of present study were to conduct phytochemical screening of giloy stem powder and to evaluate the sensory properties and proximate composition of GSP incorporated sweet balls with control sweet balls. Ethanolic and water extracts were found to have the presence of alkaloids, glycosides, phytosterols, saponins, resins and tannins. GSP incorporated sweet balls were prepared using nine different blends of RBGF: SS: GSP:: 100:0:0, 90:5:5, 80:10:10, 70:15:15, 60:20:20, 50:25:25, 40:30:30, 30:35:35 and 20:40:40 with other ingredients *viz*. jaggery, rice flakes, ghee, cardamom, jaggery and water. Results of sensory evaluation revealed that sweet balls prepared with roasted bengal gram flour had the highest sensory score for all parameters namely; colour, appearance, taste, hardness, after taste and overall acceptability. A significant difference (p<0.05) was observed for all the parameters as the incorporation GSP increased alongwith sesame seeds. Further, control roasted bengal gram flour sweet balls and sweet balls prepared with RBGF: SS: GSP:: 70:15:15 blend were evaluated for proximate composition and results revealed that GSP and sesame seeds incorporated sweet balls have considerably higher crude fat, total ash, crude protein and energy content when compared with roasted bengal gram flour sweet balls.

Keywords: Giloy, sweet balls, phytochemical screening, giloy stem powder, sensory evaluation, proximate composition

Introduction
Medicinal plants are defined as "plants in which one or more of their parts contain compounds that can be used for treatment purposes or which are precursors for the preparation of competent drugs" by the World Health Organization (WHO), and it is estimated that 70–80 percent of people in developing countries rely on traditional medicine. Giloy (*Tinospora cordifolia*) belongs to the Menispermaceae family and is known in India by different names such as giloy, guduchi, and amrita. It is well known in Ayurveda and traditional medicine for its wonderful therapeutic efficiency (Sankhala *et al.*, 2012) [1]. Guduchi is an Indian shrub which includes roughly 70 genera and 450 species found in low tropical regions (Spandana *et al.*, 2013) [2]. In Indian Ayurveda Pharmacy, it is a well-known medicinal herb. The substance has been the focus of substantial phytochemical, pharmacological, and clinical research during the last two decades (Nagaprashanthi *et al.*, 2012) [3]. Guduchi is an important element in Ayurvedic medicine, and it is used to cure fever, liver and spleen disorders, bleeding sickness, heat burning feeling, aphrodisiac treatment, and oligospermia. Its roots, stems, and leaves have all been utilised medicinally in Ayurvedic tradition for thousands of years. Because of its ability to impart juvenility, liveliness, and long life, it is referred to as "Nectar of Immortality." *Tinospora cordifolia*, which grows on neem trees (*Azadirachta indica*), has a high medicinal potential and is known as 'Neem giloy' because of its synergistic impact (Mittal *et al.*, 2014) [4]. Different parts of giloy *viz*. root, stem, and leaf have been identified to have good amount of crude fiber, minerals, and bioactive phyto-ingredients exerting antioxidant and antimicrobial effects (Tyagi *et al.*, 2020) [5]. The stem is a more widely utilised and beneficial component of the plant than the leaves (Sarala *et al.*, 2012) [6], and its extract has been shown to be an excellent source of antioxidant for nutraceutical purposes, providing protection against cardiovascular disease, premature ageing, and cancer (Ilaiyaraja and Khanum 2011) [7]. Giloy contains a wide range of components from several classes, including alkanoids, di-terpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic chemicals, and polysaccharides, and it has no adverse effects or toxicity when used appropriately (Singh *et al.*, 2003) [8]. The global trend of "Return of Nature" (Shivatare *et al.*, 2013) [9] is a transition

from synthetic to natural. Giloy (*Tinospora cordifolia*), a valuable common herb and versatile medicinal plant known as "everlasting nectar," is one of nature's blessings. Ladoo is a classic sweet that is enjoyed by people of all ages and is a high-energy snack. Ladoos are easy to eat and made using a variety of local ingredients. Supplementing ladoo with substances like medicinal plant extract or powder can improve its nutritional and therapeutic characteristics (Reddy *et al.*, 2005) [10]. *Tinospora cordifolia* contains a variety of components, including alkaloids, di-terpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic chemicals, and polysaccharides (Singh *et al.*, 2003) [8]. Many researchers have laboured to characterise the *Tinospora cordifolia's* medicinal and outstanding positive properties, but only a few studies have been conducted with food containing giloy stem.

Supplementing with medicinal herbs not only provides therapeutic benefits, but it can also boost the nutritional value of the product while also providing functional health benefits. Therefore, the present study was planned to: 1)phytochemical screening of giloy stem powder, 2) evaluate the potential of GSP to be used as a food source by its incorporation into sweet balls based on sensory evaluation and 3) assess the proximate composition of GSP incorporated sweet balls and control roasted bengal gram flour sweet balls.

Materials and Methods

Collection of Plant material

MRDC (Medicinal Plant & Research Development Centre), Haldi provided the Giloy.

Preparation of extraction

The extracts were made by soaking 5 g of giloy stem powder powder in 100 ml of each of methanol, ethanol, petroleum ether, and water and shaking thoroughly. The solution was allowed to sit at room temperature for 72 hours before being filtered through filter paper. The filtrate was taken and used for additional phytochemical analysis. Standard phytochemical screening methods were used. (Raaman, 2006) [11].

Phytochemical screening of Giloy stem powder

1. **Detection of Alkaloids**

a. **Mayer's test:** Mayer's reagent was added to five ml extract. The presence of a creamy white precipitate in the sample indicated the presence of alkaloids.

b. **Dragendroff's test:** Five ml of extract were mixed with two ml of Dragendorff's reagent. The presence of alkaloids is established by the reddish brown colour precipitation.

c. **Wagner's test:** To the 5 mL of extract, two drops of Wagner's reagent were added. Presence of alkaloids is indicated by a reddish brown precipitate.

d. **Hager's test:** To 5 ml of extract, two mL of Hager's reagent (saturated aqueous solution of picric acid) was added. The presence of alkaloids is indicated by the presence of yellow precipitate.

2. **Detection of Glycosides**

a. **Borntrager's test:** Filtered (2 mL) extract was mixed with 3 mL CHCl3 and 10% ammonia solution, yielding a pink colour that indicated glycoside presence.

b. **Legal's test:** The presence of glycosides was determined by mixing five mL of plant extract with pyridine, sodium nitroprusside, and 10% NaOH, and the result was pink.

3. **Detection of Saponins**

a. **Froth Test:** 20 mL distilled water was used to dilute the extracts. For around 15 minutes, the diluted extracts were shaken in a graduated cylinder. Saponins are present when a one-centimeter layer of foam forms.

b. **Foam Test:** In a test tube, around 0.5 gram of the extracts were diluted with 2 ml of water. Saponins were detected by the presence of foam that lasted for around ten (10) minutes after being shaken.

4. **Detection of phytosterols**

a. **Salkowski's test:** In a test tube, around 0.5 gram of the extracts was diluted with 2 ml of water. Saponins were detected by the presence of foam that lasted for around ten minutes after being shaken.

b. **Liebermann-Burchard's Test:** A few drops of concentrated H_2SO_4 were added to five mL of filtrate (Shaken well and allowed to stand). The presence of sterols was revealed by the presence of red colour in the upper layer.

5. **Detection of tannins:** After dissolving the plant extract in 5mL of distilled water, 1 per cent gelatin solution and 10% NaCl were added. The presence of tannins was revealed by the formation of white precipitate.

6. **Detection of Resins:** One ml of extract was dissolved in acetone before being added to distilled water. The presence of resins was suggested by the presence of turbidity.

Optimization of sweet balls

For optimization of GSP incorporated sweet balls, nine different blends of RBGF: SS: GSP 100:0:0, 90:5:5, 80:10:10, 70:15:15, 60:20:20, 50:25:25, 40:30:30, 30:35:35, 20:40:40 were taken. Amount of rice flakes (10g), ghee (9g), cardamom (1g), jaggery (50g) and water (10ml) were kept constant for all combinations. Blend mixture alongwith rice flakes and cardamom was mixed thoroughly. Jaggery and water was mixed and boiled to one thread consistency and all other ingredients were added to the jaggery syrup. The sweet balls were shaped and kept in room temperature for sensory analysis.

Sensory evaluation of Giloy stem powder incorporated sweet balls

A semi-trained panel of 15 individuals from the department of Foods and Nutrition, G.B.P.U.A.T., Pantnagar, evaluated all of the sweet balls for sensory quality. Based on several sensory criteria, a nine point hedonic scale was utilised to select the most acceptable sweet balls (Meilgaard *et al.*, 2007) [12].

Nutritional quality evaluation of control Giloy stem powder incorporated sweet balls

Based on the results of sensory evaluation control and the most acceptable GSP incorporated sweet balls were evaluated for their crude fiber, crude protein, crude fat, ash, crude fibre, carbohydrate and physiological energy content on calculation basis.

Results and Discussion

Phytochemical screening of ethanolic, methanolic, petroleum ether and water extract of giloy stem powder

The results pertaining the phytochemical screening of giloy stem powder are presented in Table 1. Presence of alkaloids and glycosides was detected in ethanolic, methanolic and water extract whereas they were lacking in petroleum ether extract. Saponins were present in ethanolic and water extract, however methanolic and petroleum ether extract were found to be lacking for saponins. Presence of tannins and resins were also detected in ethanolic, methanolic and water extract and petroleum ether extract was found to be lacking of the both phytochemicals. Ethanolic, methanolic, petroleum ether and water extract had the presence of phytoesterols. Saedhara and Gopal, 2013 [13] estimated the phytochemicals in giloy stem and reported that alkaloids are present in ethanolic extract whereas their absence was found in petroleum ether and water extract. Also, tannin were reported in ethanolic and water extract and found to be missing in petroleum extract. Dahanayake *et al.,* 2020 [14] reported that saponins, tannins, alkaloids, glycosides and phytosterols all were present in hot methanolic and water extract of the giloy stem, however the amounts varied among the both extracts.

Table 1: Phytochemical screening of ethanolic, methanolic, petroleum ether and water extract of giloy stem powder

Phytochemicals	Ethanolic extract	Methanolic extract	Petroleum ether extract	Water extract
Alkaloids				
Mayer's test	+ ve	+ ve	- ve	+ ve
Dragendroff's test	+ ve	+ ve	- ve	+ ve
Wagner's test	+ ve	+ ve	- ve	+ ve
Hager's test	+ ve	+ ve	- ve	+ ve
Glycosides				
Borntrager's test	+ ve	+ ve	- ve	+ ve
Legal's test	+ ve	+ ve	- ve	+ ve
Saponins				
Froth Test	- ve	- ve	- ve	+ ve
Foam Test	- ve	- ve	- ve	+ ve
Phytosterols				
Salkowski's test	+ ve	+ ve	+ ve	+ ve
Liebermann-Burchard's Test	+ ve	+ ve	- ve	+ ve
Tannins				
Gelatin test	+ ve	- ve	- ve	+ ve
Resins				
Acetone-Water test	- ve	- ve	- ve	+ ve

Sensory evaluation of sweet balls prepared using different blends roasted Bengal gram flour (RBGF), sesame seeds (SS) and giloy stem powder (GSP)

Control sweet balls and different combinations of GSP incorporated sweet balls were evaluated for various sensory parameters *viz*; colour, flavour, taste, appearance, hardness, after taste and overall acceptability using nine- point Hedonic scale (Table 2). The results of sensory evaluation revealed that a significant difference ($p< 0.05$) was observed in the olour, flavour, taste, appearance, hardness, after taste and overall acceptability attribute for all the blend when compared to control, however this can be attributes to the bitter taste of the giloy stem powder which led to the lower acceptability of giloy stem powder in sweet balls. RBGF:SS:GSP::70:15:15 was selected for proximate evaluation with the control sweet balls, even though hardness and after taste parameter was effected, the taste parameter was not that effected, which can be perceived as the addition of the taste profile of sesame seeds. Indu and Awasthi, 2018 [15] evaluated the sensory acceptability of ashwagandha supplemented cereal-legume based ladoo where no significant difference was found in the acceptability of product containing 3%, 4% and 5% ashwagandha. Tyagi *et al.,* 2020 [5] prepared giloy stem powder incorporated cookies where at 10% and 12% levels, the cookies showed a significant unacceptable change from control cookies in all sensory attributes. It means that the addition of TC in cookies beyond an 8% level decreased the liking. Sensory values at 8%, significantly different from control but not significant with 4%, and 6%, were in an acceptable range. In the case of all attributes, TC-8% scored very near to control samples. Hence, supplementation of *Tinospora cordifoila* stem powder at an 8% level was found to be acceptable in cookies without causing any adverse effect on the sensory attributes and considered as optimized cookies.

Table 2: Sensory evaluation of sweet balls prepared using different blends roasted Bengal gram flour (RBGF), sesame seeds (SS) and giloy stem powder (GSP)

Blends	Colour	Taste	Appearance	Hardness	After taste	Overall acceptability
RBGF:SS:GSP::100:0:0	8.68 ± 0.23^{a}	8.40 ± 0.52^{a}	8.70 ± 0.62^{a}	8.43 ± 0.43^{a}	8.53 ± 0.45^{a}	9.05 ± 0.54^{a}
RBGF:SS:GSP::90:5:5	8.46 ± 0.27^{b}	8.28 ± 0.63^{ab}	8.42 ± 0.47^{b}	8.24 ± 0.53^{b}	8.41 ± 0.48^{b}	8.85 ± 0.37^{b}
RBGF:SS:GSP:: 80:10:10	8.36 ± 0.40^{bc}	8.22 ± 0.36^{b}	8.37 ± 0.28^{b}	8.10 ± 0.23^{c}	8.33 ± 0.17^{c}	8.77 ± 0.29^{c}
RBGF:SS:GSP::70:15:15	8.38 ± 0.35^{bc}	8.04 ± 0.23^{c}	8.26 ± 0.29^{c}	7.78 ± 0.67^{d}	8.17 ± 0.51^{d}	8.62 ± 0.15^{d}
RBGF:SS:GSP::60:20:20	8.32 ± 0.52^{c}	7.76 ± 0.26^{d}	8.20 ± 0.52^{cd}	7.66 ± 0.53^{de}	8.09 ± 0.42e	8.22 ± 0.35^{e}
RBGF:SS:GSP::50:25:25	8.27 ± 0.45^{c}	7.68 ± 0.26^{d}	8.14 ± 0.43^{d}	7.64 ± 0.46^{e}	7.85 ± 0.33^{f}	8.08 ± 0.28^{f}
RBGF:SS:GSP::40:30:30	7.86 ± 0.36^{d}	7.40 ± 0.47^{e}	7.79 ± 0.56^{e}	7.26 ± 0.42^{f}	7.79 ± 0.38^{f}	7.84 ± 0.41^{g}
RBGF:SS:GSP::30:35:35	7.88 ± 0.22^{e}	7.25 ± 0.18^{f}	7.73 ± 0.73^{e}	7.21 ± 0.49^{fg}	7.55 ± 0.19^{g}	7.49 ± 0.27^{h}
RBGF:SS:GSP::20:40:40	7.74 ± 0.64^{e}	7.18 ± 0.37^{f}	7.51 ± 0.33^{f}	7.14 ± 0.33^{g}	7.47 ± 0.43^{h}	7.24 ± 0.61^{i}
CD value at 0.05	0.154	0.164	0.132	0.156	0.088	0.101

All values are mean of fifteen observations ± standard deviation
Means having different superscripts differ significantly in each column ($p < 0.05$)
RBGF: roasted Bengal gram flour; SS: sesame seeds; GSP: Giloy stem powder

Proximate composition of control sweet balls and GSP incorporated sweet balls on calculation sweet balls

Table 3 presents the proximate composition of control roasted bengal gram flour and sweet balls prepared with the blend combination of RBGF:SS:GSP::60:20:20**,** on the calculative basis. It was observed from the table incorporation of 15% sesame seeds and 15% giloy stem powder in sweet balls had 7% increase in crude protein (14.15% and 15.19%) content and 3.7% increase in energy content (305.38kcal and 316.67kcal). However, there was highly significant increase in total ash (0.75% and 1.67%) content and crude fat content (3.23% to 6.91%).

Table 3: Nutritional composition of control sweet balls and GSP incorporated sweet balls (calculation basis)

Nutrients	Control sweet balls RBGF:SS:GSP::100:0:0	Giloy stem powder incorporated sweet balls RBGF:SS:GSP::70:15:15
Crude protein (%)	14.15	15.19
Crude fiber (%)	9.29	9.17
Crude fat (%)	3.23	6.91
Total ash (%)	0.75	1.67
Carbohydrate (%)	53.42	48.43
Energy (kcal)	305.38	316.67

Tyagi *et al.*, 2020 [5] formulated giloy stem powder cookies by incorporation of GSP level of 2%, 4%, 8%, 10%, and 12%, there was a slight decrease in crude fiber (9.29% to 9.17%) content of 1% and carbohydrate (53.42% to 48.43%) content decreased 10%. The addition of giloy significantly increased the crude protein (5.42 ± 0.07 to $5.98 \pm 0.01\%$), ash (0.64 ± 0.04 to $0.84 \pm 0.02\%$) and fiber (1.67 to 4.67) in cookies when compared to control cookies, whereas the percentage of crude fat (17.42 ± 0.13 to $16.64 \pm 0.16\%$) significantly decreases. The mean values of total energy estimated by bomb calorimeter for TC cookies and control cookies were 21.05 and 20.79 kJ/g respectively. Indu and Awasthi, 2018 [15] prepared ashwagandha supplemented cereal-legume based ladoo where ashwagandha root powder was incorporated at a level of 3%, 4% and 5%. Proximate analysis of control and 3% and 5% incorporated ladoo led to the non- significant increase in increase in total ash (3.10a ± 0.57, 3.35b ± 0.14 and 3.60b ± 0.02) and crude fiber content (3.53a ± 0.14, 3.60a ± 0.49 and 3.73a ± 0.11) whereas there was a non- significant decrease in crude fat content (2.27a ± 0.21, 2.30a ± 0.15 and 2.34a ± 0.00), energy content (333.03 ± 8.50, 331.90 ± 3.08 and 330.10 ± 0.41), crude protein content (14.09 ± 0.90, 13.95 ± 0.12 and 13.89 ± 0.94), carbohydrate content (64.25a ± 0.50, 62.85b ± 0.82 and 63.67a ± 0.47) which is in line with our results.

Conclusion

Medicinal plants with many heath and therapeutic benefits can be inculcated in daily dietary practices by incorporating it sweet balls and also have the added benefit of being sensory accepted Numerous investigations have been conducted on the various parts of plant to this plant to sort out the apparent pharmacological and bioactive components and further mechanisms of utilizing of these various parts. Recently, giloy has been of great interest to researchers across the globe because of its reported medicinal properties like anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities. However during COVID-19 pandemic, giloy was brought into highlight as an immune booster. This medicinal plant can be utilized for production of new food products which are not only nutritious but also health promoting. Giloy is one of the most abundantly found plant which has variety of properties but at the same time is least exploited herb which can be used to produce such food products which can be a part of daily dietaries. The present study revealed that GSP incorporated sweet balls were highly acceptable as the incorporation of giloy stem powder (GSP) up to 15% was being accepted sensorially when compared to control RBGF sweet balls. Moreover, incorporation of GSP remarkably increased crude fat, total ash, crude protein and energy content of sweet balls. Therefore, giloy stem powder, an important medicinal herb, may be successfully used for preparation of sweet balls at household as well as commercial level without compromising the sensory acceptability.

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