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Development of a Functional Chocolate using Microwave Assisted Extract of Giloy Stems

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ABSTRACT

Background: The present study aimed at formulating a confectionery product namely chocolate using the extract obtained from giloy stems.

Methods: Green technology of microwave assisted extraction (600 W, 60°C for 10 min) have been employed in obtaining giloy extract. The extract was investigated for its *in vitro* antioxidant properties and antidiabetic properties prior to its application in food product

Result: The DPPH radical scavenging activity (RSA) of the extract was found to be 82.26% suggesting its strong antioxidant potency. The extract inhibited the activity of α-amylase (*in vitro*) by 14.63% indicating its antidiabetic potency. The extract was incorporated in formulation of chocolate at the concentrations *viz*. 0.0131 ml/g, 0.0156 ml/g, 0.0181 ml/g. Chocolate formulated with 0.0131 ml/g giloy extract exhibited appreciable *in vitro* antioxidant efficacy (72.36% RSA) and anti-diabetic efficacy (5.68%). This novel giloy extract incorporated chocolate is a promising functional food and could be further utilized in formulation of other functional foods**.**

Key words: Antidiabetic activity, Antioxidant activity, Chocolate, Giloy extract, Microwave assisted extraction.

INTRODUCTION

Tinospora cordifolia plant, commonly known as giloy and guduchi, belongs to the menispermaceae family. At higher altitudes, this plant is a climbing shrub with heart- shaped leaves that are greenish yellow in color. The herb has a long history of being used in traditional system of medicines and by ayurveda practitioners in India to treat numerous disorders or diseases. Almost all parts of the plant are used in ayurvedic formulation owing to its medicinal properties such as immunomodulation, antidiabetic, antifungal, anticancer, anti-HIV potential and antitoxic effect, to name a few (Saha and Ghosh, 2012; Yoggeta *et al*., 2021).

Owing to the medicinal properties of giloy, it has gained interest among researchers. It has been reported that several bioactive components such as alkaloids, diterpenoid lactones, steroids, glycosides, polysaccharides, phenols and sesquiterpenoids are present in giloy extract. These chemical compounds possess prominent antioxidant, antidiabetic, anticarcinogenic and immunity boosting properties (Saxena and Rawat, 2019). Reportedly, fresh plants are more efficacious than dried plants due to their efficiency of their nutrients and chemical composition (Tyagi *et al*., 2020).

Solvent extraction methods using solvents such as methanol, ethanol and water are commonly employed for extraction of components from giloy stems (Satija *et al*., 2015). Few studies have reported on extraction of bioactive components from giloy stems using ultrasound-assisted extraction and microwave assisted extraction (MAE) (Satija *et al*., 2015; Sahoo *et al*., 2023; Kumar *et al*., 2023) MAE has been proved to be beneficial in terms of solvent consumption and low extraction time (Satija *et al*., 2015). In addition to that, MAE has also been found advantageous for extracting terpenoids along with other bioactive components (Sahoo *et al*., 2023). Present study focuses of employment of MAE to obtain extract from fresh giloy stems.

Apart from employing various extraction methods, giloy extracts had also been used in development few food products such as sweet balls, cookies, herbal lassi and amla pulp candy (Joshi and Awasthi, 2022; Tyagi *et al*., 2020; Nirmal *et al*., 2022). There are studies reporting development of confectionery product such as chocolate using jackfruit seeds and ash gourd seeds (Pallavi *et al*., 2018; Molu *et al*., 2021). However, no research finding on formulation of confectionery products especially chocolate using giloy extract has been reported till date. Therefore, the current study forayed into formulation of confectionery product namely chocolate using giloy extract. The extract would be obtained employing MAE method followed by product formulation using the same and subsequently characterization of the newly formulated chocolate for its sensory, physicochemical and functional properties namely *in vitro* antioxidant, antidiabetic properties.

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MATERIALS AND METHODS

Materials

Fresh giloy stems and compound chocolate were procured from the local market of Loni Kalbhor, Pune. Specialty chemicals namely DPPH and α- amylase were procured from Sigma, India. All chemicals used in this work were of AR grade. Compound chocolate was obtained from a local supermarket in Pune, India. The packaging material *i.e.*, metallized pouches were sourced from the local market of Pune, India.

MAE extraction from giloy stems

A 28 L microwave oven (CE104VD, M/s Samsung) with power of 600 W was employed for MAE of giloy stems. The fresh giloy stems were cut into small pieces followed by addition in conical flasks containing ethanol. The weight of the giloy stems and the volume of ethanol were varied at different proportions (Table 1). The conical flasks were then subjected to MAE at 60°C for 10 min (10 s of break at an interval of 20 s). Each flask was placed inside a bowl containing ice to maintain the temperature at 60°C during the extraction process. The extract obtained was further subjected to rotary vacuum evaporator to remove the excess ethanol. The concentrated extracts were then investigated for the in vitro antioxidant properties. The extract exhibiting the highest antioxidant property was further analyzed for its *in vitro* antidiabetic efficacies. Fig 1 shows the process flowchart for MAE extraction from giloy stems.

Table 1: *In vitro* DPPH radical scavenging activities (%RSA) of MAE extracts of giloy at varying extraction conditions.

Time (Min)	Weight of giloy stem (g)	Ethanol (ml)	% DPPH RSA*
1.5	5	25	49.43±0.020
3	10	50	57.86±0.002
5	15	75	68.47±0.017
8	20	100	76.86±0.001
10	25	125	82.26±0.002
12	30	150	72.57±0.003

* Each value indicates mean±SD of three independent samples

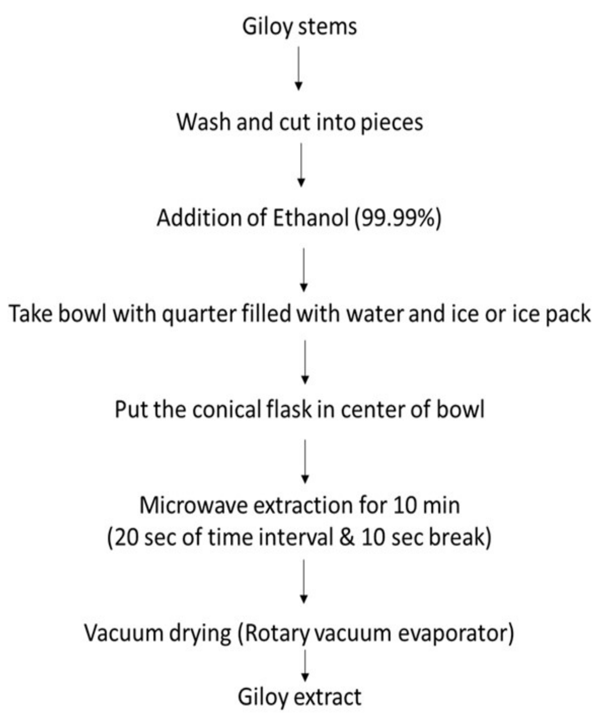

Fig 1: MAE from giloy stems.

Formulation of chocolate using MAE extract of giloy

The chocolate was formulated by using compound chocolate in equal proportion (dark and white, w/w) followed by incorporation of the standardized MAE giloy extract (Fig 2). In accordance with the preliminary trials conducted, the percentage of giloy extract has been kept in the range of 0.6 to 1.2% in this study. Three samples were prepared using giloy extract at three different concentrations (Table 2). The chocolate samples were then subjected to sensory analysis in terms of color and appearance, texture flavour, taste and overall acceptability. 20 semi-trained panellists evaluated the chocolate samples using a 9-point Hedonic scale (from Like extremely to Dislike extremely). The sample with the highest sensory scores was subjected to further analyses.

Analyses of the giloy extract incorporated chocolate

The sample chocolate with the highest sensory scores, was subjected to analyses of its proximate parameters, *in vitro* antioxidant properties and *in vitro* antidiabetic properties. The analyses were conducted in accordance with the standard methods reported by Paul and Bhattacharjee (2018) and Beniwal *et al*. (2022).

Storage study

Samples of giloy extract incorporated chocolate were packaged in aluminium laminated packaging and stored at room temperature for up to 3 months at room temperature (25±2°C), in accordance with the methods reported by Mokbul *et al*. (2023). The samples were investigated for their microbiological quality in terms of total plate count (TPC) and yeast-mold count at regular intervals. Additionally, the physicochemical properties of the chocolate, including moisture content, crude fat, total protein, crude fibre, ash, carbohydrate, were analyzed at the end of the storage study.

Statistical analysis

The experimental results are expressed as means of the experimental data obtained from three independent samples. A value of $p \leq 0.05$ was considered significant to establish differences in all tests. All statistical tests were performed by STATISTICA 8.0 software (Statsoft, Oklahoma, USA).

RESULTS AND DISCUSSION

Standardization MAE extraction from giloy stem

In the present study, weight of the giloy stems and volume of solvent along with the extraction time was standardized in terms of DPPH radical scavenging activity (Table 1). MAE extraction of 25 g of giloy stems using 125 ml of ethanol for 10 min exhibited highest antioxidant activity. In a study by Satija *et al*. (2015), the authors have employed the MAE technology (480 W, 3 min) to obtain extract from giloy stems. However, they have optimized the extraction parameters in terms of berberine content in the extract. The results in that study revealed the efficiency of the MAE method to be superior to the conventional methods in terms of yield of extract and extraction time (Satija *et al*., 2015).

In vitro antioxidant activity of MAE giloy extract

MAE extract from giloy exhibited prominent DPPH radical scavenging activity of 82.26% suggesting its high antioxidant efficacy. The findings are in good agreement with the values reported by Upadhyay *et al*., (2014) who reported 84.62% in methanolic extract of giloy. According to the literature reports, extracting solvent plays a significant role in yield of extract as well as bioactive compounds (Ngo *et al*., 2017).

Table 2: Formulation of chocolate with MAE giloy extract.

Components	Control	S_1	S_2	S_3
Dark compound	50 g	50 g	50 g	50 g
White compound	50 g	50 g	50 g	50 g
Giloy extract	00	0.62 ml	0.83 ml	1.25 ml

Thus, the antioxidant activities of the extracts vary with the extracting solvent used in extraction (Satija *et al*., 2015). The strong antioxidant property of the extract was owing to the presence of antioxidants (such as polyphenols, tannins) present in giloy stem. Further antioxidant potency of the giloy extract could be due to the presence of several glycosides and sesquiterpenoids present in the giloy stems (Gupta *et al*., 2024; Singh *et al*., 2003). Thus, the extract obtained from the aforesaid extraction conditions were subjected to further analyses.

In vitro α-amylase inhibition by MAE giloy extract

The extract obtained from giloy inhibited the activity of *in vitro* α-amylase by 14.63%. The inhibition of the enzyme by the extract indicates its *in vitro* antidiabetic efficacy. This is the first-time report on *in vitro* antidiabetic efficacy of MAE extract of giloy stems. However, Sharma *et al*. (2021) have reported appreciable *in vitro* α-amylase inhibition by ayurvedic formulation of giloy. Alkaloids namely berberine present in giloy stems reportedly possess antidiabetic properties (Cicero and Tartagni, 2012). Satija *et al*. (2015) have reported that MAE extraction of giloy stems resulted in significant yield of berberine in the plant extract. Therefore, the aforesaid findings attest the *in vitro* antidiabetic result of current study on MAE extract of the giloy stems. The results are strongly suggestive of the extract to be a potential alternative to the

Table 3: Proximate composition of a giloy extract incorporated chocolate.

Sample	Proximate composition (%)						
	Moisture	Fat	Protein	Crude fiber	Ash	Carbohydrate	Energy (kcal)
S_1	5.16±0.024	29.24±0.031	2.39±0.013	9.37±0.081	1.95±0.022	59.95±0.12	512.32±0.19
S_2	5.11±0.021	29.22±0.023	2.35±0.021	9.23±0.053	1.93±0.051	59.87±0.11	511.23±0.35
S_3	4.98±0.011	29.19±0.018	2.32±0.022	9.17±0.049	1.90±0.019	59.79±0.23	510.74±0.45
SE	0.026±0.001	0.024±0.001	0.026±0.001	0.023±0.001	0.040±0.002	0.024±0.001	0.042±0.001

* Each value indicates mean±SD of three independent samples.

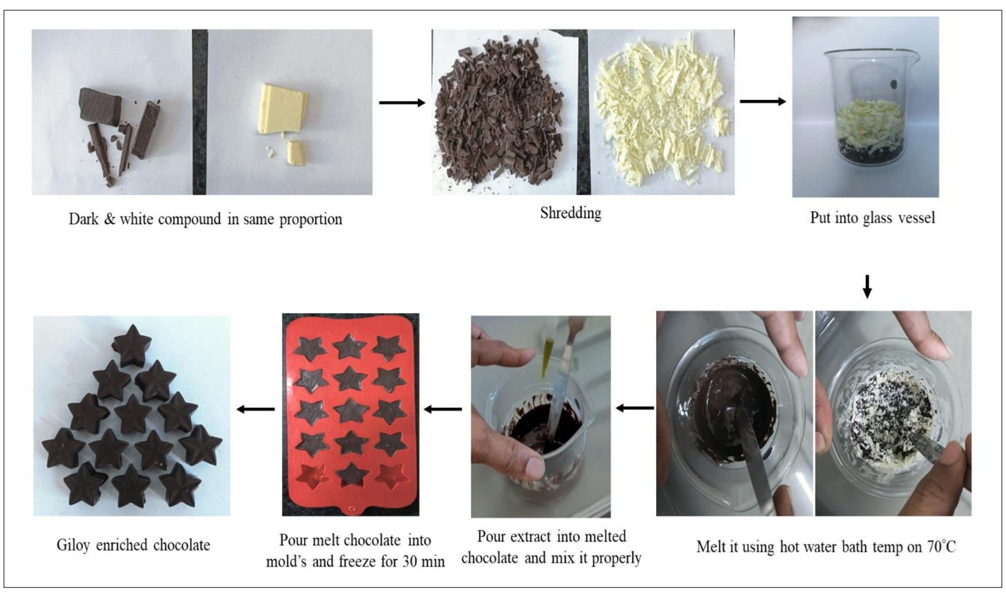

Fig 2: Process flow sheet for formulation of chocolate employing MAE giloy extract.

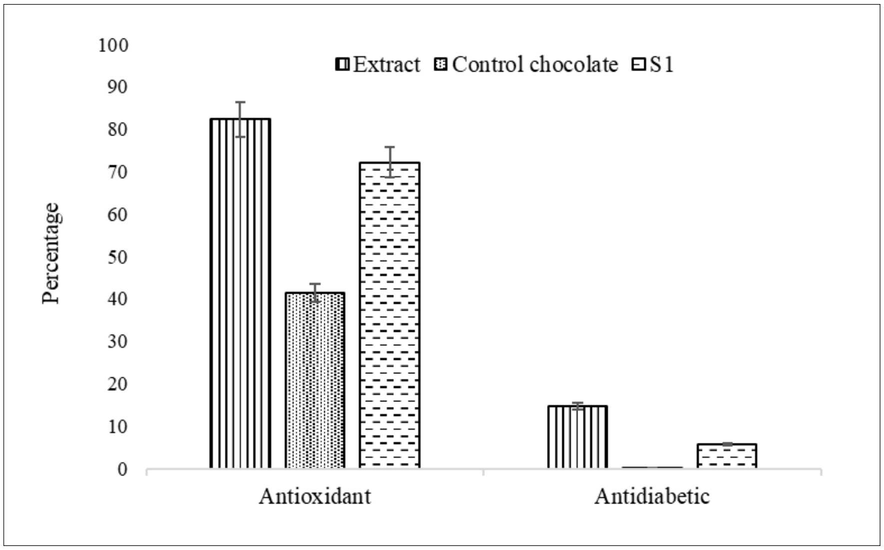

Fig 3: Comparative representation of antioxidant and antidiabetic activities of native giloy extract (MAE) and the developed food product.

Table 4: TPC and Yeast-mold count of the giloy extract incorporated chocolate.

Storage period (days)	Total plate count (cfu/g)	Yeast and mold count (cfu/g)
0	ND	ND
15	ND	ND
30	ND	ND
45	ND	ND
60	ND	ND
75	1.8×10^3	ND
90	2.5×10^3	ND

* Each value indicates mean of three independent samples.

synthetic drugs used for diabetes and can be further utilized in formulation of food product.

Sensory evaluation of giloy extract-rich chocolates

The sensory scores of the sample S_1 were highest whereas the scores were lowest for S_3. These findings agreed well with those reported by Nirmal *et al*. (2022) where the sensory scores decreased with increase in giloy extract in the candy samples. Thus, the said sample (S_1) was well accepted in sensory analysis and was subjected to further analyses.

In vitro antioxidants activity giloy extract incorporated chocolate (S_1)

Sample S_1 exhibited 72.16% RSA whereas control chocolate sample had scavenged DPPH by 41.39% (same amount of both samples were considered). The antioxidant potency of the control chocolate sample was owing to the polyphenols present in cocoa (Oracz and Żyżelewicz, 2020). The results clearly indicate that incorporation of giloy extract has significantly (p=0.02) increases the antioxidant potency of the formulated chocolate (Fig 3). These findings are in good agreement with studies that revealed potent DPPH radical scavenging activity giloy extracts (Upadhyay *et al*., 2014; Onkar *et al*., 2012). Sharma *et al*., (2019) suggested that the antioxidant capacity of giloy extract could be compared with the standard antioxidant ascorbic acid. The antioxidant potency of the giloy extract could be due to the presence of alkaloids and terpenoids present in the giloy stems (Gupta *et al*., 2024). Singh *et al*., (2003) also reported the presence of several glycosides and sesquiterpenoids in the giloy stem which could be responsible for antioxidant activity. Owing to the aforesaid findings, giloy-rich chocolate developed in this study, could be an excellent alternative of conventional chocolate that can lower the chances of metabolic disorders of the consumers (Frei, 2004; Uttara *et al*., 2009; Tan *et al*., 2018).

In vitro α-amylase inhibition by giloy extract incorporated chocolate (S_1)

The sample S_1 exhibited 5.73% α-amylase inhibition assay whereas control sample had 0.08%. The results are clearly indicative that the incorporation of giloy extract has increased in the antidiabetic potency of the formulated chocolate (Fig 3). The inhibition of α-amylase depends on the amount of the giloy extract incorporated in the chocolate samples. However, increasing the giloy extract would have an adverse effect on the sensory properties of the food product. This is the first report on the *in vitro* antidiabetic efficacy of giloy extract (MAE) incorporated food product. The antidiabetic potency of the extract could be further validated by *in vivo* animal study.

Shelf life of giloy extract incorporated chocolate

Table 4 shows that susceptibility of the chocolate sample (S_1) to microbial growth during storage time in terms of total plate count (TPC) and yeast and mold count (YMC).

TPC and YMC were negligible up to 90 days of storage as the giloy extract incorporated chocolate owing to its low moisture content (Table 3). Thus, in accordance with the storage study, a standardized giloy extract incorporated chocolate can be kept at room temperature for 90 days when packaged in metalized pouches.

CONCLUSION

MAE of giloy extract was obtained at 60°C at 600 W using 25 g of giloy stems and 125 ml of ethanol for 10 min extraction time. The extract exhibited the high antioxidant activity (82.80% in terms of DPPH RSA) as well as prominent antidiabetic activity (14.38% α-amylase inhibition activity). The chocolate was formulated using the said extract (0.62 ml/ 100 g). The developed chocolate revealed prominent antioxidant and antidiabetic properties. The shelf-life study of the chocolate suggested that the food product could be consumed up to 90 days when stored at room temperature (25±2°C). The findings are strongly indicative of the development of a functional chocolate product using MAE giloy extract with potent antioxidant and antidiabetic properties. The *in vitro* results on therapeutic efficacy of the chocolate developed with MAE giloy extract could be further validated by *in vivo* rat model studies and the potency of this food product as a novel functional food can be established.

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Disclaimers

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Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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