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Formulation and evaluation of herbal syrup analogous of ginger, basil and stevia

Snehal Jadhav[1], Pawan Kumar[2], Ramalakshmi K[3], Yogita chavan[4], Sahoo AK[5]
[1, 3] Department of Food Technology, Bannari Amman Institute of Technology, Tamil Nadu, India
[2] Research and development department, Mother Dairy Fruits and Vegetable India Pvt. ltd, Delhi, India
[4] Department of Food Technology, MIT College, Pune, Maharashtra, India
[5] Department of Food Technology, Shivaji University, Kolhapur, Maharashtra, India

Abstract
The objective of the study was to develop analogous herbal syrup using ginger, basil and stevia (GBS Syrup) as the main ingredients and evaluate for its antimicrobial, antioxidant activity and storage study of the syrup. The focus was on Ginger, Basil and Stevia to formulate a healthy option to synthetic syrups. For this purpose, extract of ginger and basil were withdrawn and tried at different level, to match the sweetness level of traditional syrup (65^0Brix), Different concentration of Stevia was used in order to match the viscosity. Different levels of stabilizer were also explored. The developed syrup was analyzed for its storage study using various physico-chemical and microbial parameter and evaluated for anti-oxidant and anti-microbial activity against *Basillus subtilis, Staphylococcus aureus, Salmonella typhi, Echerichia coli, Aspergillus niger* and *Saccharomyces cerevisiaea*. Product acceptability of this syrup to consumer was evaluated and it shows satisfactory results.

Keywords: ginger extract, basil leaves extract, stevia powder, herbal syrup analogues, antimicrobial activity, Antioxidant activity

1. Introduction
Herbal syrup is one that offers the consumer additional perceived health benefits besides its primary function of taste enhancement these plants are the potent source of natural bioactive components. Herbs are present ubiquitously but they are often used commercially in combination for formulation of syrup. As people are getting more health conscious, so there is need of formulating natural syrup will be easy to handle and use even at commercial platform.

Tradition Sugar (Sucrose) Syrup contains 65oBrix TSS in general, 25% juice and 1.5% acidity [1]. Sweetening power of stevia is higher than sucrose (300 times) due to presence of stevioside [2]. Addition of xanthan gum limit in syrup is maximum up to 0.5% [1]. The addition of xanthan gum for development of syrup using high sweetening power reported in scientific literature [3].

The extract of ginger and basil leaves showed good source of polyphenols. Previous reports on Ginger (Zingiber officinale) revealed that ginger contains -gingerol, 6-shogaol, 8-gingerol, and 10-gingerol. Pungency in ginger is mainly because of gingerol and zingerone [4]. In Ginger α-pinene, borneol, camphene and linalool are responsible for its antimicrobial activities [5]. The anti-inflammatory and anti-oxidant properties of ginger helps to relieve various inflammatory disorders like gout, osteoarthritis, and rheumatoid arthritis. Another study suggests that ginger can reduce cell death and restore motor function in a rat spinal cord injury [6].

In similar way Basil (Ocimum sanctum) leaves also contains various components like cirsilineol, circimaritin, isothymusin, apigenin and rosameric acid, and appreciable quantities of eugenol possess good activity [7]. Basil contain oleanolic acid, ursolic acid, rosamarinic acid, eugenol, carvacrol, linalool, elemene, germacrene as chemical constituents that have antimicrobial activity [8]. Various part of Basil like leaves, flowers and stems are being used in the treatment of various disorders such as skin disease, cold, cough, fever, vomiting, swelling etc. It also posses anti-cancer, antimicrobial, antiseptic, antispasmodic, antifungal, antiviral, anti-inflammatory, analgesic and immuno-stimulatory properties. The major chemical constituents present in basil are eugenol, methylcinnamate, camphor and thymol [9].

Stevia is a sweetener and sugar substitute extracted from the leaves of the plant species Stevia rebaudiana, native to Brazil and Paraguay. The active compounds are steviol glycosides (mainly stevioside and rebaudioside), which have 30 to 150 times sweetness of sugar are heat-stable, pH-stable, and not fermentable. Sweetening power of stevia is higher due to presence of stevioside [2]. Recent interest of food and beverage industry has driven their focus towards natural high-potency sweeteners due to the increasing awareness of obesity problem and the health impacts associated with certain artificial sweeteners. Hence, many soft drink manufacturers are trying to reduce calories by introducing natural non-caloric sweeteners into their systems. The increasing consumption of sugar (sucrose) has resulted in several nutritional and medical problems, such as obesity. Therefore, low caloric sweeteners have been investigated to substitute sugar. Daily consumption of specific nutrient's in diet will surely promote good health [10]. The development of commercial product that can either substitute or replace the sugar aimed at tackling the ill-effects that can come from sugar.

2. Materials and methods

2.1 Raw material

Fresh Rhizome of ginger (Aurangabadi Variety) procured from Satara, Fresh herbs of Basil-Krishna basil (Department of Botony, Shivaji university, Kolhapur), Steviol glycoside 95 (Devson Pvt. Ltd, Mumbai), Stabilizer-Xanthan gum (Cp Kelco Pvt Ltd, Mumbai), Citric acid
and Sodium benzoate (Acid India Pvt.ltd) and fresh syrup were used for other estimation.

2.2 Composition analysis

Ginger and Basil leaves extract was analyzed for their proximate analysis such as moisture, ash, protein, fat, fiber etc content by standard method [11].

2.3 Analysis of bioactive compounds

Samples were extracted by 70% methanol at a solvent to sample ratio of 4:1 (v/w). The mixture centrifuged at 5,000 rpm for 15 min. This procedure was repeated for three times. All supernatants were then combined and concentrated using a rotation evaporator at 40°C. The final extracts were stored in a freezer until analysis [12].

2.4 Antioxidant Activity

The total antioxidant property of ginger and basil extract and final syrup was determined by 2, 2-Diphenyl-1-picrylhydrazil (DPPH) in terms of % radical scavenging activity. DPPH solution (1 mg/ml) was made by dissolving DPPH in methanol. DPPH solution (100µl) was diluted to 5 ml and absorbance was taken at 535nm in UV-Spectrophotometer. The absorbance was taken as control absorbance. The extract (100µl) was made by dissolving required extract in methanol; then it was added with 100µl of 1mg/ml of DPPH solution. Then it was diluted to 5 ml by methanol then it was incubated at room temperature for 30 min. Then absorbance was measured at 535 nm in UV spectrophotometer. The absorbance was taken as sample absorbance [12]. Following formula was used to calculate the antioxidant activity.

$$\%RSA = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} * 100$$

2.5 Total Phenolic Content (TPP)

TPP was determined using the calorimetric Folin–Ciocalteu method 0.2 ml of diluted extracts were mixed with 1 ml of 1:10 diluted Folin–Ciocalteu reagent and reacted for 5 min. 0.8 ml of 7.5% sodium carbonate was added to the mixture, and incubated for 30 min in the dark at 27± 2ºC. Absorbance was measured at 765 nm on the spectrophotometer. Gallic acid was used as a standard. The standard graph was prepared by using gallic acid in the range with different concentrations gave a regression equation Y =0.006X + 0.039 (R2= 0.999)

2.6 Anti-microbial activity

Ginger extract, basil extract and developed syrup were checked against 9 different microorganisms namely Basillu subtilis, Staphylococcus aureus, Salmonella typhi, Echerichia coli, Aspergillus niger, Saccharomyces cerevisiae. Three different concentrations of juice were tested for antimicrobial activity using agar well diffusion method standardized by National committee for clinical laboratory standards. The microorganisms were incubated in petri plate at 37 ºC for 24 hrs. Media was prepared using nutrient agar, test microorganisms were spread over the solidified plates and wells bored using sterile borer of 4 mm diameter. These wells were then filled with 25, 50,100µl of juice. Streptomycin antibiotic (1mg/ml) was use for comparative study (control sample). These plates were incubated at 37 to 48 ºC according to optimum temperature required for microorganisms. Antibacterial activity was obtained by determining the zone of inhibition around the well [13].

2.7 Sensory evaluation of syrup

GBS syrup were diluted at 1:4 (Syrup: water) for evaluation. Sensory evaluation was carried out by semi trained panel members. Hedonic rating test was employed using 9-point hedonic scale. Sensory parameter such as color, taste, texture and overall acceptability was evaluated [11]. The following were numerical score assigned. Like extremely (9), Like very much (8), Like moderately (7), Like slightly (6), Neither like for dislike (5), Dislike slightly (4), Dislike moderately (3), Dislike very much (2), Dislike extremely (1).

2.8 Storage study of GBS Syrup

Storage study was carried out after every 15 days i.e. 0,15,30,45,60,75,90 days by keeping syrup at refrigeration temperature (4±1oC). During these intervals syrup evaluated for physico-chemical parameter l (TSS, Acidity, pH, Brix, Viscosity (cP), sensory evaluation and microbial analysis like Total plate count (TPC) and Yeast and mold count [11].

3. Results and discussion

3.1 Dosage level of Stevia syrup

Several trials of stevia syrup for equivalent sweetness to sugar syrup (650B) were conducted at lab scale at the level of stevia at 0.3%, 0.4%, 0.5%and 0.6% and the sensory evaluation was carried out against sugar syrup (650B) of using various sensory parameters like sweetness, color, after taste, overall acceptability. The stevia concentration of 0.4% was selected based on sensory feedback, which carried out, by semi-trained panel [3].

3.2 Dosage level of Xanthan gum

The addition of different level of xanthan gum for development of syrup using high sweetening power reported in scientific literature [3]. Trials on addition of xanthan gum in stevia syrup was conducted to achieve proper consistency as of sugar syrup (650B). Therefore, the range for addition of xanthan gum was selected from 0.10%, 0.20%, 0.30% and 0.40% and the analysis and sensory feedback showed that 0.30 % xanthan concentration attaining the equivalent viscosity of Sugar syrup (650B).

From the Table 1, it was observed that GBS syrup was prepared by mixing ginger extract, basil extract, Stevia powder in different concentrations and compare with control sample (Sugar syrup-J). Ginger extract, Basil extract and Stevia concentration were mixed 50:50:0.4 (K), 40:60:0.4 (L), 30:70:0.4(M), 20:80:0.4(N) respectively in ratios. Out of this sample containing 30 % ginger extract, 70 % basil extract and 0.4% stevia i.e. sample M was selected on the basis of sensory feedback as shown in (Figure.1) Whereas the sample containing ginger extract more than 30 % gave spicy taste and basil extract beyond 70% suppressed the taste of ginger and flat taste observe which was no so appealing.

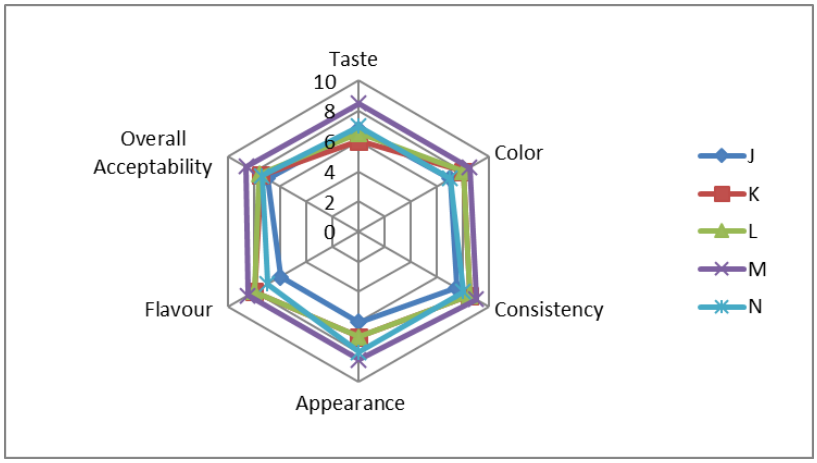

Fig 1: Sensory evaluation of GBS Syrup

Table 1: Sensory Evaluation of GBS Syrup

Sample code	GE: BE: SP	Taste	Color	Consistency	Appearance	Flavour	Overall Acceptability
J	Sugar syrup(65^0B)	6.8	7	7.5	6	6	7
K	50:50:0.4	6	8	8.5	7	8	7.5
L	40:60:0.4	6.5	8	8.5	7	8	7.6
M	30:70:0.4	8.5	8.5	9	8.5	8.5	8.6
N	20:80:0.4	7	7	8	8	7	7.4

GB ginger extract, BE Basil extract, SP Stevia powder

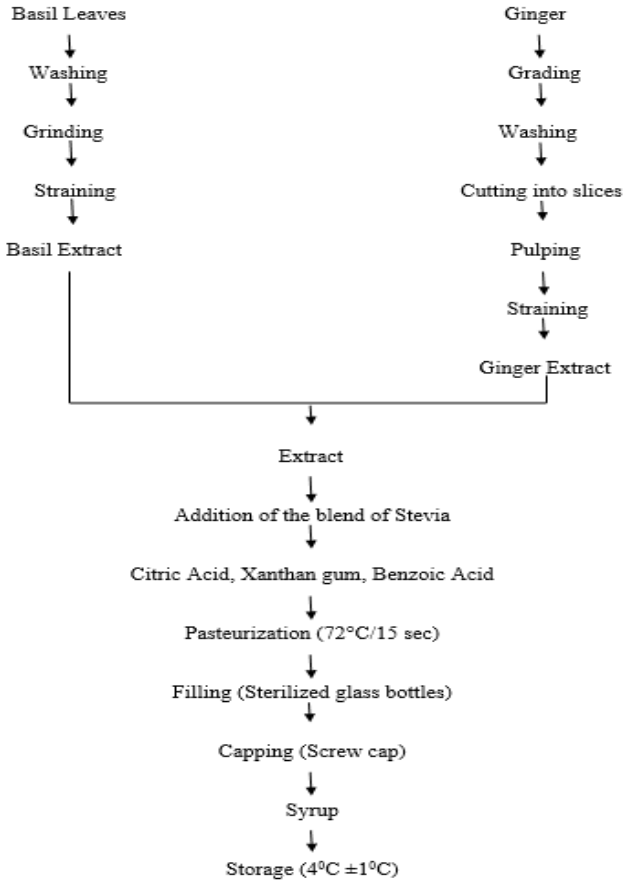

Fig 2: Flow chart for preparation of GBS syrup

From the Table 2, it was observed that the results obtained for ginger and basil extract similar to results reported by [15]. The proximate analysis of GBS syrup also carried out and compared with sugar syrup (650B) sample. The result showed ginger and basil leaves extract are good source of polyphenols and anti-oxidant which is similar to the result reported by [16]. We observed that GBS syrup is the richest source of TPP and antioxidant activity.

Table 2: Chemical composition of raw material (Ginger and Basil extract) and GBS Syrup

Parameter	Ginger Extract	Basil Extract	GBS Syrup
Moisture (%)	90.52	91.96	90.64
Ash (%)	0.9	0.6	0.11
Protein (%)	1.2	0.1	0.654
Fat (%)	0.2	0.01	7.75
Crude fiber (%)	0.12	0.13	0.10
Carbohydrate	7.06	7.23	0.75
Acidity (%)	0.12	0.12	1.5
Vit.C (mg/100gm)	3.6	3.6	3.6
T.S.S (^{0}Brix)	3	3	3
PH	5.6-6.0	5.5-5.8	4.5
Viscosity (cp)	----	----	60
TPP (mg/ml)	2.916	1.540	206
DPPH (% RSA)	79.41	68.92	62.86

TPP Total polyphenol, RSA Radical scavenging activity

Table 3, shows that ginger extract exhibited maximum inhibitory activity against Escherichia coli. Basil extract and GBS syrup showed no effect against Aspergillus niger, while anti-microbial activity against Basillus subtilis, Staphylococcus aureus, saccharomyces sereviciae, Escherichia coli found to be moderate. This study showed that the extracts of ginger and basil possess antimicrobial compounds which could be used as substitutes for the antibiotics [17]. Our result compared with the findings of [18], [19]. Antimicrobial activity of it indicative that the presence of broad spectrum of anti-biotic compound in GBS syrup.

Table 3: Anti-microbial activity of Ginger extract, Basil extract and GBS syrup

Name of organism	Concentration (µl)	Zone of inhibition (mm)			
		Standard (Streptomycin)	Ginger Extract	Basil Extract	GBS Syrup
Staphylococcus aureus	25	16	14	7	8
	50	20	17	9	10
	100	20	19	10	12
Bacillus subtilis	25	14	10	8	10
	50	18	12	10	12
	100	20	15	15	13
Escherichiea coli	25	17	11	9	9
	50	20	13	9	9
	100	22	15	10	10
Salmonella typhi	25	8	13	10	10
	50	17	14	12	12
	100	18	16	15	15
Aspergillusniger	25	9	9	---	---
	50	11	14	---	---
	100	14	20	---	---
Saccharomyces cerevisiae	25	15	11	7	8
	50	17	12	9	10
	100	18	13	10	12

Changes during refrigeration storage of GBS syrup is presented in table 4. The T.S.S of GBS syrup was 3ºBrix at 0 day and observed slightly increase during storage. Increase in total soluble solid may be due to break down of polysaccharides into monosaccharide and oligosaccharides while decreased may be due to fermentation of sugar into ethyl alcohol, carbon dioxide and water [20]. The acidity of GBS syrup was 1.5% at 0 day and continuously increased during storage. Acidity increased may be due to breakdown of pectin into pectenic acid or due to the formation of acid by the breakdown of polysaccharides or oxidation of reducing sugars [20]. Viscosity was observed constant whereas sensory score decreased during storage.

From the table 4. It was seen that TPC and Yeast and mold count of syrup was analyzed for 90 days and it was found that syrup was in good condition upto 90 days that we conclude from regulation of "The Thai Industrial Standards Institute Ministry of Industry" (2003) requires that the total plate count and the yeast and mold count in syrups should not be more than 5x102 cfu/ml and 100 cfu/ml, respectively [21]. Furthermore, refrigeration storage (4±10C) restricted or delayed the growth of micro-organisms and thus reduced potential for acid production and spoilage., osmophilic yeast may survive after heating as sugar can protect its spore as reported by [22]. Thus the growth of osmophilic yeast is another factor affecting the decrease of pH values and increases in the total acidity of syrup during storage as mentioned previously [23].

Table 4: Physico-chemical and microbial change in GBS syrup during storage study

Days	TSS	Acidity	pH	Viscosity(cP)	Sensory evaluation	TPC (cfu/ml)	Y&M count(cfu/ml)
0	3	1.5	4.5	60	8.6	$1*10^1$	$0*10^1$
15	3	1.5	4.5	60	8.6	$2*10^1$	$0*10^1$
30	3	1.7	4.6	60	8.5	$10*10^1$	$1*10^1$
45	3	1.8	4.6	60	8.4	$12*10^1$	$1*10^1$
60	3	1.9	4.7	60	8.4	$16*10^1$	$2*10^1$
75	3.1	2	4.8	60	8.3	$19*10^1$	$2*10^1$
90	3.2	2.1	4.9	60	8.3	$25*10^1$	$4*10^1$

Cp centipoises, TPC Total plate count, Y&M Yeast and mold count

4. Conclusion

The present investigation was under taken to prepare herbal syrup analogus using natural sweeteners, mixture of ginger and basil extract for development of GBS syrup a ratio of Ginger extract: Basil extract: Stevia powder is (30:70:0.4) respectively. The selected ingredients ginger, Basil are the richest source of phytonutrients. Developed GBS syrup showed highest source of total polyphenol content and % radical scavenging activity 206 mg/ml and 62.86 % respectively. GBS syrup posses the antimicrobial activity moderately against Basillus subtilis, Staphylococcus aureus, saccharomyces sereviciae, Escherichia coli. Syrup can be potentially used as food supplement. Storage study of the syrup was carried out at refrigeration temperature using physico-chemical and microbial parameter and found that developed syrup were good for consumption upto 90 days. It can be a good syrup for diabetic patient, weight reduction. This indicates that GBS syrup should be further investigated in terms of possible applications in developing functional food products.

5. Acknowledgement

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