International Journal of Science, Environment and Technology, Vol. 6, No 4, 2017, 2258 – 2270
ISSN 2278-3687 (O)
2277-663X (P)

PREPARATION OF PROBIOTIC GUDUCHI WHEY BEVERAGE

[1]Nandakumari Kamble*, [2]D.B. Puranik and [3]M.K. Salooja
[1]PhD Student, School of Agriculture (SOA), IGNOU, New Delhi-10068
Permanent address: No.6-22-287, Gouthamnagar, Nizamabad-503001
[2]Professor and Head, Dept. of Dairy Technology, Dairy Science College, Bangalore
[3]Professor, School of Agriculture, IGNOU, New Delhi

Abstract: Whey is a by-product of the dairy industry and an excellent beverage base and genuine thrust quencher, nutritious and possesses medicinal properties but treated as waste by-product. Utilization of whey helps to control the pollution and on the other hand adds value to the products and it is good carrier of probiotics. Guduchi (*Tinospora cordifolia*) is known as amrit, and it is one of the most valued herbs in the Ayurvedic pharmacy and had proven antioxidant activity along with number of other health benefits. Probiotics are living microorganisms that, when taken by mouth imparts health benefits by improving the balance of bacteria in the intestines. The *Lactobacillus acidophilus* is the most commonly used Probiotic or "good bacteria". As such an attempt has been made in the present study to develop a value added beverage by combining the Whey, Guduchi Extract and *Lactobacillus acidophilus* as probiotic. In order to prepare Guduchi Whey Beverage, the extracts of Guduchi Leaves and Stem are used separately in different ratio as 90(Whey):10(Guduchi), 85:15 and 80:20 level and sugar at constant rate of 10 per cent is used to mask the bitterness of Guduchi. The influence of the different levels of Guduchi extracts on sensory characteristics was studied. Further the Whey beverages with 90(Whey):10(G1) and 90(Whey); 10(G2) were selected to produce Probiotic Whey Beverage, starter culture of *Lactobacillus acidophilus* was used at different levels as 1%, 1.5%, and 2.0%. Basing on the sensory studies, the Probiotic Guduchi Whey beverage with 1.5% Probiotic was selected for antioxidant studies. The Whey beverages with both the extracts are proven their antioxidant activity by *in vitro* antioxidant models such as DPPH scavenging model, Metal Chelating model and Reductive activity. In conclusion it could be said that it is possible to prepare a value added Probiotic Guduchi Whey beverage with the addition of aqueous extracts of Guduchi leaves and stem separately at 90(Whey):10 (Guduchi).
Keywords: *Tinospora cordifolia*, Extracts, *Lactobacillus acidophilus,* antioxidant activity.

INTRODUCTION

Recent literature reviews in clinical nutrition have amply proved that there is a strong interrelationship between the type of food intake and human health. Man's inquisite desire to relish tasty food has now changed to food that has therapeutic and curative properties.

Whey is a byproduct obtained in manufacturing of Paneer, Chhana and Cheese. It constitutes about 80 to 90 percent of the volume of milk used for paneer and Chhana. It retains 45 to 55 percent of the milk nutrients comprising serum proteins; lactose, minerals, vitamins and whey

*Received June 13, 2017 * Published Aug 2, 2017 * www.ijset.net*

protein which are rich in essential amino acids and whey is good carrier for Probiotics (Koushik and Rajorhia 1998).

Medicinal plants used since long back times and have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparations as those described in ancient texts such as the Vedas and the Bible and obtained from commonly used traditional herbs and medicinal plants have been traced to the occurrence of natural products with medicinal properties. Guduchi (*Tinospora cordifolia*) is a large, glabrous, deciduous climbing shrub belonging to the family menispermaceae. It is distributed throughout tropical Indian subcontinent and China and described as '*the one who protects the body against disease*' with several health benefits (Pandey *et al.,* 2012). Guduchi is a widely used shrub in folk medicines and Ayurvedic medicinal systems. It is reported to possess anti-spasmodic, anti-inflammatory, anti-allergic, anti-diabetic, anti-oxidant properties (Singh *et al*., 2003).

Probiotic Dairy Products containing probiotic bacteria have received increasing attention in recent decades including the expansion of the market for functional foods and research into the development of probiotic foods (Karimi *et al.,* 2011). Probiotics are defined as a dietary supplementation of beneficial bacteria, such as *Lactobacillus acidophilus spp.* and *Bifidobacterium spp.* (Tannok *et al.,* 1995). The *Lactobacillus acidophilus* is proven to produce healthy byproducts that protect the stomach the gut from harmful bacteria and it is best known species of *Lactobacillus* complex in the LAB group and can be grown in lower pH below 5.1 (Parvaneh and Ebrahimi 2011).

MATERIALS & METHODS

Plant material:

Fresh Leaves and Stem of Guduchi (*Tinospora cardifolia)* were collected from the campus of Dairy Science College, Mahagoan, Gulbarga.

Authentication of Plant Materials

The Plant material i.e., leaves and Stem of the plant identified and authenticated by the Herbarium in Botany Department Osmania University, Hyderabad according to the "Illustration on the Flora of the Tamilnadu Carnatic, Vol-II published by K.M. Matthew on 28-12-1982. A specimen voucher No.19 was deposited in the Department.

Other materials

Distilled water, Guduchi leaf extract and Stem extract, Whatman No.1, Paneer whey, Sucrose (Sugar), Thermometers, Probiotic culture of *Lactobacillus acidophilus* NCDC014, Skim Milk powder, MRS agar etc.

FLOW DIAGRAM.

Collection of Paneer Whey
↓
Addition of Sugar @10%
(Ref: Chavan *et al* 2015 and Manasi *et al*2013))
↓
Preparation of Guduchi Extracts-Phytochemical Screening
(Proximate Analysis of Extracts & Total antioxidants)
↓
Addition of Guduchi Extract separately to sweetened whey
at different levels
↓
Heat treatment of Guduchi Whey at 70°C for 35 min (Chavan *et al 2015)*
As Guduchi extract can with stand at this temperature (Ramya and Laxmidevi 2010)
↓
Selecting the level of Guduchi Extract in Whey beverage based on Sensory score
↓
Inoculation of Probiotic Culture of *L.bacillus acidophilus*
at different levels @1%, 1.5% and 2.0% (Ref: Manasi *et al* 2013)
↓
Incubation at OGT 37º C for 5 hours
(*Ref:* Toqueer *et al* 2006*,* Sasi 2015)
↓
Selecting the Probiotic beverage based on the viable probiotic count
For further studies
↓
Storage studies at 4 +/-1ºC
↓
Study of Anti oxidant activity of Developed Probiotic Guduchi whey beverage

OPTIMIZATION OF LEVEL OF GUDUCHI IN WHEY BEVERAGE

Collection and Analysis of Paneer Whey

Paneer Whey is collected from Milk Products Factory, APDDCF Ltd, Hyderabad-17. The Physical and chemical analysis of whey are conducted by standard methods. Results are given below

Composition of Whey:

Fat 0.4±0.1%, Protein 0.850±0.005%, Titratable acidity 0.220±0.005%, Ash 0.408±0.006, Total solids 6.66+0.04%, pH-5.12±0.03 Colour greenish yellow.

Preparation of Aqueous Extract of Guduchi by Maceration Process:

The aqueous extract prepared according to the procedure adopted by Jyothi *et al* (2015). Collected Plant material washed under running tap water to remove dust and foreign matter and then dried under shade at room temperature for 15 days. The material was crushed well into fine powder, packed into air tight polythene bags and stored at room temperature as base stock material for further use. The aqueous extract is prepared by soaking 50 grams of crushed material of leaves and stem separately in 200ml of water and shaken well. The solutions left at room temperature for 72 hours and then filtered with the help of Whatman No.1 filter paper. The filtrates/ Extracts of the selected plant material were used for further study. The Extracts are coded as G1 and G2.

G1- Extract prepared from Guduchi leaves

G2- Extract prepared from Guduchi Stem.

The Extracts of the selected plant material were taken and used for further studies. The prepared Extracts subjected to the Preliminary phytochemical screening.

Preliminary Phytochemical Screening of the Guduchi extracts

The Preliminary phytochemical analysis gives primary idea about presence of phytochemicals in the extract**.** The Preliminary phytochemical screening was carried out for both the extract as per standard methods described by Brain & Turner (1975), Evans (1996) and results are given in Table 1.

Detection of Alkaloids

***Mayer's test*:** The extract was added with Dil. HCl and Mayer's reagent, cream colored precipitate formed which indicated the presence of alkaloids. The test results shown positive in both Leaf and Stem Extracts.

***Wagner's test*:** Test for alkoloids by this method found positive for both Leaf and Stem extracts. The extract was added with Dil. HCl and Wagner's reagent, reddish brown precipitate indicates presence of alkaloids

Dragendorff's test: To the extract add dil. HCl and Dragendorff's reagent, reddish brown precipitate indicates presence of alkaloids.

Detction of Flavonoids:

Shinoda test for flavonoids detection: 5 ml of the extract is taken and added few magnesium burnings and concentrated hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue color appears after few min.

Alkaline reagent test: To the extract add few drops of sodium hydroxide solution, intense yellow color is formed which turns to colorless on addition of few drops of dilute acid indicate presence of flavonoids.

***Zinc hydrochloride test*:** To the extract add a mixture of zinc dust and conc.Hcl . It gives red color after few minutes.

Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates that the presence of flavonoids.

Specific chemical tests for tannins

Test for Gallotannins: To the extract add KI solution. Pink color (free gallic acid shows orange).

Test for Ellagitannins: To the extract add acetic acid and conc. HNO3. Pink at first then turns to purple to blue.

Gum& Mucilage: The test found positive for both Leaf and Stem Extracts which indicates presence of Gums and Mucilage in Aqueous extract

Volotile Oils& fats*:* The test showed Negative in both the extracts.

Test for Carbohydrates: Fehling's Test for carbohydrates shown positive in both extracts

Test for Amino acids: Milion's test Ninhydrin's Test for Aminoacids of both the extracts found positive.

Test for Phenolic Compounds

Ferric chloride test: The extract treated with ferric chloride solution if blue color appears that indicates hydrolysable tannins are present and green color appears if condensed tannins are present.

Test for Steroids & Terpenoids:

Libermann-Burchard test: Treat the extract with few drops of acetic anhydride, boil and cool. The concentrated sulphuric acid added from the side of the test tube, brown ring is formed at the junction in two layers and upper layer turns green which shows presence of steroids and formation of deep red color indicates presence of tri-terpenoids.

Salkowaski test: Treat the extract with few drops of concentrated sulphuric acid, red color at lower layer indicates presence of steroids and formation of yellow colored lower layer indicates presence of terpenoids.

Test for Sapponins

Froth formation test: Place 2 ml solution of drug in water in a test tube, shake well, stable froth (foam) is formed if the sapponins positive test.

Haemolysis test for tannins: 0.2 ml of extract added to 0.2 ml of blood in normal saline and mixed well after this centrifuge and note the red supernatant compare with control tube containing 0.2 ml of 10 % blood in normal saline diluted with 0.2 ml of normal saline.

Detection of Glycosides

***Borntrager's test for Glycoside detection*:** Boil the extract with 1 ml of sulphuric acid in a test tube for 5 minutes. Filter while hot. Cool the filtrate and shake with equal volume of dichloromethane or pet ether. Separate the lower layer of dichloromethane or pet ether and shake it with half of its volume of dilute ammonia. A rose pink to red color is produced in the ammoniac layer. The above re-corroborate with the observations found by Dipti *et al* (2013) and Parham *et al* (2013) during the study of Phyto chemical Analysis of *Tinospora cordifolia* stem and leaf extracts.

Table 1. Preliminary Phytochemical Analysis of Guduchi extracts

S.No.	Name of the Test	G1 (Leaf Extract)	G2 (Stem Extract)
I.	**Test for Alkoloids**		
	i). Mayer's test:	+	+
	ii). Wagner's test:	+	+
	iii). Dragendorff's test:	+	+
II.	**Test for Glycosides**		
	i).Borntrager's test for Anthraquinone Glycosides	+	+
	ii). Cardiac gycosides	+	+
III.	**Test for specific Tannins**		
	i). Test for Gallotannins	+	-
	ii). Test for Ellagitannins	+	-
IV.	**Gum& Mucilage**	+	+
V.	**Volotile Oils& fats**	-	-
VI.	**Fixed oils & fats**	-	-
VII.	**Proteins**		
	i).Xanthoproteins	+	+
VIII.	**Carbohydrates**		
	i). Fehling's Test	+	+
IX.	**Amino acids**		

	i).Milion's test	+	-
	ii). Ninhydrin's Test	+	-
X	**Test for Phenolic Compounds**		
	i). Ferric chloride test	+	+
XI.	**Steroids &Terpenoids**	**+**	**-**
XII	**Sapponins**	**+**	**+**
XII	**Test for Flavonoids**	**+**	**+**

*Phytochemical Test: '+' Present, ' –'Absent
*G1-Aqueous Extract of Guduchi Leaves prepared by maceration process.
*G2-Aqueous Extract of Guduchi Stem prepared by maceration process.

Quantitative Analysis of Guduchi Extract

Determination of Total Phenols: The total phenols content is estimated by method of Folin Ciocalteau. 0.5grams of dried powdered Guduchi Stem and leaf are grinded separately grinded in 20ml of 80% ethanol. The homogeneous mass was centrifuged at 10,000 RPM for 20min. The supernatant was transferred to a glass beaker and dried. Then the residue was dissolved in 20ml of distilled water. From this 0.2ml of samples were taken in test tube and volume made up to 3ml with distilled water added 0.5ml of Folin Ciocalteau reagent. Further 2 ml of 20% Na_2CO_3 solution was added after 3 minutes to each tube, mixed thoroughly, placed in boiling water for exactly 1 min, cooled and absorbance was taken at 650 nm against blank. By using different concentration of catechol the standard graph was prepared and the phenols were calculated from the standard graph.

Determination of Total Flavonoids: The total Flavonoids were determined by alluminium chloride colorimetric technique. The samples of both leaf and stem extract weighed 0.5grams and kept 95% ethanol for 24hours. The mixer was filtered and volume was make up to 25ml by using 80% ethanol. From this 0.5ml of filtrate was mixed with 1.5ml of 95 percent Ethanol and 0.1ml of 10% $Alcl_3$, 0.1ml of potassium acetate and 2.8ml water. The tubes have incubated in room temperature for 30minutes and absorbance was measured at 415nm. The flavonoids content of the sample is calculated from the standard graph of quartering.

Determination of Total Alkaloids: 0.5 grams of extract into a 250ml of conical flask and 200ml of 10% acetic acid in ethanol was added. The conical flask covered with lid and kept without disturbing it for 10 hours. This is subjected for filtration process then the extract was subjected to concentration on a water bath to one quarter of the original volume then

concentrated ammonium hydroxide was added drop by drop. Addition of Ammonium hydroxide precipitation takes place till the complete precipitation takes place. The whole solution allowed to settling and the precipitate was collected, washed with dil. Ammonium hydroxide followed by filtration. The remaining residue was collected, dried and weighed for alkaloid content.

Determination of Cardiac Glycosides: 5 grams of sample was taken and 100ml of distilled water added. After mixing well 10grams of Conc. Sulphuric acid (Prediluted with 10ml of Water) was added. It was refluxed for 6-8 hours cooled and extracted with Chloroform (2x25ml). The Chloroform layer has washed with distilled water till it become acid free. Then it was transferred into a pre weighed beaker and dried in an Hot air oven till constant weight.

Percentage of Cardiac Glycosides = (B-A) X 100 X 2 Weight of the sample

Where B=Weight of the beaker with Content

A= Weight of the empty Beaker.

Determination of Saponins: 2grams of sample weighed into a conical flask and 25ml of 20% ethanol was added to it. It was heated over a hot water bath for four hours at 55 º C. The mixture allowed for filtration and the residue subjected to the Re-extraction with another 25ml of 20% ethanol. The combined extracts were reduced to 15 ml over a water bath at 90 º C. The concentrate was transferred into a 250 ml separating. 25 ml diethyl ether was added to it and shaken vigorously While the ether layer discarded the aqueous layer was recovered. The aqueous layer was further separated by 60ml (2 X30) of n-butanol. The combined butanol were washed with 10ml 5% sodium chloride for twice.The extract was then transferred to pre-weighed to beaker and dried in oven to a constant weight.

$$\text{Percentage of Saponins} = \frac{(\text{B-A})\ \text{X}\ 100}{\text{Weight of Sample}}$$

Where, B = Weight of beaker with content, A = Weight of empty beaker.

Addition of Sugar in Whey beverage:

Guduchi extract in both ways is bitter and the bitterness is not acceptable by the all individuals. Hence Sugar is used to mask this bitterness at constant sugar10% level on the basis of whey (Manasi *et al* 2013) which can mask the bitterness.

Addition of Guduchi extract to the Whey at different proportions and Sensory studies:

Guduchi extracts G1 and G2 are added separately to sweetened whey at different levels as Whey: Guduchi extract ratio. The samples were analyzed for physico-chemical parameters

and sensory attributes. Basing on the Hedonic scale the product with high Sensory Score with high acceptability has been selected for further study.

G1A-Whey: Guduchi Leaf extract ratio-90:10 (Guduchi leaf extract at the level of 10%).

G1B-Whey: Guduchi Leaf extract ratio-85:15 (Guduchi leaf extract at the level of 15%).

G1C-Whey: Guduchi Leaf extract ratio-80:20 (Guduchi leaf extract at the level of 20%).

G2A-Whey: Guduchi Stem extract ratio-90:10 (Guduchi Stem extract at the level of 10%).

G2B-Whey: Guduchi Stem extract ratio- 85:15 (Guduchi Stem extract at the level of 15%).

G2C-Whey: Guduchi Stem extract ratio -80:20 (Guduchi Stem extract at the level of 20%).

Sensory evaluation of the Guduchi Whey Beverage to select the Guduchi level:

The samples of Guduchi Whey beverage were evaluated as described by Larmond (1977) for their sensory characteristics namely colour and Appearance, Consistency, Flavour and Overall Acceptability by Hedonic scale. Ten judges (8 male and 2 female) were selected to detect differences among the beverages using the Triangle test (n=30). The samples with highest mean score were selected for further study. The beverage at ratio of Whey: Guduchi 80:10 had highest mean score in both the ways i.e., using Leaf and Stem extract.

According to the Overall acceptability among the all beverages Guduchi Whey Beverage with 10% of Guduchi Leaf Extract i.e., G1A-80(Whey):10(Leaf Extract) and 10% of Guduchi Stem Extract i.e., G2A-80(Whey):10(Stem Extract) are selected for further study. Among these two the Guduchi Whey with Stem Extract (G2A) had highest sensory score of colour &appearance, Consistency, flavor and Overall acceptability.

According to this work and tables given it is clearly indicating that as the Level of Guduchi Extract increased the overall acceptability of the beverage reduced due to intensive bitterness. Further G1 B- 80(Whey):10GSE and G1A- 80(Whey):10GLE scored highest mean values of all the attributes including Overalll acceptability. Hence the Whey Beverage with 10% both Guduchi Extracts selected for further course of study.

Table 2: Effect of level of Guduchi Leaf Extract (GLE) on sensory score of Whey Beverage

Whey : Guduchi leaf extract ratio	Colour& appearance	Cosistency	Flavour	Overall Acceptability
G1A-80:10	8.09	8.02	8.18	8.10
G1B-85:15	7.91	7.98	7.14	7.14
G1C-90:10	6.70	7.98	4.81	4.69

Table 3: Effect of level of Guduchi Stem Extract(GSE) on sensory score of Whey Beverage

Whey : Guduchi Stem extract ratio	Colour& appearance	Cosistency	Flavour	Overall Acceptability
G2A-80:10	8.20	8.15	8.30	8.25
G2B-85:15	8.00	7.98	7.20	7.25
G2C-90:10	6.90	7.98	4.90	4.71

*Average of Five Replicates.

STANDARDISING THE PROCESS PARAMETERS IN THE FORMULATION OF PROBIOTIC GUDUCHI WHEY BEVERAGE.

Optimization of level Probiotic Culture of *L.acidophilus in Guduchi Whey Beverage:* :

The selected Guduchi whey beverage inoculated with Probiotic Starter culture of *Lactobacillus acidophilus* NCDC014 which have the activity of 10^{19}cfu/ml. The Guduchi Whey beverage inoculated with the above said Probiotic culture at different levels as 1.0%, 1.5% and 2.0% to G!A and G2A then incubated at 37º C for 5 hours (Manasi *et al* 2013).

After 5hour of fermentation the beverage samples were analysed for pH, Titratable acidity, Total Viable counts and Sensory characteristics later based on the viable count and sensory characteristics the Probiotic Guduchi Whey Beverage Selected for further study.

STUDY OF THE PHYSICO-CHEMICAL, SENSORY ATTRIBUTES AND MICROBIOLOGICAL QUALITY OF THE FORMULATED BEVERAGE

The Probiotic Guduchi Whey Beverage with 1.5% inoculum scored highest mean Sensory score and viable Probiotic Count is above 10^6 which required for commercial Probiotic beverage. Hence the Probiotic whey beverages with 1.5% inoculum are used for Shelf life study and antioxidant activity.

Table 5. Effect of level of Probiotic Culture on Physico chemical attributes of Guduchi whey beverage prepared with Stem Extract.

Parametrs	1.0% Inoculum	1.5% Inoculum	2.0% Inoculam	Probiotic Whey without Guduchi Extract
pH	4.82	4.76	4.62	4.42
Titratable acidity	0.360	0.415	0.432	0.428
Total Viable Count	$6.5x10^6$	$8.0 x10^7$	$8.5x10^8$	$9.25x10^8$
Colour& appearance	8.10	8.22	8.22	8.20
Cosistency	8.12	8.18	8.00	8.10
Flavour	8.11	8.25	8.10	8.15
Overall Acceptability	8.15	8.28	8.00	8.20

*****Incubation Temperature 37±1 for 5 hours

*Mean of four Replicates/Trials.

The Probiotic Guduchi Whey beverage with 80 (Whey):10(GLE) and 80(Whey): 15(GSE) at 1.5% inoculam are subjected to the Proximate analysis and shelflife studies.

The antioxidant activity of the formulated beverage is carried out by different by in *Vitro* antioxidant models

1). DPPH radical scavenging activity.

2). Reduction Potential Assay (Total Reducing Power).

3). Metal Chelating activity.

THE STORAGE STABILITY OF FORMULATED PROBIOTIC GUDUCHI WHEY BEVERAGE AT LOWER TEMPERATURES

The Guduchi whey beverage with 10% of both G1 and G2 with 2.5% *L.acidophilus* are selected for further study and shelf life studies at room and refrigeration temperatures are carried out The Probiotic Guduchi whey beverage with 10% Guduchi leaves extract (G1A) and 1.5%*L.bacillus* culture is acceptable for 1 month at refrigeration temperature without much change in its sensory characteristics.

Table 6. Analysis of Probiotic Guduchi Whey Beverage with G1 and G2

Parameters	**G1A**			**G2A**		
Culture	***1.5%***	***2.0%***	***2.5%***	***1.5%***	***2.0%***	***2.5%***
Fat%	0.4±0.05	0.4±0.05	0.4±0.05	0.4±0.05	0.4±0.05	0.4±0.05
Protein%	0.853±0.03	0.858±0.03	0.856±0.04	0.855±0.04	0.858±0.03	0.856±0.03
Titratable acidity%	0.38±0.04	0.40±0.05	0.45±0.03	0.32±0.04	0.41±0.05	0. 43±0.03
Ash%	0.418±0.025	0.418±0.028	0.412±0.025	0.416±0.025	0.414±0.028	0.413±0.02
Total solids%	15.80±0.03	15.60±0.03	15.60±0.03	15.82±0.04	15.62±0.06	15.81±0.34
pH	4.60±0.5	4. 60±0.5	4. 58±0.3	4.65±0.4	4. 64±0.5	4. 60±0.5
L.aciiphilus count in MRS agar (CFU/ml)	No colonies in 10^6 But growth observed as $5x10^5$	$1x10^6$	$4x10^6$	No colonies in 10^6 But growth observed as $5x10^5$	No colonies in 10^6 observed as But growth $5x10^5$	$6x10^6$
Sensory characteristics	No bitterness/ Liked very much	No bitterness/ Liked very much	No bitterness/ Liked very much	No bitterness/ Liked very much	No bitterness/ Liked very much	No bitterness/ Liked very much

Antioxidant activity of Probiotic Guduchi Whey Beverage:

Antioxidant activity of the Guduchi whey beverage formulated with G1 (GLE) Guduchi Leaf Extract shown higher than the Beverage prepared with stem the reason may the leaves are

rich flavonoids, alkaloids and glycocides which are prominent antioxidants (Priyanka *et al* 2014) .

Conclusion

The sensory evaluation of the prepared beverage revealed that the product is comparable to the control Probitic whey beverage without any bitterness and can be stored for 30 days at 4±1ºC without changing the sensory attributes. The Developed Probiotic Guduchi Whey Beverage proved the antioxidant activity by different *in vitro* antioxidant models.

References

[1] Brain, K.R., and T.D. Turner, 1975. The practical evaluation of phytopharmaceuticals, Wright- science technical, 1 st Ed, Bristol Britain., 144.

[2] Chavan RS, Shraddha RC, Kumar A, Nalawade T (2015) Whey Based Beverage: Its Functionality, Formulations, Health Benefits and Applications. J Food Process Technol 6:495. doi:10.4172/2157-7110.1000495.

[3] DeptiGawai, Das G, and G.R. Rout (2013), Phytochemical Screening and compative analysis of antimicrobial activity of root and leaf extracts of *Tinospora cordifolia*, *Phyllanthus* niruri and *Abrus recatorious,* important medicinal plants, J.of Medicinal Plant Research &(29):2208-2213, http://www.academicjournals.org/JMPR.

[4] Evans, W.C., 1966. Trease Evans Pharmacognosy., 14 th Ed, London: WB Saunders Ltd, 119- 159.

[5] Jyothi Rani, Lalitha Singh, Holishar Singh, Manish Kapoora and Gagandeep Singh (2015): Priliminary Phytochemical Analysis of Diferent Solvent Extracts From Leaf and Stem of Tinospora Cordifolia, International Journal of Phytotherapy, Vol 5 , issue3-124-128

[6] Kavitha B.T., Shruthi S.D, Padmalatha Rai S and Ramachandra Y.L (2011): Phytochemical Analysis and hepatoprotective properties of Tinospora cordifolia against carbon tetrachorode-induced hepatic damage in rats, J. of Basic and Clinical Pharmacy (2):3,369-142.

[7] Lormond E (1977) Laboratory methods for sensory evaluation of foods, Department of Agriculture. Ottawa, Canada.

[8] Manasi Shukla, Yogesh Kumar Jha and Shemelis Admassu (2013) Development of Probiotic Beverage from Whey and Pinapple Juice, Journal of Food Porcessing & Technology, Vol-4, Issue 2, 1000206. Open access http://dx.doi.org/10.4172/2157-7110.100026.

[9] Pradhan D, Ojha V and PAndey AK, (2013) Phytochemical analysis of *Tinospora cardifolia* (willd) Miers ex.Hook.F & Thoms stem of varied thickness. *Int. J. Pharm Sci Res::*4(8):3051-3056.

[10] Prashanth Tiwari, Bimlesh kumar, Mandeep Kaur, Gurpreeth Kaur, Harleem Kaur (2011), Phytochemical Screening and Extraction:A Review, International Pharmaceutical sciencia, Vol1, Issue 1, P.No 98-106.

[11] Priyanka Mishra, Preya Jamdar, Sharan Desai, Dhara Patel and Dhananjay Mesharam 2014: Phytochemical Analysis and assessment of in vitro antibacterial activity of *Tinospora cordifolia:* 3, pp.224-234.

[12] Ramya Premnath andLaxmidevi N 210: Studies Studies on Antioxidant activity of *Tinospora cordifolia* (Miers.): Journal of Amercan Science 6(10), 736-743.

[13] Sasi Kumar R (2015), Development, Quality Evaluation and Shelf Life Studies of Probiotic Beverages using Whey and Aloe vera Juice, J.Food Process Technol 2015, volume6, Issue 9-1000486.

[14] Toqeer Ahmed, Rashida Kanwal and Najma Ayub (2006) Influence of Temperature on Growth Pattern of Lactococcus lactis, Streptococcus cremoris and Lactobacillus acidophilus Isolated from camel Milk, Biotechnology, 5:481-488.