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Formulation development and real time stability studies of herbal oral liquids containing natural sweetener

Vijay R Salunkhe* and Satish B. Bhise
**Govt. College of Pharmacy, Vidyanagar,Tal. Karad- 415 124, Dist. Satara, (M. S.) India.*

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ABSTRACT

The present research work supports formulation, development of herbal oral liquids containing *Withania somnifera*, *Asparagus racemosus*, *Ipoamea purga*, Glycyrrhiza glabra, *Terminalia chebula*, *Curcuma zedoria*, *Tinospora cordiofolia*, *Cyperus rotundus*, *Tribulus terrestris*, and *Sida cardifolia* as active ingredients and *Stevia rebaudiana* as natural sweetener. Standardization was carried out using applicable parameters like color, odor, general appearance, taste, pH, viscosity, surface tension, clarity, specific gravity and other additional parameters like microbial count, TLC profile, HPTLC fingerprint, determination of heavy toxic metal ions and pesticide residue. Sweetness potency was determined by taste evaluation method. Effect of aqueous extract of natural sweetener in the formulation was determined by standardization. Use of this natural sweetener is most convenient, acceptable and palatable in sweet formulations.

Keywords: Formulation, Standardization, Oral Liquids, *Stevia rebaudiana*.

INTRODUCTION

At present there is an increasing interest in development and evaluation of herbal formulations. However several problems like safety, efficacy and stability are associated with herbal formulations containing synthetic sweeteners[1]. Due to the toxic effect of synthetic sweeteners in many sweet formulations, demand of safety profile from consumer and health care professionals renders the formulation with alternative sources to synthetic sweeteners. Current status of this project orients the designing of herbal formulations with nutraceuticals, which can promote health by providing nutritional supplement and treating diseases. Research into the health benefits of food has been rising during the last five to seven years. Recent scientific research increasingly support[2] that the foods and food ingredients has many potential health benefits. The present research work is associated with development and evaluation of sweet herbal formulation with *Stevia*.

The present research work is aimed at the development of herbal formulation with nutraceuticals by using natural sweetener, which is an alternative to artificial sweetener (as Aspartame, Sucrose, Sodium saccharin, cyclamate). One such plant is *Stevia rebaudiana*[3], native to South America, popularized as sweet herb of Paraguay. Stevia is a wonder drug with several medicinal properties. It has been known as "Madhupatra" in India. The Institute of Himalayan Bioresource Technology (IHBT), Palampur introduced accessions of *Stevia* to evaluate production feasibility of this crop under agro-climatic conditions. This crop can be best cultivated through out India due to high tolerance. The Sweetener in *Stevia* is attributed due to the presence of 10 diterpene glycosides[4] which are water soluble with compounds viz. stevioside and rebaudioside are the major components being 300 times as sweet[5] as Sugar. The mixture of these compounds in the form of white powder is a commercial product. These medicinal plants act as nutraceutical due to presence of vitamins, amino acids, minerals, fats, proteins, iron, fibers etc. The *Stevia* is used[6] in Japan, China, Korea, Brazil and Paraguay in Sweetened dietaries, Soft drinks, coffee and tea. The sweetener is an ideal substitute for the patient suffering from diabetes and /or obesity divided to using cane sugar.

Guidelines[7] suggest approach of physiochemical properties to evaluate stability several studies[8] have indicated that TLC or HPTLC fingerprint serves a highly useful purpose in evaluating the changes in chemical composition during storage. It is also known that changes in TLC/ HPTLC finger print pattern do not necessarily reflect an appreciable change in bioactivity. Manufactures may take another approach of monitoring TLC/ HPTLC finger printing including assays of markers and generate data by determining the bioactivity of bacterial / fungal strains or in vitro bioactivities including those involving cell lines also provide excellent stability data. In fact studying biological activity data using validated *in vitro* methods[9] would be an ideal method, throughout suggested in these guidelines with a view to make the guidelines simple and adoptable by large sections of Ayurvedic industry.

The present research work was carried out for the development of sweet formulations using aqueous extract of *Stevia rebaudiana* and real time stability study by applicable and critical parameters. This research work provides a new scientific data for benefit of industry as well as safe use of this product for the society. Raw Materials used in herbal oral liquids are *Withania somnifera*, *Asparagus racemosus*, *Ipoamea purgra*, *Glycyrrhiza glabra*, *Terminalia chebula*, *Curcuma zedoria*, *Tinospora cordiofolia*, *and Cyperus rotundus*.

Withania somnifera is used as brain tonic and immunomodulatory agents[10]. It has been reported as a health promoter. *Aspararus racemosus* is used as a health tonic[11] and common Indian remedy used a rejuvenator, promoter of strength. *Glycerriza glabra* has been used[12] medicine as memory stimulant and antioxidant. *Tinospora cordiofolia*

***Corresponding author.**
E-mail: vrsalunkhe@rediffmail.com

is one of the most versatile rejuvenating herb [13]. It promotes longevioty hence called *Vayastha*. It protects the body against diseases. *Terminalia chebula* have anticarcinogenic activity, antioxidant activity, adaptogenic [14] and antianaphylactic activity, immunomodulator activity. It is highly regarded as a universal panacea in the Ayurvedic medicine. *Bacopa monnierra* is used [15] as a brain tonic to enhanced memory development, learning and concentration. These plants have also been used as a cardiac tonic and digest. *Curcuma zedoria* has been used [16] as immunomodulatory and antioxidant agents. These crude drugs contain mainly flavonoids and phenolic moieties which are reported to possess antioxidant potency [17]. Crude materials containing all these principles have been generally used for the preparation of *chyawanprash, churna*, granules and herbal tablets. But our intention was to prepare herbal oral liquids by using these active principles which are used as immunomodulatory agents and tonic. Generally Ayurvedic and other herbal market survey reveals that the herbal preparations contains honey on syrup as sweetening agent.

People especially Diabetic consumers, who are consuming these preparations, are less satisfied due to the use of syrup or sugar. Some herbal marketed preparations also contain sugar and other artificial sweeteners. Due to the utilization of carbohydrate and carcinogenic effect of synthetic sweeteners [18], not only diabetic patients but also all of us are trying to get herbal products with low or zero calorie substance. The best alternative is aqueous extract of *Stevia rebaudiana* which acts as antidiabetic [19] and antihyperlipidemic [20], antihypertensive [21] and antioxidant [22]. Different concentrations of aqueous extract of *Stevia* are used as a sweetening agent and stability enhancer in oral liquids in different oral liquid samples.

Materials and Method:

Material:

All these above crude materials were procured from *Ayurvedic Arkshala,* Satara and authenticated by Botanist, Dr. S. K. Patil, Botany Dept., Y. C. College of Science, Karad, Satara.

Method:

Different Samples of herbal oral liquids were prepared with various concentrations of *Stevia rebaudiana* extract as natural sweetener and nutraceutical.

Herbal oral liquids were obtained as per the procedure [23] given in Ayurvedic Pharmacopoeia and Ayurvedic Formulary of India and standardized as per the procedure given in pharmacopoeia for Ayurvedic formulations.

As per the guidelines given in different traditional systems of medicines and as per the modern pharmaceutical practice, oral liquids were obtained by maceration process. The ground plant material is called the macerate. The macerate is soaked in a liquid referred as the menstrum in order to extract the active ingredients. The powdered materials of active ingredient were treated with purified water with occasional shaking at room temperature for seven days.

Aqueous extract of dried leaves of *Stevia rebaudiana* was obtained by cold maceration method [24]. The dried powdered extract of this natural sweetener was used in different concentrations in 5 samples of oral liquids. 0.25%, 0.5%, 0.75%, 1% and 1.25 % aqueous extract of *Stevia* was used in samples respectively.

Standardization was carried out [25] using applicable parameters like color, odor, general appearance, taste, pH, viscosity, surface tension, specific gravity and other additional parameters like microbial count, antioxidant potential, TLC profile, HPTLC fingerprint, determination of heavy toxic metal ions and pesticide residue. Sweetness potency was determined by taste evaluation method.

Five samples of herbal oral liquids were prepared as follows:-

Herbal oral liquid 1:- 500 ml of aqueous filtrate with active ingredient and 0.25% aqueous extracts of *Stevia rebaudiana* as natural sweetener.

Herbal Oral liquid 2:- 500 ml aqueous filtrate with 0.5% *Stevia* extract.

Herbal oral liquid 3:- 500 ml aqueous filtrate with 0.75% *Stevia* extract.

Herbal oral liquid 4:- 500 ml aqueous filtrate with 1% *Stevia* extract.

Herbal oral liquid 5:- 500 ml aqueous filtrate with 1.25% *Stevia* extract.

Different samples of herbal oral liquid containing natural sweetener were stored at $\pm$ 30^{0}C at room temperature. Stability study of herbal oral drug was carried out [26] by following parameters at an internal of 0, 3, 6, 12 18, 24 months and keeping the packs of formulations at 30 $\pm$ 2^0 C and 65% RH.

Determination of pH [27]:

The pH of herbal oral liquids was obtained by potentiometer. The pH method was calibrated using distilled water, buffer (at pH 4 and 9) pH till constant reading.

Determination of Viscosity [28]:

Ostwald viscometer was used to determine the viscosity of all samples of oral liquid. The method was followed as per the standard procedure.

Determination of R. I. [29]:

Abbey's refractometer was used to determine R. I. of oral liquids the method was followed as per the procedure described.

Determination of surface tension [30]:

All the samples were evaluated for surface tension, by stalagnometer. The method was followed as per the procedure mentioned.

Determination of specific gravity [31]:

Pcynometer was used to determine the specific gravity at 25^{0}C. It was determined dividing the weight of sample (expressed in 9ms) by the weight of water (in gm).

Microbiological study [32]:

Different samples of herbal oral liquids were tested for their presence or absence of total viable aerobic count, yeast and mold count, *E. Coli*, *Salmonella*, *S. aureus* and other pathogens.

Total viable aerobic count of oral liquids was done by plate count method as per the WHO guidelines. Petridishes used of 9-10 cm in diameter. To one dish added a mixture of 1ml of the pre treated material and about 15 ml of liquefied casein soybean digest agar at a temperature not exciding 45^{0}C. Alternatively, spread the pretreated material on the surface of the solidified medium in a Petridish. The material was diluted as described above to obtain an expected colony count of not more than 300. Prepared two dishes using the same dilution and incubate them at 30-35^{0}C for 5 days unless a more reliable count is obtained in a shorter period of time. Counted the number of colonies formed and calculated the results using the plate with the largest number of colonies, up to a maximum of 300.

Yeast and mold count was determined by standard method as per the WHO guidelines. Petridishes used of 9-10 cm in diameter. To one dish added a mixture of 1ml of the pre treated material and about 15 ml of

liquified sabroud and glucose agar with antibiotics at a temperature not exceeding 45^{0}C Alternatively, spread the pretreated material on the surface of the solidified medium in a Petridish. Diluted the pre treated material as described above to obtain an expected colony count of not more than 100. Prepared at least two dishes using the same dilution and incubated them at 20-25^{0}C for 5 days, unless a more reliable count is obtained in a shorter period of time. 60 united the number of colonies formed and calculated the results using the dish with not more than 100 colonies.

Microbial study with respect to *E. coli, Salmonella, S. aureous* and *pseudomonas* was carried out by the standard method as per the quality control of herbal product.

Antioxidant activity[33]:

Antioxidant activity of herbal oral liquid was studied by different *in vitro* chemical models as-

DPPH assay:

To 1ml extract of different concentrations, 1ml solution of 0.1mm of DPPH (1, 1-diphenyl -2-picryl hydrazyl) was added. An equal amount of methanol and DPPH solution serve as control. After 20 min of incubation in the dark, absorbance was measured at 517 nm. The experiment was performed in triplicate and the percentage scavenging was calculated. The ascorbic acid was used as a standard.

Iron Chelating activity:

1ml of each extract was treated with an equivalent amount of reaction mixture which contains 1ml , .05 5 0- phenanthroline in treated compound was incubated at ambient temperature for 10 min and the absorbance of same was measured at 510 nm. The experiment was performed in triplicate. The ascorbic acid was used as a standard.

Total antioxidant capacity:

1 ml of extract of different concentrations was treated with 1 ml of reagent solutions (0.6 M sulphuric acid, 28 mm sodium phosphate and 4 mm ammonium molybdate) in eppendorff's tube. The tubes were capped and incubated in thermal block at 95^{0}C for 90 min. After cooling to room temperature the absorbance was measured at 695 nm against blank. The ascorbic acid was used as standard. The experiment was performed in triplicate.

Scavenging of superoxide radial by riboflavin – NBT system.

The assay was based on the capacity of the sample to inhibit blue formation by scavenging the superoxide radicals generated in the riboflavin NBT system. The mixture contains 50 mm phosphate buffer, pH 7.6, 20 gm riboflavin, 12 mm NBT. Reaction was started by illuminating the test samples of the extract. The absorbance was measured at 590 nm. Ascorbic acid was used as positive control. The experiment was performed in triplicate.

Hydroxyl radical scavenging activity:

Extracts of different concentrations were taken in different test tubes and evaporated on water bath. To these, 1ml of fe – EDTA, 0.5 ml of EDTA and 1 ml DM 50 were added and the reaction was initiated by adding 0.5 ml as cupric acid to each to the test tubes. Test tubes were capped tightly and heated on water both at 80^{0}C-90^{0}C for 15 mm. then the reaction was terminated by addition of ice-cold TCA (17.5 % w/v) to the test tubes and kept aside for 5 min. The formaldehyde formed was determined by adding 3 ml Nash reagent (75 gm ammonium acute 3 gm glacial acetic acid, 2 ml acetyl acetone was mixed and raised to 1 liter with distilled water). This reaction mixture was kept aside for 15 min for color development Intensity of yellow color formed was measured spectrophotometrically at 412 nm against reagent blank. The experiment was performed in triplicate.

Scavenging of nitric oxide radical:

Nitric oxide was generated from sodium nitroprusside and measured by Griss reagent as described previously 19, 20. Sodium nitroprusside (5 mm) in Standard phosphate buffer saline solution (0.025m, pH 7.4) was incubated with different concentrations of (200 mg/ml, 300 mg, 500 mg/ml) of the samples of oral liquid were dissolved in phosphate buffer saline (9.925 m, pH 7.4) and the tubes were incubated at 25^{0}C for 5 hrs. Control experiments without test compounds but with equivalent amount of buffer were conducted in identical manner. After 5 hrs, 0.5 ml of solution was removed and diluted with 0.5 ml of Griss reagent (1% napthyl ethylene diamine dihydrochloride. The absorbance of chromophore founded during diazotization of and its subsequent coupling with napthyl ethylene diamine was read at 546 nm. The experiment was performed in triplicate.

Rapid screening for antioxidant compounds by using TLC.

To make a semi quantitative visualization possible, samples of oral liquids were applied on a TLC plate and developed in solvent system consisting of ethyl acetate: formic acid: glacial acetic acid water (14.28 :1.42:1.42:2.85 v/v/v/v). The plate was then dipped in a 0.2% solution of DPPH in ethanol. The yellow colored spots on stationary phase are an indirect measure of antioxidant activity. The experiment was performed in triplicate.

TLC Profile[34]:

TLC plates were developed by using mobile phase as toluene: ethyl acetate: formic acid: ethanol (6: 4: 0.3: 0.4) that shows good resolution for standard Gallic acid. Chloroform: methanol: water (85: 25: 4) solvent system was developed for identification of stevioside in S*tevia* extract. One dimensional thin layer chromatography was performed with and without chamber saturation. Spot was observed when visualized by iodine vapours. and stevioside as well as samples. Rf value was calculated by the ratio of the distance traveled by the spot to the distance traveled by the solvent i.e. Rf = a/b

HPTLC Profile[35]:

Much of the crude materials used in herbal oral liquid contain Gallic acid and Quercetin as chemical markers. Therefore Gallic acid and Quercetin were used as standards for HPTLC determination. A simple, sensitive HPTLC method developed for the analysis of Gallic acid, Quercetin which are present in herbal oral liquid formulation. Apparatus used was Camag Linomat IV- semiautomatic sampler applicator, Camag TLC Scanner III, CATS software (V 3.15, Camag).

Stationary Phase used was pre coated silica Gel 60 F_{254}. The developed mobile phase was ethyl acetate: formic acid: glacial acetic acid water (14.28: 1.42: 1.42: 2.85 v/v/v/v). Methanolic extracts of 30, 60, 90,120,150 and 180 µg/ml of oral liquids were prepared by diluting with methanol. The same concentrations of Gallic acid and Quercetin were prepared as a standard. Microliter of standard solution of mixture (Gallic acid and Quercetin) was applied on prey coated TLC. Silica gel 60 F_{254} plates using a Camag Linomat IV sample applicator. The plate was developed with a twin trough chamber to a distance of 10 cm. After removal from the chamber the plate was dried in air for 15 min, was scanned and quantified at 540 nm using a Camag TLC Scanner III. Data of peak area of each bond was recorded. Standard curve for Gallic acid in the range of µg/ml and Quercetin in the range of microgram per ml was generated by plotting the peak area against concen

Table 1. Real time stability data of herbal oral liquids containing natural sweeteners.

Sr. no.	Parameters	Observations including percentage content and values					
		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Mean ± S.D
1	Color	Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown	-
2	Odor	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	-
3	Taste	Slightly sweet	Just sweet	Sweet	Suitable sweet	More sweet	-
4	General Appearance	Clear Liquid	Clear Liquid	Clear Liquid	Clear Liquid	Clear Liquid	-
5	pH	5.2	5.3	5.2	5.4	5.3	5.28 ± 0.032
6	Viscosity	1.08	1.04	1.06	1.08	1.07	1.066± 0.0064
7	Tension	138.14	138.12	137.97	137.95	138.05	138.0± 0.032
8	Specific gravity		1.33	1.28	1.28	1.32	1.32 1.30± 0.11
9	Refractive Index		1.523	1.523	1.521	1.521	1.523 1.522± 0.0005
10.	Microbial count						
	Total aerobic plate count		4400 CFU/g	4392 CFU/g	4241 CFU/g	4198 CFU/g	4112 CFU/g 4268.6±50.96
	Yeast count	10 CFU/g	9 CFU/g	8 CFU/g	7 CFU/g	6 CFU/g	8 ± 0.6
	E. coli	Absent	Absent	Absent	Absent	Absent	-
	Salmonella	Absent	Absent	Absent	Absent	Absent	-
	S. aureus	Absent	Absent	Absent	Absent	Absent	-
	P. aruginosa	Absent	Absent	Absent	Absent	Absent	-

Fig. 1. Chromatogram of standard Gallic acid throughout the period of stabilitv.

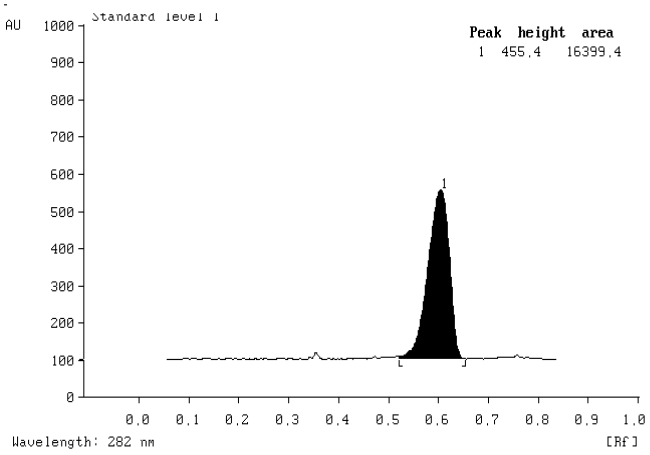


Fig. 2. Chromatogram of standard Quercetin throughout the period of stability.

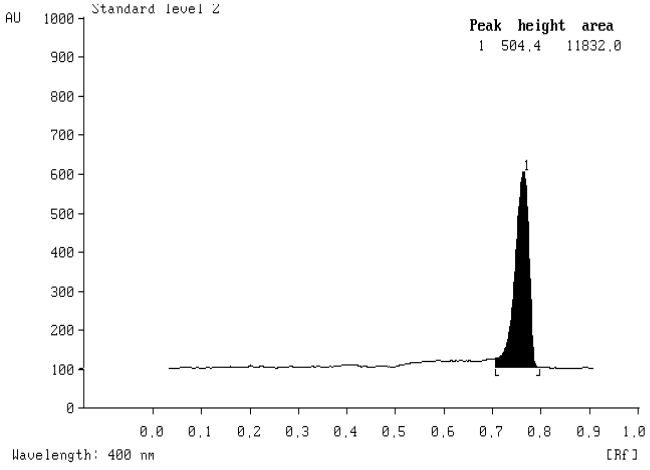


Fig. 3. HPTLC fingerprint of herbal oral liquid indicating stability at an interval of 0 month.

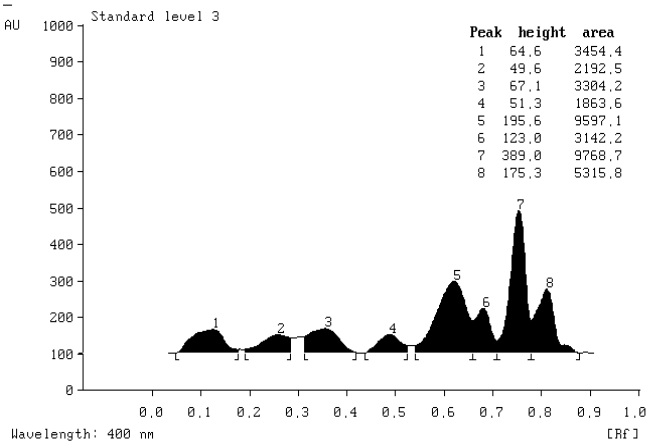


Fig. 4. HPTLC fingerprint of herbal oral liquid indicating stability at an interval of 3 month.

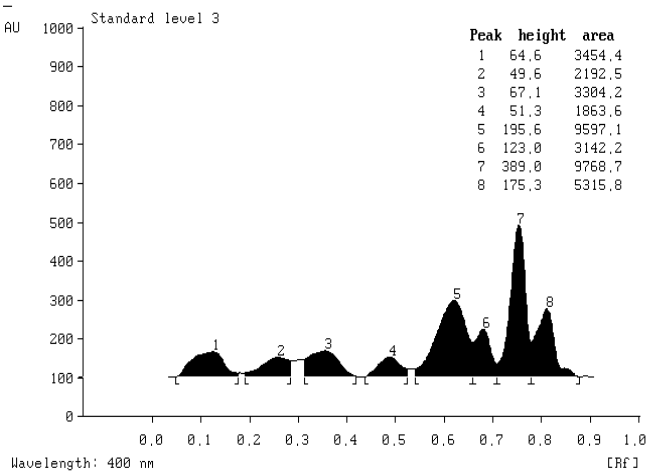


Fig. 5. HPTLC fingerprint of herbal oral liquid indicating stability at an interval of 6 month.

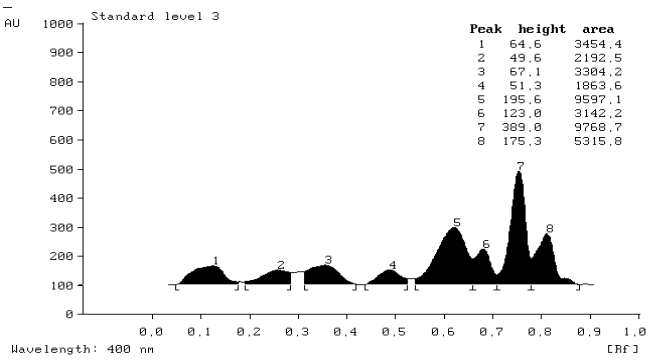


Fig. 6. HPTLC fingerprint of herbal oral liquid indicating stability at an interval of 12 month.

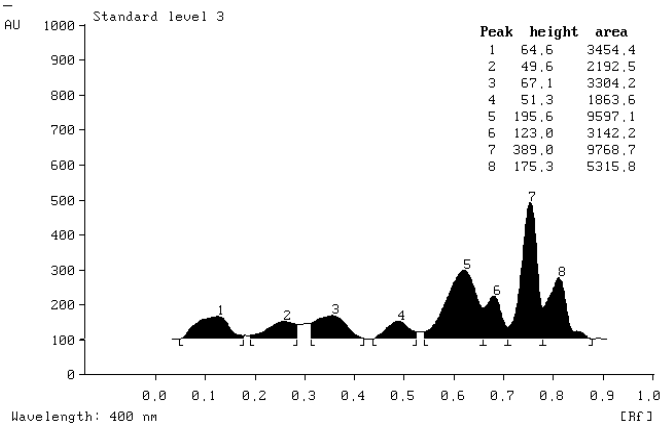


Fig. 7. HPTLC fingerprint of herbal oral liquid indicating stability at an interval of 18 month.

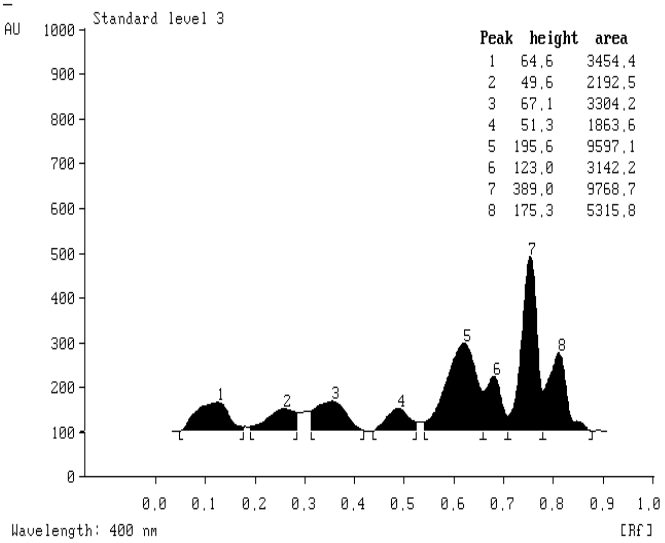
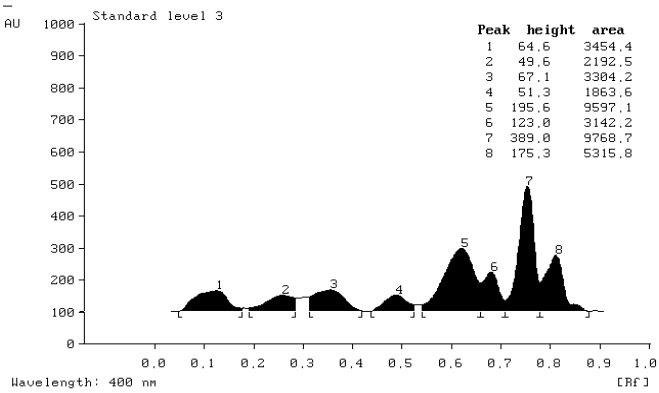


Fig. 8. HPTLC fingerprint of herbal oral liquid indicating stability at an interval of 24 month.



tration of standards.

Developed oral liquid sample was extracted with methanol. The extract was filtered through Whattman filter paper no 41 and again the residue was washed with 10ml of methanol. The extract and washing were transferred to a 100 ml volumetric flash and the volume was made up to 100 ml with methanol. Five microliter from the sample solution was spotted in triplicate on a prey coated silica gel G 60 F_{254} TLC plates. The plate was developed as the same procedure and standard and the peak areas were recorded. Amount of Gallic acid, Quercetin in the foundation was estimated using the calibration curve for standards.

Sweetness potency[36]:-

Sweetness potency of oral liquids containing natural sweetener was evaluated by taste evaluation method. The healthy human volunteers of age 25 to 35 years were used for taste masking and informed consent was obtained from all of them. It was determined by a panel of ten experts. The sample equivalent to normal dose was held in a mouth for 10 sec by movements of the tongue and swallowed. Volunteers opinion for sweetness was considered and the potency was calculated by using the sweetness of sucrose as a standard.

Qualitative analysis [37] for the detection of heavy toxic metal ion (As, Pb, Hg, Cd) and pesticide residues (Chloride and phosphates) was carried out.

Results and Discussion:

Five samples of 500 ml of herbal oral liquids developed with various concentrations of *Stevia rebaudiana*, studied throughout the period by its applicable parameters and it reveals that there was no any major change in values as shown in table 1 that proves a good stability.

The yellowish brown colored oral liquids with pleasant odor having appropriate sweet taste were obtained. A clear solution was obtained and passes through an appropriate filter.

The pH value of herbal oral liquids was obtained by potentiometer. The pH of all samples was in the range of 6.2 – 6.5. Refractive Index was in the range of 1.25 – 1.28. The specific gravity of all the samples was in the range of 1.35 to 1.55. The viscosity of herbal oral liquids when tested by Ostwald viscometer was in the range of 1.04 to 1.08 cps throughout the period of stability. Surface tension of all samples of herbal oral liquids was in the range of 137.95 to 138.14. Real time stability studies at an interval of 0, 3, 6, 12, 18 and 24 months highlights the values with a negligible difference of ± 0.05% that are shown

in table no. 1.

Antioxidant potential of bitter and sweet herbal oral liquid was studied in terms of percentage scavenging by oral liquid and IC_{50} values also determined. The maximum percentage scavenging of each concentration of extract is shown by DPPH assay and Riboflavin – NBT system as compare to other *in vitro* modules. The maximum inhibitory concentration (IC_{50}) in all models namely viz. DPPH assay, Iron chelating activity, Total antioxidant activity, Riboflavin-NBT system, Hydroxyl radical scavenging activity, Nitric Oxide radical activity was found to be12.5, 12.8, 11.9, 13.2, 12.6 & 12.8 by bitter herbal oral liquid while 11.13, 9.62, 10.31 , 12.5, 8.4 & 9.66 by sweet herbal oral liquids.

The IC_{50} was calculated using formula-

$b = Sx . y / Sx^2$.

$A = y-bx$

$IC_{50} = a + b (50)$

Where b = regression coefficient of x on g, a = Intercept of the line, x mean of scavenging. X mean of concentration,Y mean of scavenging

The sample showed better activity in quenching nitric oxide radicals with an IC_{50} value 9.64, 10.01 µg/ml and DPPH radicals with an IC_{50} value of 11.12, 11.42 µg/ml . However the extract also showed encouraging responses in generating superoixde with IC_{50} value of 12.95, 12.41 µg/ml .The activity was moderate in remaining antioxidant models. The results showed that sweet herbal oral liquid has a potent scavenging activity with increasing percentage inhibition. Heavy toxic metal ions (As, Pb, Hg, and Cd) and pesticide residues (Chloride & Phosphates) were totally absent.

TLC profile of sweet Herbal oral liquid was studied by using silica gel G activated on glass plate as a stationary phase and toluene: ethyl acetate: formic acid: ethanol (6: 4: 0.3: 0.4) as a mobile phase. Rf values of standard Gallic acid, Quercetin and stevioside were 0.6, 0.7 and 0.2 respectively. The same Rf values of different sweet herbal oral liquids were detected which are significant[38] comparable to that of standard. This TLC profile of different sweet herbal oral liquids indicates the presence of active phytoconsitituents through out the period of stability. This chromatographical aspects pointed out that when the herbal preparations are formulated with stevia as a natural adjuvant then it can not affect or treat with other constituents so as to chemical degradation. The active marker remains stable.

Qualitative HPTLC analysis of different sweet herbal oral liquid shows the same Rf values with that of standard Gallic acid and Quercetin and UV spectra at 254 nm when scanned denistometrically as shown in figure no. 1 to 8. The identity of the Gallic acid bands in sample chromatograms was confirmed by the chromatogram obtained from the sample with that obtained from the reference standard solution and by comparing retention factors of Gallic acid from sample and standard solutions. The peak corresponding to Gallic acid from the sample solution had same retention factor as that from the Gallic acid standard (Rf 0.6).

This fingerprint reveals that the sample contains active chemical entities throughout the period of stability and no degradation of chemical composition takes place within the period.

Different types of oral liquids containing crude drugs as active ingredients are available in market. Many sweet products contain honey, sugar or syrup as sweetener. Due to high caloric substance, there is problem of safety and efficacy of final product. The present research project supported a scientific data about effectiveness of oral liquids containing *Stevia* as natural sweetener. From the result, it reveals that the developed products are stable for certain period as there was no any major change in values when calculated by applicable physicochemical parameters. Due to the use of aqueous extract of *Stevia* on oral liquids, antioxidant potential of final product was increased. Microbial study indicates that the total aerobic count is within limit of standard and harmful microbes were absent. As*Stevia* act as antibacterial & antifungal, use of such natural sweetener is versatile adjustment for development the sweet product.

Real time stability at an interval of a 3, 6, 12, 18 and 24 month highlight the values by different parameters with a negligible difference of ± 0.05% that are nearly constant throughout the period as shown in table 1.

Formulation development of any sweet herbal products with *Stevia* has better advantage with regard to stability in terms of microbial flora. There will be no any harmful effect in final product on addition of *Stevia* as a sweetener. Absence of heavy toxic metal ions and pesticide residue indicates the products are safe from toxic effects. Antioxidant potentials in terms of percentage scavenging by the extract of herbal oral liquid is an indication of presence of phenolic nuclei throughout the period of stability. Use of *Stevia* in sweet products may enhance and antioxidant potential. Sweetness potency of herbal oral liquids remains appropriate and sweet product within the period. Bitterness value of herbal oral liquid was totally diminished by the use of *Stevia* extract. In overall stability study, it indicates that the formulations have no any harmful or objectionable effect with various concentrations of aqueous extract of *Stevia*. This natural sweetener has a better impact on quality and stability of final product.

Conclusion:

Although all types of herbal products are effective, there is need to formulate or modify such products with adjutants or excipients. There is demand by diabetic consumers to get sweet product with low calorie or zero calorie sweetener. One of the best alternatives to sugar, sucrose or artificial sweetener is*Stevia rebaudiana*. From the present research work it is concluded that the *Stevia* has no any harmful effect to the final sweet product when standardized by different applicable and additional parameters. In future herbal formulations with *Stevia* will a valuable boon for all of us.

Stevia is one of the best alternatives in sweet product as it acts as antidiabetic, antihyertensive, antihyperlipidemic, antioxidant, antibacterial and antifungal. It provides a good stability for at least two years. It may act as stability enhancer. As it is more potent in sweetness, very low amount of such extracts could give a most acceptable, elegant and palatable sweet product. There will be commercial impact to industry if use of *Stevia* in formulation brought into focus and hence the final product will be appropriate sweet and cost effective to all consumers.

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