Topical Gel Formulation Incorporated with Hydroalcoholic Extract of *Stevia rebaudiana* Bertoni

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Abstract: **Herbal cosmeceuticals are more acceptable due to lower side effects from ages. Herb like *Stevia rebaudiana* Bertoni. is widely utilized as natural sweetener and recently extending applications in cosmeceutical industry due to its antioxidant characteristics which is acclaimed for antiaging properties. The objective of present investigation is to develop a topical gel formulation containing Stevia leaf hydroalcoholic extract. As gel provides more penetration than other conventional topical formulations, this antioxidant gel can help to achieve maximum penetration of antioxidants along with improved patient compliance. Steviosides and rebaudiosides the active ingredients of Stevia acting as natural sweeteners, also shows antioxidant activity due to presence of secondary phytoconstituents such as flavonoid's, volatile oils, diterpenes etc. Considering this benefit of stevia, antioxidant gel formulation was developed, prepared in 4 batches (F1 to F4) using different concentrations of polymers (HPMC and Carbopol) along with Acacia as a natural viscosity modifier. The prepared batches were evaluated based on various parameters and optimum batch was identified. F2 seem to be optimum batch as it shown high stable content of stevioside and rebaudioside and was well fitted in other pharmaceutical parameters indicating effective use of stevia extract in gel system which may be a promising formulation.**

Keywords: ***Stevia rebaudiana* Bertoni, Steviosides, Rebaudiosides, Sweetening agents, Topical Gel, Antioxidants, Antioxidant gel, Antiaging gel.**

1. INTRODUCTION

The skin is continuously exposed to a combination of environmental insults including UV or visible radiation and high oxygen concentration, which constantly expose to risk the integrity of cellular oxidizable structures of the skin [1]. Sun exposure has been linked to several types of skin damage including sun burn, photo immunosuppression, photo aging and photo carcinogenesis. The increasing awareness of the potentially detrimental long term side effects of chronic solar irradiation there is a general need for safe and effective photo protectants. [1][2] The beneficial effect of topical antioxidants has been suggested [1], which might be a successful strategy for declining UV radiation-mediated photo oxidative damage of the skin [3][4] Topical administration of enzymatic and non-enzymatic antioxidants represent an effective strategy as protectant for skin against UV mediated-photo oxidative damage, because it may counter react the overly produced oxidants and enrich the depleted antioxidant systems [1][5].

Stevia (*Stevia rebaudiana* Bertoni) is a perennial shrub also known as honey leaf, candy leaf or sweet leaf shown in Fig.1 and its sweet taste is due to the presence of steviol glycosides, having 100–300 times the sweetness of sucrose which belongs to the Compositae family. Native to South America, it is now cultivated in many regions of the world including Asia, Europe and North America. [6]

It has been documented that Stevia leaves contains a combination of complex phytoconstituents such as sweet diterpene glycosides, including stevioside, rebaudiosides (A, B, C, D, and E), steviolbioside and dulcoside A [7]. The dry extract obtained from stevia leaves contains sweet diterpene glycosides, water-soluble chlorophylls and xanthophylls, flavonoids, hydroxynnamic acid (caffeic, and chlorogenic, amongst others), neutral water-soluble oligosaccharides, lipids, free sugars, amino acids, essential oils, and trace elements. Major content in Stevia is steviol glycosides, e.g., stevioside and rebaudioside A shown in Fig. 2. are both very stable molecules in aqueous solution in a broad range of temperatures and pH; with high thermostability of Steviosides, which has encouraged the global commercialisation of stevia. [8]

It has been found that stevia plant possesses antioxidant properties which is due to the presence of high concentration of bioactive phytoconstituents such as phenolic compounds, tannins, flavonoids and vitamin C amongst others. [8] It was reported that the glycol-aqueous extract contained the highest number of phenols and flavonoids and together with the ethanolic extract, it showed highest antioxidant activity against the DPPH and radicals. These results show the potential of stevia extracts in its use as a source of natural antioxidants in the cosmetic and food industries. [6]

Gels are considerably a dilute cross-linked system, which exhibits no flow when it is, in the steady-state. Gels are getting more popular topical drug delivery dosage form nowadays because they are more stable and also can provide controlled release than other semisolid preparations like creams, ointments, pastes, etc. with the advantages like of better absorption characteristics, increasing the bioavailability of the incorporated drug, stability over extended period of time, washability etc. [9].

Hence, Stevia hydroalcoholic extract incorporation in the topical gel formulation was attempted and evaluated in the present research work. HPMC, Carbopol 934p, was used as hydrophilic polymers along with Acacia as a natural viscosity modifier in gel drug delivery system in this study.

2. MATERIAL AND METHOD

Material

Dried leaves of *Stevia rebaudiana* Bertoni were procured from the Arboreal Bio-innovations Private Limited, India. These leaves were previously handpicked, sundried, cleaned, graded, quality tested and hygienically packed and delivered by the manufacturer. These leaves were powdered and further used for extraction and evaluation processes. All the chemicals used for formulation and evaluation were Lab Grade.

Extraction Method

Dried leaves of *Stevia rebaudiana* Bertoni. were procured and powdered. Then weighted amount of powdered dried leaves (50 g) were macerated with water and ethanol in the ratio 3:1 [10]. After 24 h the extract is then filtered and dried by evaporating solvent by using rotary evaporator. The dried extract was used for formulating gel and evaluation process [10][11].

Preliminary Phytochemical Screening

Phytochemical screening test of the Stevia leaf extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites such as steroidal compounds, flavonoids, saponnins, tannins and anthraquinines, carbonyl group, phenolic group, acetyl group, unsaturation using standard procedure [5][6][7][12][13].

Phenol and Total Flavonoid Content Determination [12][13]

The study was carried out for evaluating the total phenols and flavonoids content in extract prepared from dried *Stevia rebaudiana* Bertoni leaves. Total flavonoid content was determined from extract (0.5 ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% $AlCl_3$ (Aluminium chloride), 0.1 ml of 1 M CH_3CO_2K (Potassium acetate) and 2.8 ml of distilled water. It was allowed to stand at room temperature for half hour. Then the spectrophotometer was used to record the UV absorbance of the mixture. Total phenolic content was expressed regarding gallic acid equivalent (1mg of dry mass), which is a common marker. Each plant extract (0.5 ml of 1:10 g ml -1) or gallic acid (Marker phenolic Compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml, 1 M). Then the mixtures were allowed to stand for 15 min, and the total phenolic content in the extract were determined by spectrophotometer [10].

Antioxidant assay by using DPPH free radical method [12]

The stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for the determination of free radical-scavenging activity of the extracts [14]. An equal volume of different concentrations of each herbal extract were added, to a methanolic solution of DPPH. After 15 min, the absorbance was recorded at 502 nm at room temperature. Ascorbic acid (Vitamin C) was used as standard controls. The concentration of the sample, which is required to scavenge 50% of DPPH free radicals was denoted as IC50 values [10]. This activity is given as the % DPPH radical scavenged, which is calculated with the equation:

DPPH radical scavenging activity (%) = [(Abs control – Abs sample)/ (Abs control)] × 100%

Physicochemical evaluation of dried powdered leaves and extract

pH: The pH of extract was recorded using pH meter.

% LOD: It was calculated by using standard procedure for % loss on drying. Weighted sample of dried leaf of *Stevia rebaudiana* Bertoni was taken and known amount of moisture was added to it and allowed to dry at specific temperature. The weight of the sample was recorded after every 5 mins till it shows the constant reading [15]. The % loss on drying was calculated by the given formula:

% Loss on drying = (Weight of water in sample /Total weight of sample) x 100

Total Ash value: Determination of Total Ash Value was carried out by weighing 3 g of the dried Stevia leaves in the crucible. Then the sample was incinerated using muffle furnace set at a temperature 450°C. Cool the crucible at room temperature. Weigh the crucible and calculate the total ash value [15].

Moisture content: Total moisture content was determined by accurately weighing the empty evaporating dish initially. Then 10 g of the powdered Stevia leaves were accurately weighed and was placed in evaporating dish. It was placed in the oven at temperature 105±1°C. Continue repeating the process of weighing and drying at 1 h interval until the difference between two constructive weighing is less than or equal to 1 mg normally. 5 h are sufficient. Cool the evaporating dish. Record the constant weight of the dish and sample [15].

Solvent Extractive Value: 5 g of powdered Stevia leaves were weighed and macerated with 100 ml of water and alcohol and mixed it gently for 3 h. Stopper the flask and allow it to stand for 24 h. The mixture was filtered by Whatman filter paper No 1. The filtrate was transfer to evaporating dish and evaporated over boiling water bath and dry at 105°C to constant weight. Later the residue was weighed carefully and values were recorded.

TLC: TLC of extract is performed by 2 using 2 solvent systems.

Solvent system 1

TLC plate used in this study was prepared by coating it with a thin layer of a solid adsorbent (silica gel-60 for TLC). The crude extracts were applied 1.0 cm above from the lower edge of the activated plate coated with silica gel. It was then placed within a solvent in a developing chamber so that only the base of the plate is in the solvent. Chloroform: methanol: water (6:2.5:1.5) has

taken as a solvent. When the solvent (mobile phase) reached to the top of the silica plate, the plate was removed from the chamber. The developed plates were air dried and the spots were identified. The Rf values were calculated [16].

Solvent system 2

The Thin Layer Chromatography (TLC) was performed using mobile phase i.e., Ethyl acetate: methanol: water (7.5: 1.5: 10) [17]

FTIR:

Fourier Transform Infrared Spectroscopy (FT-IR) was performed to detect the active functional groups in the extract by Infrared spectrophotometer (SHIMADZU IR Affinity – 1S.) (KBr pellet). Before obtaining the IR spectrum, the dried samples grind with potassium bromide and 300 kg/cm2 pressure was applied to the mixture to form a pallet. The KBr flacks were used to develop the spectrum over a range of 4,000–400 cm−1. Software connected to the equipment converted the readings into IR Spectra. [18]

HPLC:

HPLC of ethanolic extract obtained from dried leaves of *Stevia rebaudiana* Bertoni. was carried out by using Agilent 1260 Infinity LC and further following a described procedure [17][19].

Formulation Development:

Polymers (Carbopol and HPMC, both taken in a different concentrations) and purified water along with Acacia were taken in a beaker and allowed to soak till 24 h for swelling. To this required amount of drug was dispersed in water and then the mixture of polymer was neutralized with sufficient quantity of Triethanolamine. After that glycerol (moistening agent), along with methyl paraben and propyl paraben (preservatives) were added slowly with continuous gentle stirring until a homogenous clear gel was formed [19]. 4 batches of gel formulation were prepared using different concentration of Carbopol, HPMC and Acacia as shown in Table 1.

Evaluation of Gel Formulation

Formulations were evaluated for physiological parameters like appearance, colour, odour, clarity, presence of particulate matter, homogeneity etc. Skin irritation was evaluated using group of healthy volunteers. Gel was applied on area of 2 cm of these volunteers for 6 h and observed irritability.

pH: The pH of gel was determined with the pH meter. 2 g of each batch was stirred in distilled water till a uniform suspension was formed and make up the volume up to 40 ml. pH of the gel solution was measured using pH meter. The pH of topical gel should be between 3 – 9 for treating skin infections.

Percentage Yield: The weight of an empty container (W1) and the weight with gel formulation (W2) was recorded. Subtract W1 with W2 then it gives the practical yield. Then the % yield was calculated by the following formula:

Percentage Yield = (Practical Yield / Theoretical Yield) × 100

Viscosity: Gel viscosity was measured with Brookfield viscometer with L series spindle in combination with Heli path stand. The L3 and L4 series spindle was used for measuring the viscosity of gel. The factors affecting the viscosity of gel like temperature, pressure and sample size, etc. was maintained during the process. The average reading taken in 1 min was noted as viscosity of gel.

Spreadability: For estimation of spreadability, 1 g of gel was accurately weighed and was retained between two glass slides of 8 cm in length. Weight was tied to the pulley and weight at which the glass slide relocates from its original position and the time required to pull the upper slide and lead to further extension of gel to the lower slide was recorded. The measurement was noted thrice and lastly the readings for spreadability were measured from the formula:

$$S = M \times L/T$$

where S, M, L and T stands for spreadability for ethosomal gel, weight tied to the upper slide (g), distance moved by the slide (cm) and time taken by the upper slide to move downwards (s) respectively.

Extrudability: Extrudability was preceded by packing 20 g of formulated Stevia gel in collapsible tube and further pressure was applied normally. Clamp was attached to avoid the back flow of the gel through the tube. At the end, the amount of gel extruded out from the tube till the continue pressure was measured and recorded. [18]

Entrapment Efficiency [18][21]

Entrapment efficiency (% EE) is calculated so as to determine the quantity of drug entrapped within the colloidal system. Initially small amount of gel formulation was taken in Eppendorf tube and then it was subjected to centrifugation at a speed of 14,000 rpm at 4 °C for 15 min. The process was repeatedly until and unless clear supernatant was obtained by aid of ultracentrifuge (TDL 60B) which is furnished with TLA-45 rotor. The clear supernatant was obtained and the concentration of drug was measured with the help of UV-visible spectrophotometer at wavelength of 220-240 nm for ultimate result, further quantity of drug entrapped in the colloidal system, was calculated though following formula. Each sample was estimated in triplicate.

Finally, the EE was calculated by the following formula:

EE (%) =Amount of Stevia extract in sediment / Total amount of stevia Extract added×100.

Accelerated Stability Studies: Accelerated stability study of the topical Antioxidant gel was performed as per ICH guidelines. The formulated gels were filled in the collapsible tubes and stored for 3 months and the parameters like Color, Appearance,

Homogeneity, pH were checked and studied at different temperatures and humidity conditions which are, 250 C ± 20 C/ 60% ± 5% RH, 300 C ± 20 C/ 65% ± 5% RH, 400 C ± 20 C/ 75% ± 5% RH [6].

3. RESULTS AND DISCUSSION

Phenol and total Flavonoid content

It has been recorded that secondary phytoconstituents like flavonoids show significant antioxidant activity and their effects on human nutrition and health. From recent studies flavonoids and phenolic compounds of Stevia have been used for their antioxidant properties. The mechanism by which the flavonoids act is by scavenging or by chelating process. Phenolic compounds fall under the class of antioxidant agents, these act as free radical terminators. The content of total phenolic extracts expressed as mg of gallic acid equivalents/gm of extracted Stevia leaves, was noted respectively 24,786 — the number of flavonoids in analysed Stevia extract 289,954 mg Qu/g of extracted Stevia leaves. The hydroxyls, which are present in Flavonoids are responsible for the radical scavenging effect in the plants.

Antioxidant assay by using DPPH free radical method

The results of the antioxidant activity of leaf extract by testing using DPPH were shown in Table 2. These results indicate that vitamin C has lower antioxidant activity with 11.15 µg / ml compared to extracts of 47.58 µg / ml. The extracts were found to be more active compared to the vitamin C. The scavenging activity of Antioxidants plays an important role in the management of the diseases. DPPH radical method is a very simple, rapid and sensitive to check the antioxidant activity of a selected phytocompound or plant extracts. For the evaluation of radical scavenging activity of antioxidants DPPH assay is considered as an accurate and economical method as the radical compound is stable.

Physio Chemical Evaluation

Physicochemical properties of dried leaf extract of *Stevia rebaudiana* Bertoni. was evaluated on different parameters and results were recorded and given in Table 3.

Preliminary Phytochemical Screening

The Extract as well as gel formulations shown positive results shown in Table 4 for presence of secondary phytoconstituents like flavonoids, saponnins, tannins and anthraquinines, carbonyl group, phenolic group, acetyl group, unsaturation.

TLC:

The result of TLC is given in Fig. 3 and Fig. 4 below, where the separated compounds are shown using 2 different solvent systems. TLC was performed and Rf values of the separated compounds were calculated. The result of TLC is given in Table 5 and Table 6 below.

The Rf values of the separated compounds by using Solvent system 1 were found out to be 0.52 and 0.67 and the Rf values of compounds separated by using Solvent system 2 are 0.85 and 0.91. The data obtained from TLC shows the band of compounds separated. This was confirmed by comparing the Rf value of Standard with that of the sample.

FTIR:

The results of recorded IR transmittance of the dried leaf extract of *Stevia rebaudiana* Bertoni and IR Transmittance of Antioxidant Gel containing *Stevia rebaudiana* Bertoni shown in Fig. 5 and Fig. 6, respectively. The Presence of Stevioside and rebaudioside is clearly visible in the IR spectra of stevia gel formulations but more precisely observed in F2 formulation showing no compatibility issues.

HPLC:

HPLC chromatogram of *Stevia rebaudiana* Bertoni leaf extract was recorded. The result is given in the Fig. 7.

The HPLC analysis of the extract obtained from leaf of *Stevia rebaudiana* Bertoni was performed on Agilent 1260 Infinity LC. The main peaks of Steviosides and Rebaudioside A were detected at 205 nm at retention time 3.0 min and 3.5 min respectively.

Evaluation results of Antioxidant Gel

Four formulation batches were prepared by using different concentrations of polymer. F2 found out to be the optimum batch giving high yield and complying other evaluating parameters. The prepared batches were shown in Fig. 8. Evaluation of Gel was done and the results were given in Table 7 and Table 8, respectively:

From the study, it was found out that, F1 formulation shows satisfactory results but, F2 formulation shows the best optimum results, complying with all the parameters. Moreover, due to addition of Acacia in F1 and F2 along with the both polymers provided the required viscosity to the gel. On the other side, F3 and F4 shows the average results as HPMC is the only polymer used in these formulations, thus, has less viscosity.

Spreadability And Extrudability:

Spreadability indicated increase from F1 towards F4 but the extrudability of F2 was found excellent as per gel formulations are considered. The results showed that the F2 formulation is very much suitable for topical application.

Entrapment Efficiency:
Entrapment efficiency is one of the essential specifications, as it informs about the delivery capacity of the build-up formulation. The value of entrapment efficiency of different gel formulation shown as F2 > F1>F3 > F4 in this order maximum entrapment in F2 formulation. It is illustrated in Fig.9. It may be due to increase in the concentration of acacia.

Accelerated Stability studies:
The capacity of gel system to cling with the drug was estimated though accelerated stability studies. The major issues with that of gel is drainage and aggregation of drug in the lipid bilayer. Accelerated Stability Studies were performed and results were recorded at initial month and after 3 months at different conditions. The optimized formulation F2 was scrutinized for stability testing and the outcomes were listed in Table 9, Table 10, Table 11, Table 12 and graphically represented in Fig. 10 & Fig. 11.

4. CONCLUSION
Based on the results of present formulation, stevia can be incorporated in the gel formulation. It can be concluded that this study determined that extract of Stevia leaves show antioxidant activity even after incorporation in the topical gel formulation. Due to addition of Acacia as natural viscosity modifier along with Carbopol and HPMC, Gel attainted the required viscosity for topical application. The data for total phenolics and total flavonoid suggest that stevia extract may be incorporated into a range of cosmetic and other products as an Antioxidant and Antiproliferative agent. The optimized formulation F2 expressed optimum value of pH, viscosity, and spreadability which indicates good gelling property in the aspect of topical application. Accelerated Stability studies were performed and it was found out that the Gel was stable after 3 months, there was a minute changes in some parameters but the Gel can be use further for usage. Incorporation of acacia along Carbopol and HPMC played a n interesting roll for designing the formulation. The evaluation of the formulated gel showed good results and batch F2 shown the optimum results and can be of good potential for further cosmetic product development. The accelerated studies shown least modification in the F2 formulation with respect to colour, pH and appearance. The formulation results indicated that the stevia extract can be incorporated effectively in gel system which may be a promising formulation to provide prolonged protection of the skin against the photo-oxidation process. Based on the present study, future investigation can be carried out to bring gel loaded with stevia extract formulation closer to clinical realization.

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Illustrations:
Tables:

TABLE 1: FORMULATION TABLE

Formulation	Drug (mg)	Carbopol (mg)	HPMC (mg)	Water (ml)	Acacia (mg)	Isopropyl Alcohol (ml)	Methyl Paraben (mg)	Propyl Paraben (mg)	Glycerol (ml)	TEA (ml)
F1	0.2	0.25	0.75	15	0.1	0.4	0.01	0.005	1 ml	0.4 ml
F2	0.2	0.25	0.75	15	0.2	0.4	0.01	0.005	1 ml	0.4 ml
F3	0.2	-	1	15	-	0.4	0.01	0.005	1 ml	0.4 ml
F4	0.2	-	1	15	0.1	0.4	0.01	0.005	1 ml	0.4 ml

TABLE 2: THE IC50 VALUE OF STANDARD VS. SAMPLE EXTRACT

Sample	IC50 (µg/ml)
Vitamin C	11.15
Stevia rebaudiana Bertoni leaf extract	47.58

TABLE 3: RESULTS OF PHYSIO – CHEMICAL PARAMETERS

Sr. No.	Parameters	Results
1.	pH	6.8
2.	% LOD (% w/w)	1.31 % w/w
3.	Solubility	Good solubility in Hydro-alcoholic solution
4.	Moisture Content (%)	9.608 %
5.	Ash Value (% w/w)	2.906 % w/w
6.	Melting Point (^{0}C)	≈199^{0}C
7.	Solvent Extractive Value (% w/w) ➢ Aqueous Soluble ➢ Alcohol Soluble	 28.372 % w/w 30.673 % w/w

TABLE 4: PHYTOCHEMICAL SCREENING OF *S. REBAUDIANA* BERTONI LEAVES

Sr. No.	Phytochemical screening	Test name/ reagents	Results
1.	Carbonyl group	2,4-dinitrophenylhydrazine and HCL	+
2.	Unsaturation	Baeyer's test	+
3.	Phenolic group	Ethanolic FeCL3 solution	+
4.	Acetyl group	Sodium bicarbonate	+
5.	Steroids	Salkowski s test	+
6.	Sugar molecules	Fehling s solution-I and II	+
7.	Tannins	Phlobatannins	+
8.	Saponins	Froth test Ferric chloride test	+
9.	Flavonoids	Free flavonoids test	+

(-): Negative result, (+): Positive result.

TABLE 5: RESULT OF TLC (SOLVENT SYSTEM 1)

Sr No.	Compound	Mobile phase	Detecting agent	Rf value
1.	Solute A [Pale yellow]	Ethyl-acetate: Methanol: Conc.H2So4(7.5:1.5:10)	Conc.H2SO4: ethyl alcohol (1: 10)	0.52
2.	Solute B [Green]	Ethyl-acetate: Methanol: Conc.H2So4(7.5:1.5:10)	Conc.H2SO4: ethyl alcohol (1: 10)	0.67

TABLE 6: RESULT OF TLC (SOLVENT SYSTEM 2)

Sr No.	Compound	Mobile phase	Detecting agent	Rf value
1.	Solute A [Green]	Chloroform: Methanol: H_2O (7.5:1.5:10)	Iodine chamber	0.85
2.	Solute B [yellow]	Chloroform: Methanol: H_2O (7.5:1.5:10)	Iodine chamber	0.92

TABLE 7: PHYSICAL EVALUATION OF ANTIOXIDANT GEL FORMULATION

Sr. No.	Test	Results
1.	Appearance	Greenish to light brown Gel
2.	Clarity	Clear gel
3.	Odour	Aromatic
4.	Homogeneity	Homogenous
5.	Skin Irritation	Non-Irritant

TABLE 8: EVALUATION OF GEL

Formulations	% Yield of Gel (%)	pH of Gel	Spreadability (cm^2)	Viscosity (cp)	Extrudability
FI	99.30%	7.8	4 cm^2	97466	Good
F2	112.5%	7.2	3 cm^2	74812	Excellent
F3	93.85%	8.2	15 cm^2	2971.85	Average
F4	113.45%	8.5	19 cm^2	2952.94	Average

TABLE 9: PHYSICAL EVALUATION OF ALL FORMULATIONS (INITIAL MONTH)

Formulations	Colour	Appearance	pH of Gel
FI	Greenish Brown	Clear and Homogenous	7.8
F2	Green	Clear and Homogenous	7.2
F3	Green	Clear and Homogenous	8.2
F4	Green	Clear and Homogenous	8.5

TABLE 10: 25^0C ± 2^0C/ 60% ± 5% RH (3RD MONTH)

Formulations	Colour	Appearance	pH of Gel
FI	Greenish Brown	Clear and Homogenous	7.8
F2	Green	Clear and Homogenous	7.2
F3	Green	Clear and Homogenous	7.8
F4	Green	Clear and Homogenous	8.2

TABLE 11: 30^0C ± 2^0C/ 65% ± 5% RH (3RD MONTH)

Formulations	Colour	Appearance	pH of Gel
FI	Greenish Brown	Clear and Homogenous	7.5
F2	Greenish Brown	Clear and Homogenous	7.0
F3	Green	Clear and Homogenous	7.8
F4	Greenish Brown	Clear and Homogenous	8.1

TABLE 12: 40^0C ± 2^0C/ 75% ± 5% RH (3RD MONTH)

Formulations	Colour	Appearance	pH of Gel
FI	Greenish Brown	Clear and Homogenous	7.5
F2	Greenish Brown	Clear and Homogenous	7.0
F3	Green	Clear and Homogenous	7.8
F4	Greenish Brown	Clear and Homogenous	8.1

Figures:

Fig. 1: Stevia leaf

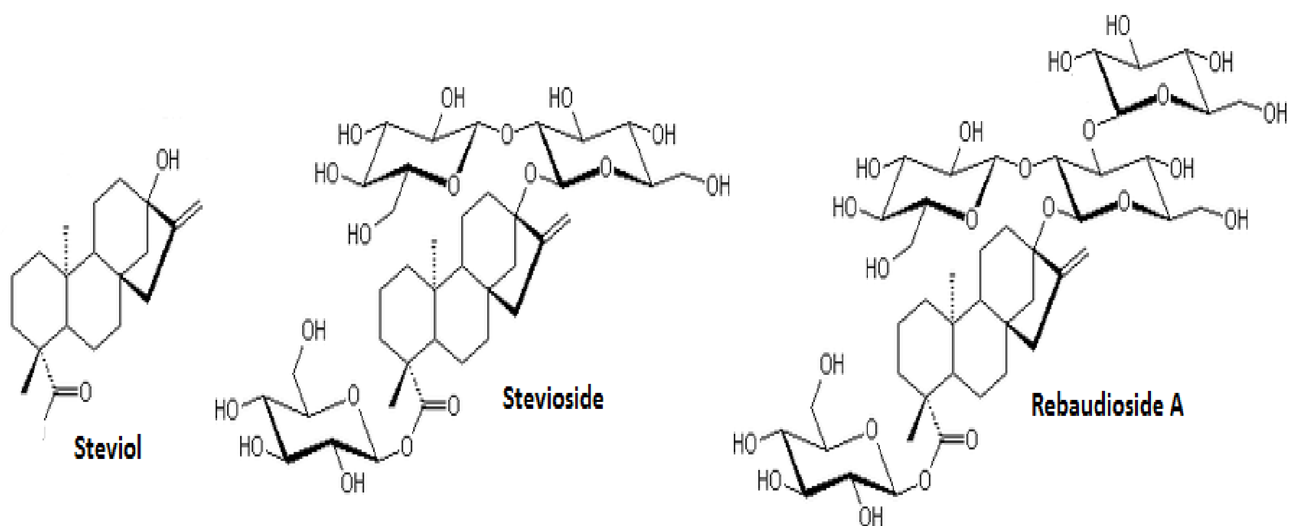

Fig. 2: Structural representation of Phytoconstituents present in Stevia

Fig. 3: Separated Phytoconstituents

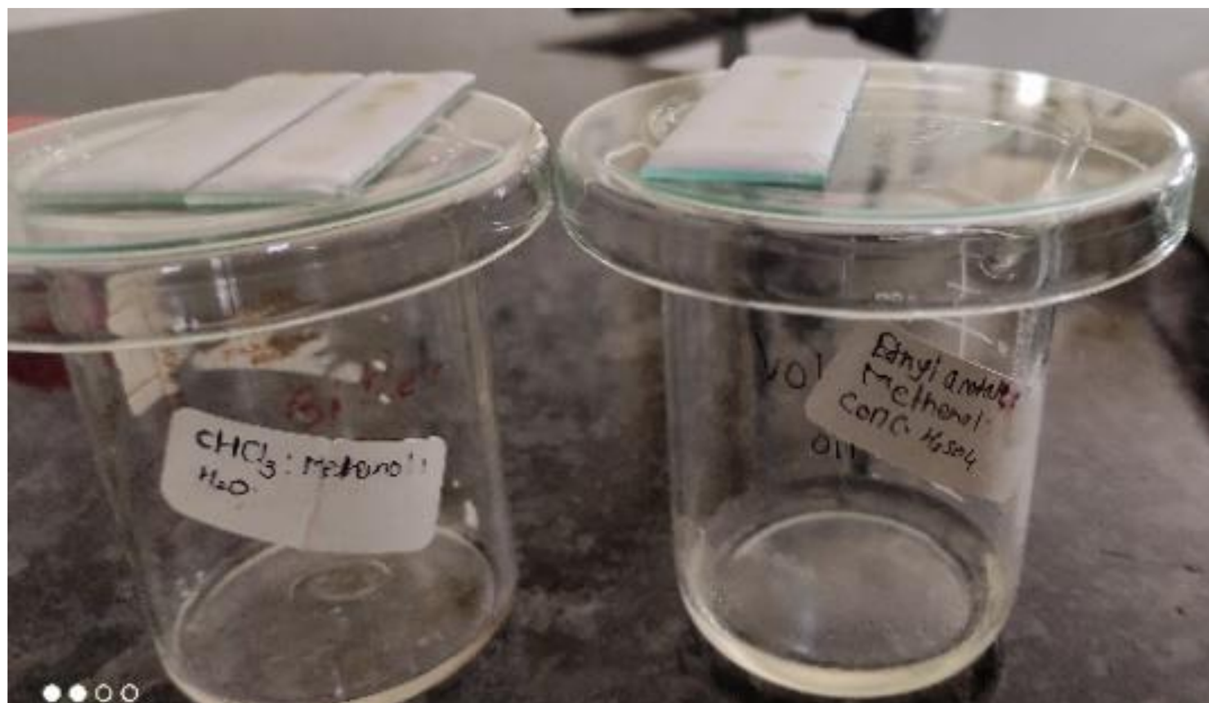

Fig. 4: Solvent systems used for TLC

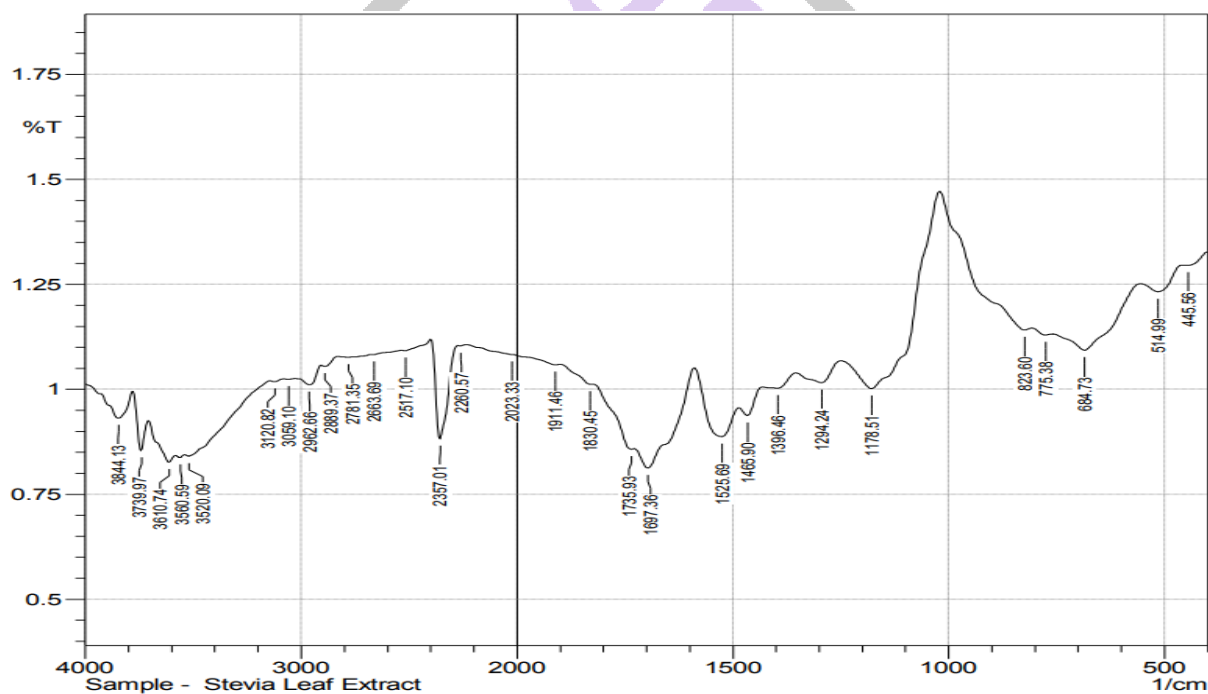

Fig. 5: IR Transmittance of dried leaf hydroethanolic extract of *Stevia rebaudiana* Bertoni

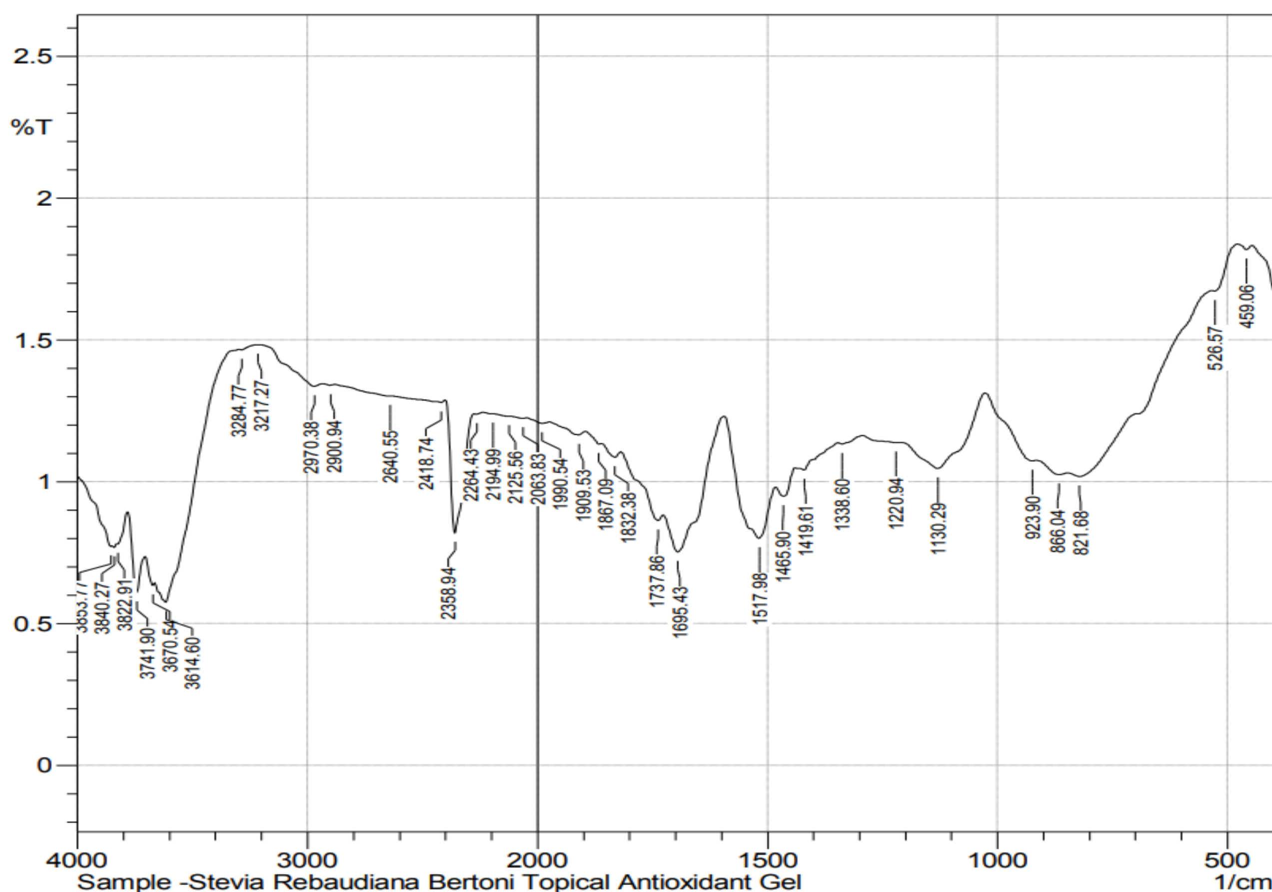

Fig. 6: IR Transmittance of Antioxidant Gel containing *Stevia rebaudiana* Bertoni

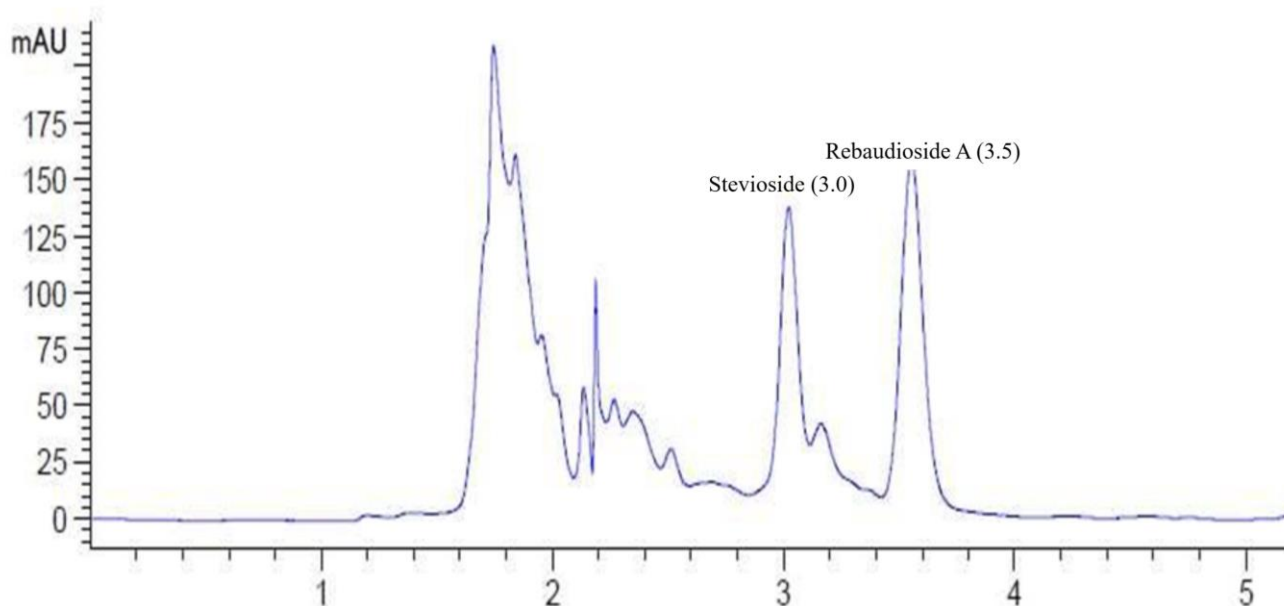

Fig. 7: HPLC chromatogram of *Stevia rebaudiana* Bertoni leaf was recorded at 205 nm. Stevioside and rebaudioside A were detected at retention time 3.0 min and 3.5 min, respectively.

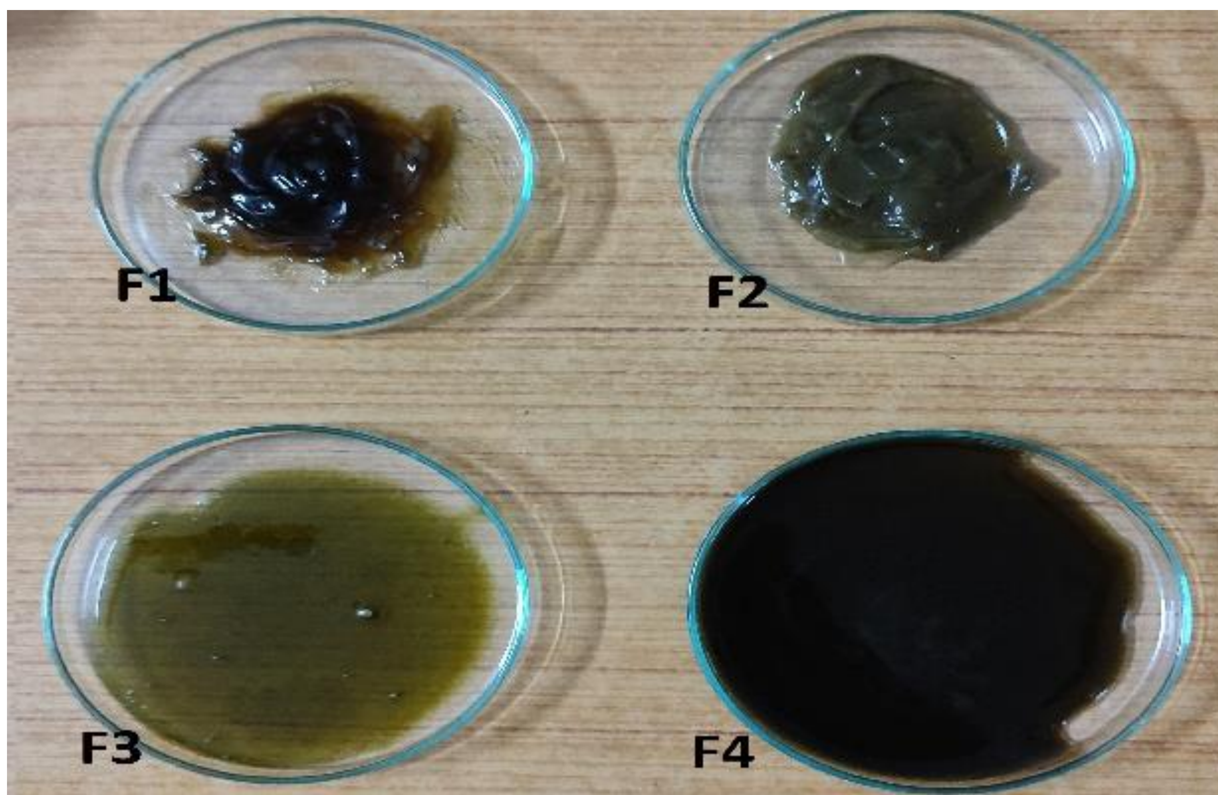

Fig. 8: Shows 4 Batches prepared by using different concentrations of Polymer Stevia Gel formulations

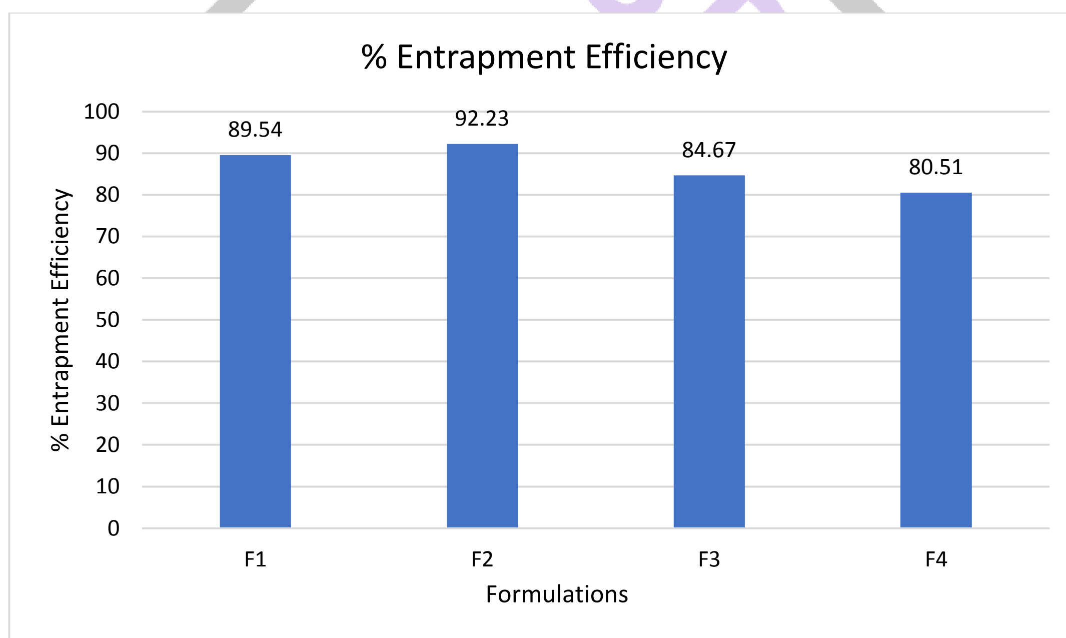

Fig. 9: %EE for Stevia Gel formulations

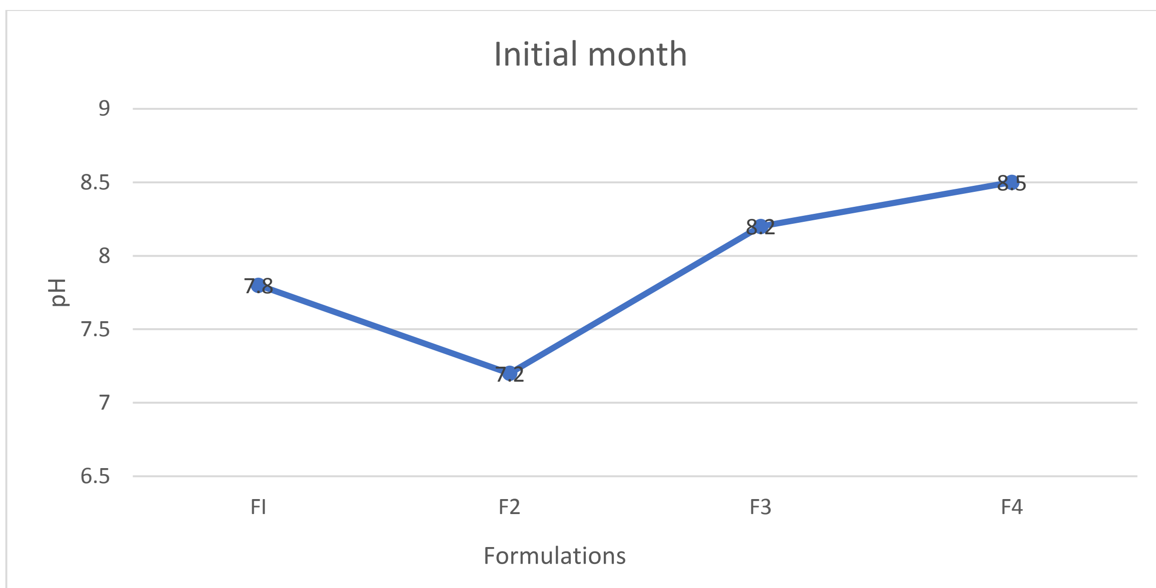

Fig. 10: pH recorded in Initial month for Stevia Gel formulations

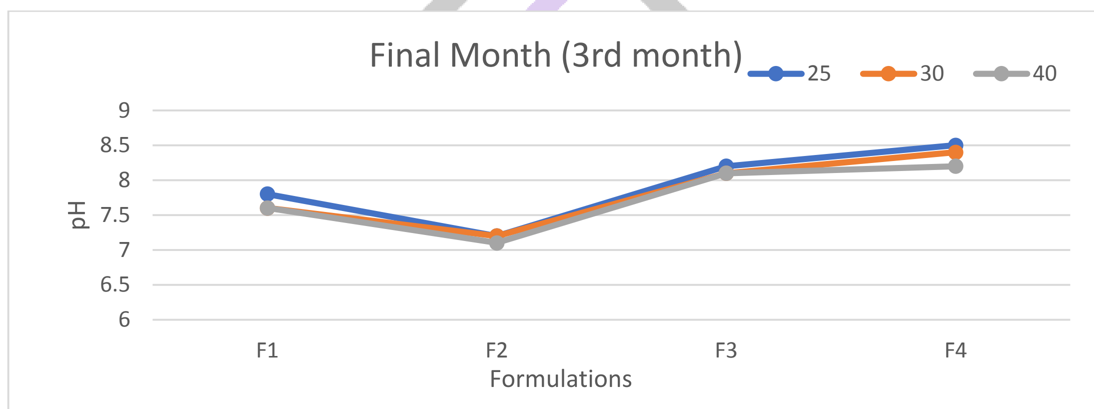

Fig. 11: pH recorded in Final month (3rd month) for Stevia Gel formulations