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Formulation and Evaluation of Herbal Lipstick from Broccoli Flower Extract and Analytical Bioactive Characterization and Quantification

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Abstract:- Herbs are obtained from natural sources. Now a days they are being incorporated into cosmeceutical formulations due to the presence of various properties like anti-bacterial, anti-viral, anti-oxidant and anti-inflammatory activities. The goal of the present study was to formulate and evaluate herbal lipstick, screening of phytochemicals and to provide in-vitro evidence for radical scavenging activity of Ethanolic flower extract of Brassica Oleraceae var. Italica, characterization of bioactive compound by chromatographic techniques and its quantification using analytical technique. Phyto-chemical screening of extract was performed which indicated the presence of many active constituents. Lipstick was formulated into five different formulations and evaluation tests were performed. The evaluation test results shown that fifth formulation has good stability than others when compared to marketed product. The broccoli extract had shown good antioxidant activity when compared with standard ascorbic acid. The total phenolic content of broccoli extract was determined and expressed in gallic acid equivalents (36.2 ± 0.9). TLC was performed using quercetin as standard. The bioactive component was detected as quercetin based on the proximity of Rf value of sample extract and Rf value of standard quercetin. A simple, rapid and sensitive method was developed for HPTLC quantitative analysis and results shown that 100mg of broccoli extract contains 29.6mg of quercetin. The herbal lipstick BLISS, formulated by us using broccoli flower extract containing quercetin, a flavanoid which being an antioxidant, reduces oxidative stress and prevents photoaging of lips keeping it supple and luscious in the long run.

Keywords:- *Broccoli, Lipstick, Photoageing, Antioxidant activity, Quercetin, HPTLC Analysis.*

I. INTRODUCTION

Herbal cosmetics are presently in trend and now-a-days there are no cosmetics without herbal ingredients. Herbs contribute to special cosmetic benefits and therapeutic benefits like skin elasticity, delay in aging and protection against UV etc. The usage of herbal cosmetics is increasing widely due to its friendliness to our skin and less side effects[1,2].

Lipstick is the one of the most important cosmetic products used by a woman to enhance her looks and to protect her lips from the detrimental environmental effects. The colors used in synthetic lipsticks are obtained from coal tar dyes & which are toxic in nature, possessing carcinogenic potential. Hence human endeavor has been to formulate herbal lipsticks[3].

Broccoli contains high levels of antioxidants which can stop the growth of cancer cells, it can reduce the risk of cell damage caused by free radicals and also reduces skin blemishes, skin ageing, dark spots etc[4].

Method development by HPTLC is one of the most important steps for qualitative and quantitative analysis. It increases resolution for accurate quantitative analysis[5].

II. DRUG PROFILE

IUPACName: 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxyphenyl)-3,5,7-trihydroxy-4H chromen-4-one.

Molecular Formula: $C_{15}H_{10}O_7$

Molecular weight: 302.236 g/mol

Structure:

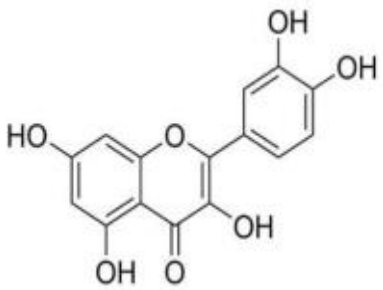

Fig 1:- Structure of Quercetin

Category: Antioxidant

Solubility:
a) Very soluble - ether, methanol
b) Soluble - ethanol, acetone, pyridine, acetic acid
c) Water - 60 mg/ml at 16°C

Pharmacokinetics:
Bioavailability- 0-50%
Half-life- 1-2hrs[6,7].

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III. MATERIALS AND METHODS

➢ *Collection and identification of plant:*

The plant material of 2kg was collected in and around Hyderabad, the collected material washed with tap water then the floral part was peeled and kept for shade drying, made it into coarse powder and stored in a well closed air-tight container for further use.

Weight of the powder obtained = 95gms

➢ *Authentication of plant:*

The herbarium of plant material was authenticated by P.V. Prasanna (Scientist in Botanical Survey of India) herbarium no. BSI/DRC/2018-2019/Tech/628.

➢ *Extraction of Plant :*

The powder material of 84gm was weighed and was subjected to soxhlet apparatus for extraction with 400ml of Ethanol. The extract obtained was filtered, solvent &evaporated by using rotary evaporator yielding the crude extracted compound.

➢ *Characteristics of Crude Extract:*

Color, odour, consistency and solubility of the extract were observed.

➢ *Phytochemical Screening:*

Preliminary phytochemical analysis were carried out on Broccoli flower extract using standard procedure to identify phytoconstituents[8].

➢ *Formulation of Lipstick:*

Herbal lipstick was formulated into five different formulations using following ingredients with varying formulas as shown in table 1:

S. No	INGREDIENS	F1	F2	F3	F4	F5
1	Bees wax	1.8	2	2	2.3	2.3
2	Paraffin wax	0.80	0.65	1	1.5	1.5
3	Lanolin	1	1	1	1	1
4	Almond oil	1	1,5	1	1	1.5
5	Broccoli extract	0.25	0.25	0.50	0.75	**1**
6	Raspberry	q.s	q.s	q.s	q.s	q.s
7	Lemon juice	1	1	1	1	1
8	Vanilla essence	q.s	q.s	q.s	q.s	q.s

Table 1:- Formulas of five different formulations

❖ *General procedure for formulation of herbal lipstick:*

➢ All the raw ingredients like bees wax, paraffin wax, lanolin, broccoli flower extract were melted on water bath.
➢ The raspberry coloring agent was mixed and heated with almond oil. Lemon juice, rose essence of small amounts were added at 30°C.
➢ Then the mixture was transferred into lipstick molds in excess amount and kept on ice bath.
➢ After solidification of lipstick, excess amount on mold was scrapped with blade and lipstick was removed and fitted into lipstick container and was kept for evaluation[9].

➢ *Evaluation of Herbal lipstick:*

The quality control of lipstick is more important to ensure the safety and efficacy of the product and raw materials used. The evaluation parameters of herbal lipstick were carried out using standard procedure as described by Anju Varghese (2017)[10].

❖ *Evaluation of In-Vitro Antioxidant activity*

➢ *Total Phenolic Content (FC Method):*

Total phenolic content in plant extract was determined by using UV-spectrophotometer. Sample extract of concentration 1000µg/ml and standard (gallic acid) of concentrations 10, 20, 30, 40, 50 µg/ml were prepared in methanol. 10% Folin-ciocalteu reagent and 7.5% $NaHCO_3$ were prepared in water. The reaction mixture was prepared by mixing 0.5ml of methanolic plant extract, 2.5ml of 10% FC and 7.5% $NaHCO_3$. Reaction mixture was incubated at room temperature, absorbance was then checked at 650nm using UV-spectrophotometer. For standard gallic acid same procedure was followed, calibration line was constructed. Total phenols present in the plant extract was expressed in terms of gallic acid equivalents mg of GAE / gram extract.

➢ *Evaluation of Antioxidant activity by DPPH assay method:*

Free radical scavenging activity of the plant extract was carried out by using DPPH assay (1,1 diphenyl 2-picrylhydrazyl) assay. Sample extract and standard (ascorbic acid) of concentrations 50, 100, 150, 200, 250 µg/ml were prepared in methanol. 0.3ml of DPPH was prepared by dissolving in methanol. Reaction mixture was prepared by adding 1ml of DPPH to 2.5ml of sample extract and 2.5 ml of standard solution separately and are allowed to stand at room temperature in a dark chamber for 30min. Colour changes from deep violet to light yellow then the absorbance was checked in UV-spectrophotometer at 518nm. Decrease in the absorbance was then converted to percentage antioxidant activity (%AA) by using the following formula:

$$\%AA = \{[(Ab_{control} - Ab_{sample})/Ab_{control}] \times 100\}$$

➢ *Evaluation of Antioxidant Activity By Reducing Power Assay Method:*

The antioxidant capacity of plant extract to reduce ferric-ferry cyanide complex into ferrous-ferry cyanide complex was determined by reducing power assay method. Plant extract of concentrations 100, 200, 300, 400, 500 µg/ml were prepared in methanol. Phosphate buffer of pH 6.6 was prepared. 1% potassium ferric cyanide, 10% Tri-chloro acetic acid and 0.1% ferric chloride were prepared. The reaction mixture was prepared by mixing 2.5ml of plant extract with 2.5ml of phosphate buffer(pH-6.6) and 2.5ml of 1% potassium ferric cyanide. Then the mixture was incubated in dark chamber at 50°C for 20min. After

incubation 2.5ml of 10% TCA was added to the mixture and centrifuged for 10min at 1000g. After centrifugation 2.5ml of upper layer of solution was mixed with 0.5ml of 0.1% ferric chloride. Then the absorbance was checked at 700nm using UV-visible spectrophotometer. Increase in absorbance of reaction mixture indicates increase in reducing power. All results were done in triplicates and were expressed in mean values. Same procedure was followed for standard ascorbic acid[11,12].

❖ *Thin Layer Chromatography (TLC)*

➢ *Preparation & Activation of TLC Plates:*

TLC Silica gel was weighed and mixed with water &made into slurry. The slurry was then spread on a clean glass plate. Then the resultant plate was dried & activated by heating in an oven at 110^0C. for ten minutes.

➢ *Chromatogram development:*

TLC development was done in a TLC chamber. The optimal solvent required for the identification of compound was determined by varying the ratios of the solvent systems such as toluene, ethyl acetate, acetic acid, chloroform and methanol. Visualization was done by using spraying reagents and the color of the spots were observed. Then the retardation factor (Rf) of compounds was calculated by using following formula:

Rf = Distance travelled by solute/Distance travelled by solvent

❖ *High Performance Thin Layer Chromatography*

➢ *Sample Preparation:*

Sample extract of 1000mg was weighed and triturated with 10ml of methanol for 30 minutes and then filtered. The volume of sample was adjusted to 10ml with methanol to get a concentration of 100mg/ml. The filtrate was then used for HPTLC analysis.

➢ *Standard Preparation:*

10mg of quercetin was weighed and transferred to a 10ml volumetric flask and was made to 10ml with methanol to get a concen tration of 1mg/ml.

➢ *Selection of HPTLC Plates:*

HPTLC plates are available in the form of pre-coated chromatographic layers. In the present study pre-coated HPTLC silica gel 60 F_{254} chromatographic layers with aluminum foil support are used.

➢ *Application of Sample:*

Sample and standard solutions are applied in the form of bands of 6mm width containing 2µl for standard and sample solutions respectively in track 1 and track 2 on the plate by using CAMAG Linomat 5, which is a microprocessor controlled and programmable applicator.

➢ *Selection of Developing Medium:*

The mobile phase, Toulene: Ethyl acetate: Glacial acetic acid (4.2:6:0.3)was selected by TLC as mentioned earlier.

➢ *Scanning:*

For the present study, the chromatogram was developed in a saturated CAMAG Twin trough chamber using mobile phase. The developed chromatogram was evaluated by using CAMAG TLC Scanner at wavelength 370nm. The quantitative evaluation sequence involved raw data acquisition - integration - calibration and calculation of result generating the analysis report. For sample, integration calibration spectra were recorded and total area included in peak was observed[13,14].

IV. RESULTS AND DISCUSSION

Weight of Broccoli taken	Amount of solvent taken	Weight of BE obtained = Wt of china dish with BE - Wt of china dish	Practical Yield (%)
84.13g	400ml	124.03-115.50	10.13%

Table 2:- Calculation of practical yield:

Color	Odor	Consistency	Solubility
Dark green	Characteristic	Sticky	Ethanol

Table 3:- Characteristic features of Broccoli flower extract:

➢ *Phytochemical Screening*

Phytochemical screening was carried out for broccoli flower extract and the results are shown in table 4:

S. No	Chemical Constituents	Results
1	Acidic compounds	+
2	Alkaloids	+
3	Amino acids	-
4	Carbohydrates	-
5	Lignin	+
6	Cellulose	-
7	Fats & Fixed oils	+
8	Flavanoids	+
9	Mucilage	-
10	Inulin	+

Table 4:- Phytochemical test results

The above phytochemical test results indicates the presence of acidic compounds, alkaloids, lignin, fats & fixed oils, volatile oil, glycosides, inulin, tannins, steroids & triterpenoids, waxes.

➢ *Formulation of Lipstick*

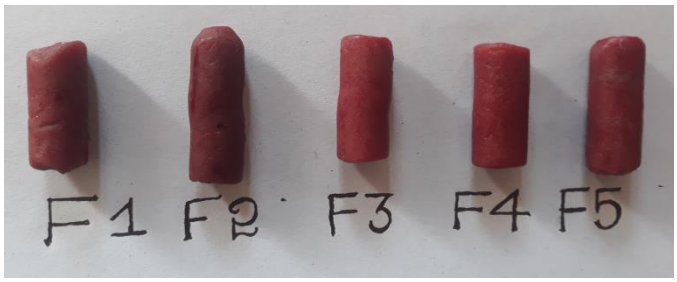

Fig 2:- Formulated Lipsticks

➢ *Evaluation Parameters:*

Parameters	F1	F2	F3	F4	F5	Standard (NIVEA)
Color	Pinkish red	Pinkish red	Pinkish red	Pinkish red	Pinkish red	Dark pink
Breaking point	31	30	32	31	30	30
Melting point	50-55	52-60	55-65	65-72	75-85	80-95
Force of application	poor	poor	poor	good	good	good
Solubility	$CHCl_3$	$CHCl_3$	$CHCl_3$	$CHCl_3$	$CHCl_3$	$CHCl_3$
pH parameter	4.5	5.2	5.5	6.3	6.7	7
Surface anamolies	no defect	no defect	no defect	no defect	no defect	no defect
Skin irritation test	no	no	no	no	no	no
Aging stability	smooth	smooth	smooth	smooth	smooth	smooth
Perfume stability	+	+	+	++	+++	+++

Table 5

➢ From evaluation test results we came to know that fifth formulation has good consistency than others when compared to standard product (NIVEA).
➢ Final lipstick was formulated using fifth formula, and the product was named as Bliss Lipstick.

Fig 3:- BLISS LIPSTICK

❖ *Evaluation of In-Vitro Antioxidant Activity:*

➢ *Total Phenolic Content:*

Concentration(µg/ml)	Absorbance at 650nm
10	0.103
20	0.172
30	0.261
40	0.339
50	0.418

Table 6:- Absorbance of Gallic acid at 650nm

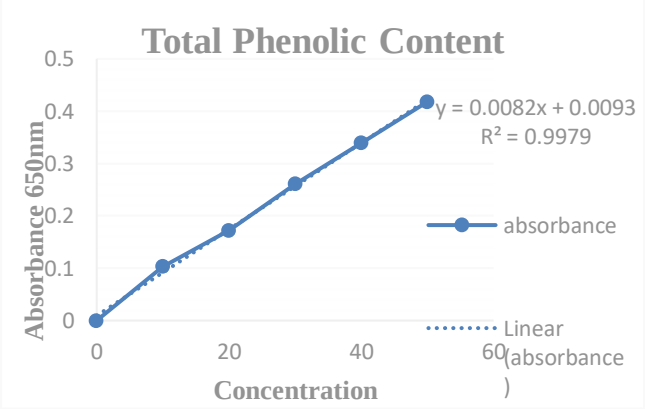

Fig 4:- Calibration curve for gallic acid standard

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Sample Conc (µg/ml)	Weight of dry extract (µg/ml)	Absorbance	GAE Conc (µg/ml)	GAE Conc (mg/ml)	T = CV/M	Mean SEM
1000	0.001	0.296	37.23	0.0372	37.2	36.2 ± 0.9
1000	0.001	0.281	35.40	0.0354	35.4	
1000	0.001	0.287	36.13	0.0361	36.1	

Table 7:- Total phenolic content expressed as GAE

➢ *Evaluation of Antioxidant activity by DPPH assays method:*

Broccoli extract exhibited a comparable antioxidant activity with standard ascorbic acid at various concentrations were tested. The percentage antioxidant activity for standard and broccoli extract was calculated [Table: 11]. The results have shown that increase in antioxidant activity was dose-dependent. The IC_{50} value was found to be 185.7µg/ml.

Concentration (µg/ml)	%AA of Broccoli Flower Extract	%AA of Gallic acid
50	16.02	27.28
100	28.49	40.36
150	41.68	58.01
200	54.25	72.71
250	65.21	84.27

Table 8:- % Antioxidant activity of Broccoli flower extract and Gallic acid

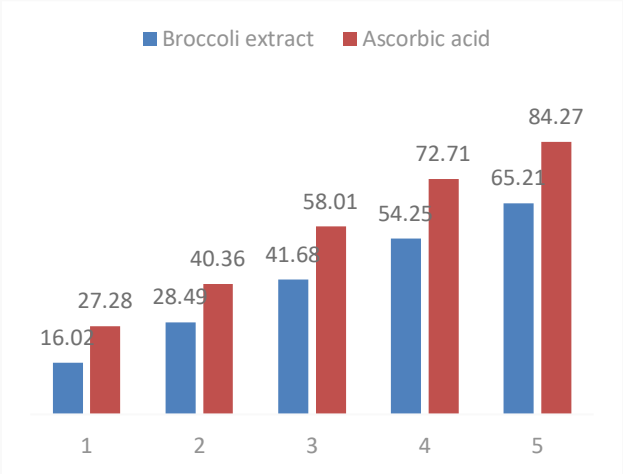

Fig 5:- % AA of BE and Ascorbic acid

➢ *Evaluation of Antioxidant activity by Reducing Power assay method:*

Reducing power assay method is based on the principle of reducing potential. In which substances react with potassium ferricyanide [Fe^{3+}] to potassium ferrocyanide [Fe^{2+}], which further reacts with ferric chloride to form ferric-ferrous complex. Fig 15: shows that how the reducing power gets increased with increase in sample concentration. The reducing power shows good linearity for both standard (R^2 = 0.9367) to sample extract (R^2 = 0.9262).

Concentration (µg/ml)	Mean ± SEM of Broccoli flower extract	Mean ± SEM of Ascorbic acid
100	0.315 ± 0.036	0.376 ± 0.049
200	0.424 ± 0.038	0.522 ± 0.049
300	0.518 ± 0.030	0.647 ± 0.032
400	0.616 ± 0.040	0.773 ± 0.039
500	0.710 ± 0.034	0.889 ± 0.051

Table 9:- Reducing power assay mean and SEM values

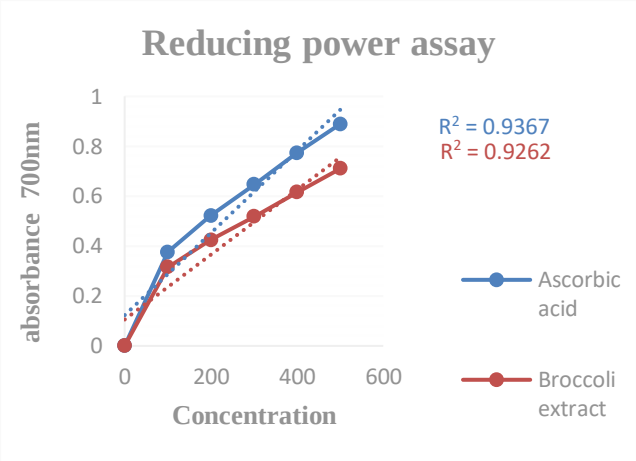

Fig 6:- Reducing power assay of broccoli flower extract

❖ *Thin Layer Chromatography*

➢ The visualization of spots on developed TLC plates was done by spraying conc.sulphuric acid (destructive method) fig 7:

➢ When the developed TLC plates were kept under UV light it showed fluorescent spots in ethanolic extraction of *Brassica oleracea.var. italica*, and results are shown in fig 8:

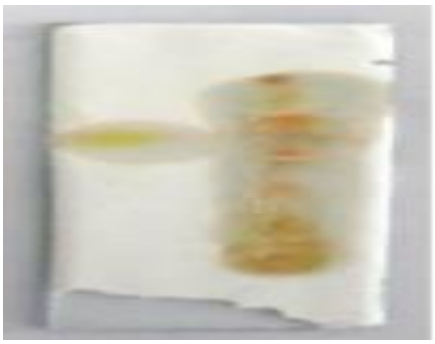

Fig 7:- Visualization of spots by destructive method

Fig 8:- TLC of broccoli under UV cabinet

➢ *RF Calculation*

S. No	No of spots obtained	Distance travelled by solute	Rf value = distance travelled by solute/Solvent front
1	Unknown A	2.3	2.3/5.6 = 0.41
2	Unknown B	2.6	2.6/5.6 = 0.46
3	Unknown C	4.1	4.1/5.6 = 0.73
4	Standard (quercetin)	2.6	2.6/5.6 = 0.46

Table 10:- Calculation of Rf value

➢ From the above calculated Rf values of unknown are compared with standard Rf value. Rf value of unknown B is nearer to the Rf value of standard (Quercetin).

➢ *High Performance Thin Layer Chromatography*

Track	Track type	Vial	Sample ID
1	Standard	1	Quercetin
2	Sample	2	Ethanolic extract

Table 11:- Evaluation sequence

Substance	Position Tracks		
	MD mm	1	2
Quercetin	27.9	A	A

Table 12:- Table depicting track position of substance

➢ *Integration Results*

Baseline correction - Lowest slope
Peak threshold min slope - 5
Peak threshold min.height - 10 AU
Peak threshold min.area - 990 AU
Peak threshold max.height - 990 AU
Track start position - 26.5 mm
Track end position - 28.7mm
Display scaling – Automatic

➢ *Calibration Results:*
Sample from vial 2 - Ethanolic extract

Substance	Rf	X(average)	CV[%]	n
Quercetin	0.32	0.0 unknown	0.000	0

Table 13:- Results via height

Substance	Rf	X(average)	CV[%]	n
Quercetin	0.32	0.0 unknown	0.000	0

Table 14:- Results via area:

➢ *Quantification results:*
Substance: Quercetin @370nm
Regression via height: single level Y= 0 + 197.8* x
Regression via area: single level Y = 0 + 2381*x

Track	Rf	Amount	Height	X(cal)	Area	Sample Id
1	0.32	2.00µg	395.53		4762.91	standard
2	0.32		16.18	<1.800	141.0	Ethanolic extract

Table 15:- Regression via height and area

Amount of quercetin = sample area/standard area x standard vol/standard conc x sample conc/sample vol x 100

= 141.0/4762.9x1/1x1/100x100 = 0.0296%

The amount of quercetin present in 100mg of sample extract = 29.6mg

The amount of quercetin present in 1gm of sample extract = 296mg

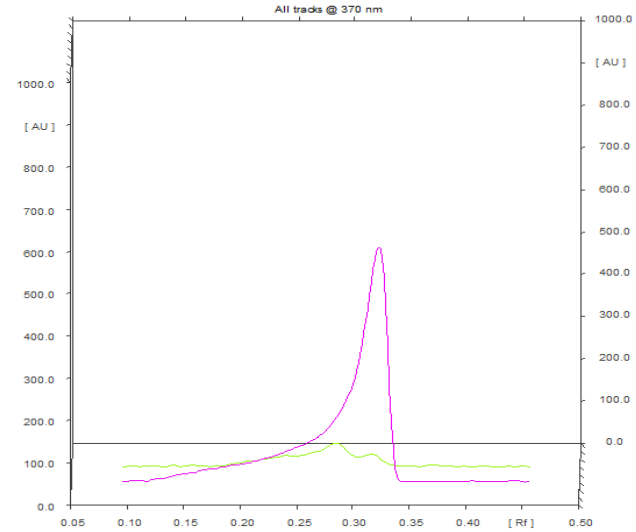

Fig 9:- Comparison peak of sample & standard (Quercetin) at 370nm

Peak	Start Rf	Max Rf	Max height	End Rf	Area	Assigned substance
1	0.30	0.32	395.5	0.33	4762.9	Quercetin

Table 16:- Track 1 Standard

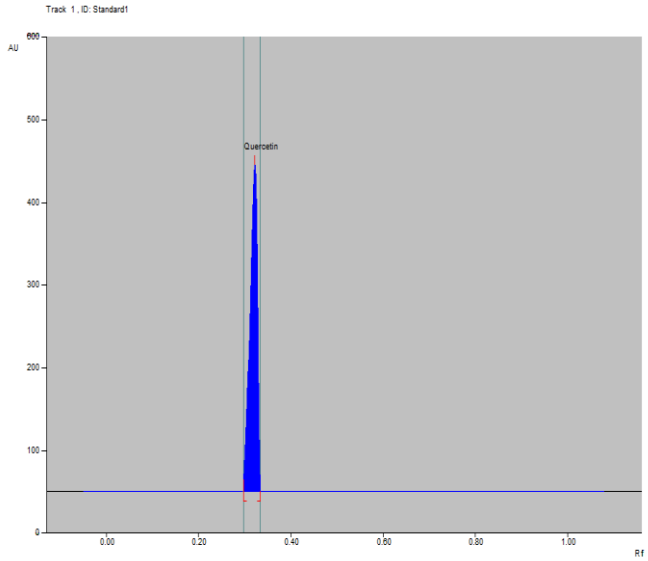

Fig 10:- Track 1 Standard densitogram

Peak	Start Rf	Max Rf	Max height	End Rf	Area	Area %	Assigned substance
1	0.30	0.32	16.2	0.33	141.0	100.00	Quercetin

Table 17:- Track 2 Sample

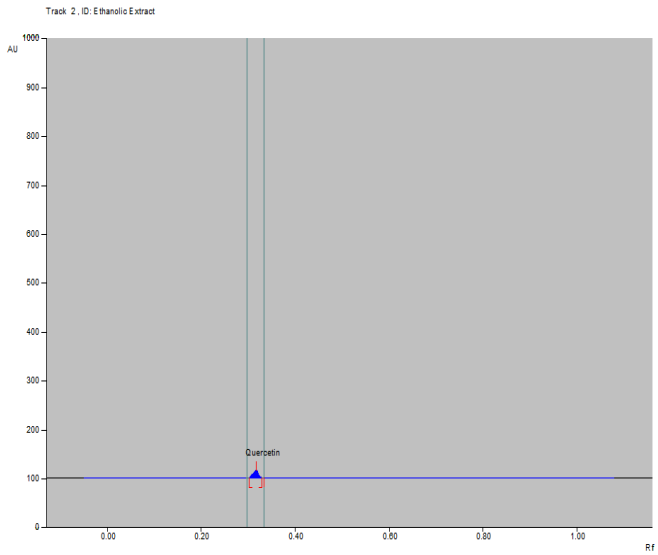

Fig 11:- Track 2 Sample densitogram

➢ *Chromatogram results:*

Chromatogram: 1
Illumination instrument - CAMAG Visualizer
Resolution - Full
Exposure mode - Automatic, digital level: 80% band
Illumination type - 254nm remission : Default correction

Chromatogram: 2
Illumination instrument - CAMAG Visualizer
Resolution - Full
Exposure mode - Automatic, digital level: 85% band
Illumination type - 366nm remission : Default correction

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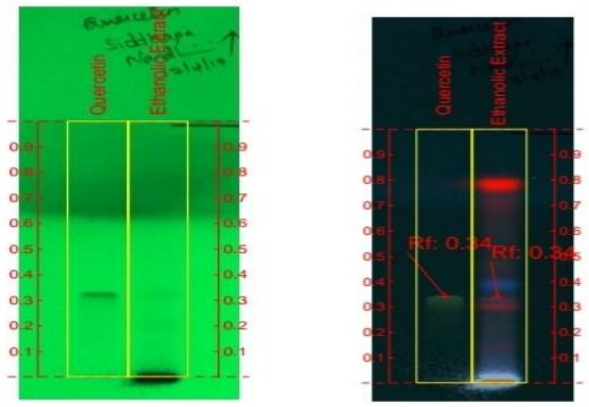

Fig 12:- Comparison of chromatogram 1 & 2 based on exposure mode

- Chromatogram 2 shows clearer spots and better visualization capture.

V. CONCLUSION

By performing the phytochemical tests we came to know that broccoli flower extract contains flavanoids, alkaloids, tannins, volatile oils, lignin, inulin, fats & fixed oils, steroids & triterpenoids and waxes. Flavanoids are the polyphenolic compounds which are responsible for antioxidant properties. Increase in oxidative stress may lead to photoaging of the lips. Hence, a herbal lipstick was formulated using broccoli flower extract in order to prevent oxidative stress. The optimized formulation was analyzed. A single level HPTLC quantitative analysis of quercetin in broccoli flower extract was carried out and HPTLC profiles were generated. So we conclude that by using lipstick containing antioxidant properties we can rejuvenate and prevent photoaging. Also the natural lipstick lacks the carcinogenic agents of synthetic products ensuring safety which should be of prime concern to the manufacturer and end user.

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