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DEVELOPMENT AND EVALUATION OF HERBAL WHITENER SKIN FORMULATION USING TYROKINASE INHIBITION PROPERTY

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ABSTRACT

Background: Cosmetic products are used to protect skin against exogenous and endogenous harmful agents and thereby enhance the beauty and attractiveness of skin. The products that are used for skin brightening directly or indirectly inhibit the synthesis of melanin, which is one of main cause of skin darkening.

Material & Methods: Herbal skin whitening topical formulation was prepared by using some herbal extract. The different ratios of herbal constituents were tried with different topical formulations. Formulations which was prepared by using *Curcuma longa* extract, Mahua oil, *Hibiscus sabdariffa* extract and *Cynodon dactylon* extract using water in oil emulsion base. Out of the prepared batches on trial and error basis, three formulation batches showed appropriate and considerable results with marketed formulation.

Conclusion: The Flavonoids having ability as a skin whitening agent so total flavonoid content was determined. The evaluation parameters was selected as preliminary phytochemical tests, pH, viscosity, spreadability, total flavonoid contents, TLC, HPTLC, FTIR, accelerated stability study which was within limit. The *Curcuma longa* extract, mahua oil, *hibiscus sabdariffa* have the tyrokinase inhibition property, which reduces melanin production.

Keywords: *Curcuma longa*, *Madhuca indica*, *Hibiscus sabdariffa*, *Cynodon dactylon*, skin whitener

INTRODUCTION

The natural ingredients present in the skin care formulation supports the health, texture and integrity of skin, moisturizing, maintaining elasticity of skin by reduction of type I collagen and protection from light etc. Skin pigmentation is influenced by several factors, including, haemoglobin in the blood vessels, carotenoids in the dermis and particularly the dark pigment, melanin in epidermis. Upon exposure of the skin to sun radiation, melanogenesis is enhanced by the activation of Tyrosinase, a melanogenesis key enzyme **[1]**. Tyrosinase, a polyphenol oxidase, can catalyze two distinct reactions, the oxidation of L-tyrosine to L-dihydroxyphenylalanine (LDOPA)and the oxidation of L-DOPA to dopaquinone. Then, dopaquinone, through a non-enzyme-catalysed process, is transformed into leukodopachrome. This compound is oxidized into dopachrome which is an extremely fast and non-enzyme-catalyzed process. Then, dopachrome is transformed to melanin through a series of chemical- and enzyme-catalyzed reactions. Thus, the referred process shows that dopachrome synthesis can be suppressed when any of the steps are inhibited. However, not all substances that can inhibit the formation of dopachrome are tyrosinase inhibitors over-activity of tyrosinase leads to over-production of melanin **[2]**. Inhibition of tyrosinase enzyme it helps in skin lightening through reduced melanin production. Tyrosinase inhibitors are antioxidants, flavonoids, vitamins such as A, B, C, E either directly or in directly serve as skin lightening ingredients **[3]**.

The plant parts used in cosmetic preparation should have varieties of properties like antioxidant, anti-inflammatory, antiseptic, emollient, antiseborrhatic, antikerolytic activity and antibacterial etc. Herbal products claim to have less side effects, commonly seen with products containing synthetic agents. Skin lightening creams are the products which work on skin by reducing a pigment called melanin in the skin. It also lightens naturally dark skin. Skin lightening products are also known as, whiteners, skin brighteners, etc. As skin is daily exposed to sunlight which causes skin pigmentation disorders such as melasma, hyperpigmentation, skin problem such as freckles, age spots, acne scars, or discoloration related to hormones. The aim of present study was to develop a novel skin lightening preparation which containing herbs. In cosmetics, Curcuma longa has an excellent potential for antiaging, cooling, healing and soothing to an irritated skin, whether caused by sun, or

the effects of a cutaneous eruption. The skin of the face can become thinned and the area around the eyes can have increased pigmentation causing a "bleach panda effect." Facial skin can even become hyper pigmented **[4]**. Tretinoin (also known as all-trans retinoic acid) **[5]**, Hydroquinone **[6],** The flavonoids, due to their ROS-scavenging activity and ability to chelate metals at the active site of metalloenzymes, present a pigment reducing action. A number of flavonoids are frequently used in skin-lightening preparation. Ex. Quercitin, Rutin **[7]**.

MATERIAL AND METHODS

The plant material was used in the research project was Curcuma longa, *Cynodon dactylon*, *Hibiscuss abdariffa* was collected from local market and authenticated from Rashtrasant Tukadoji Maharaj Nagpur University Nagpur, Botanical department while Mudhuca Oil was purchase from local market and used as it is. While other ingredients were purchase from local market and having laboratory grade.

Extraction process

The continuous hot extraction process was used for the extraction for Curcuma longa rhizomes, *Cynodon dactylon* whole plant and *Hibiscuss abdariffa flowers*. The shade dried plant material was used for extraction in coarse powder form. Approximately 250 gm of sample was taken into a thimble and placed in a soxhlet apparatus **[8]**. Each plant material was extracted separately by using different soxhlet apparatus and different solvents non polar to polar. After completion of extraction the extract was filtered and evaporated by rotary evaporator to get a crude dried extract.

Preliminary phytochemical screening **[9, 10]** was carried out for the identification of active chemical contents in the extract ie. carbohydrates, glycosides, alkaloids, fixed oils and fats, proteins and free amino acids, phenolic compounds and tannins, flavonoids etc.

Thin layer Chromatography

TLC Method of *Hibiscus sabdariffa* [11]

Hibiscus sabdariffa calyx extract and standard compounds quercetin were dissolved in separate beakerin methanol to got the concentration of 1 mg/ml. Diluted 10 mg of extract and standards were loaded into silica gel TLC plates and left for drying. Solvent system was use ethylacetate: methanol: water (10:2:1, v/v/v). The plates were dried for 15 minutes then visualized using UV light, iodine vapour. The R_f values for separated spots were calculated & compared with R_f value of standard drug.

3.2 TLC method of Curcuma *longa* [12]

TLC for *Curcuma longa* extract was performed using a pre-coated silica gel G plates (stationary phase) using mixture of

n-hexane and ethyl acetate in the ratio 7:3 as solvent system (mobile phase). *Curcumin* was use as a standard. The plates were dried for 15 minutes then visualized using UV light, iodine vapour. The R_f values for separated spots were calculated & compared with R_f value of extract *Curcuma longa.*

Fourier transform infrared spectroscopy compatibility study [13]

An infrared spectrum of isolated extracts was recorded using FTIR Spectrophotometer (IR Affinity-1, Shimadzu, Japan). The scanning was 4000 to 700cm^{-1} and the IR spectra of samples were obtained using NaCl disc method.

Formulation development as cream and gel [14-16]

After determination of flavonoids and other contents in the extract then different 25 batches were tried out of these total eighteen batches were considered for further study. Water in oil emulsion based cream was selected for further study as it released the content from the formulation as required. The emulsifier (stearic acid) and oil soluble components *curcuma longa* extract and *Mahua oil* were dissolved in the oil phase and heated 75°C. The extract of *Hibiscus sabdariffa,* extract, *Cynadon dactylon, Honey,* Triethanolamine were dissolved in aqueous phase and heated to 75°C. After that the aqueous phase was added in small portions to the oil phase with continuous trituration in porcelain mortar until a smooth cream was formed. While other formulation is based on gel base 1gm of Carbopol 934 dissolved in 100 ml of distilled Water then hydrate for 24 hrs added the triethanolamine neutral to the pH. 20gm of carbopol gel dissolved the *Hibiscus sabdariffa, Cynodon dactylon, Curcuma longa extract,* honey dissolve in *Mahua oil* and continuous trituration in porcelain morter with pestle to get smooth formulation having gel structure.

Evaluation of prepared formulation

Determination of pH [17]

.Accurately weight sample in dissolved in 25 ml water in a beaker and reading was taken by calibrated pH meter.

Determination of Viscosity [18]

The viscosity was determined by Brookfield Viscometer using spindle number 64 at 5 rpm. And reading was recorded.

Determination of spreadiability [19]

The sprediabity of prepared formulation was determined by sprediabilty apparatus by using following formula

$$S = m \times \frac{l}{t}$$

Where, *S* is the spreadability of the cream formulation,

m is the weight (g) tied on the upper plate,

l is the length (cm) of the glass plates, *t* is the time taken (s) for the plates to slide the entire length

***In-Vitro* DPPH Free Radical Scavenging Activity [20]**

DPPH radical scavenging activity of samples was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH according to the method of Blois. Briefly, 3 ml of alcoholic solution of samples was added to 1ml of DPPH (1, 1-diphenyl-2-picrylhydrazyl) as the free radical source. The mixture was shaken and kept for 30 minutes at room temperature. The degree of solution absorbance due to proton donating activity of components of each extract was determined at 517nm. The DPPH radical scavenging activity was calculated using the following formula:

$$\text{Radical Scavenging \%} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absobance of control}}$$

The antioxidant activity of the extract was expressed as $IC_{50.}$ Then % inhibitions were plotted against respective concentrations of Sample used and from the graph IC_{50} was calculated. Ascorbic acid was used as reference standard.

Total flavonoids content estimation formulation

Prepare different concentration of quercetin (10 to 100 µg/ml) in methanol. Prepare test sample in methanol, mix 0.5 ml aliquot of appropriately diluted sample solution with 2ml of distilled water and subsequently with 0.15 ml of a 5% $NaN0_2$ solution. After 6 min, add water to bring the final volume upto 5ml, mix the mixture thoroughly and allowed to stand for another 15 min. Take absorbance of the mixture at 510 nm versus a prepared water blank. Calculate total flavonoids content by using following formula.

$y=0.001x + 0.092$ $R^2= 0.979$

Microbial test [21]

Aseptically nutrient agar was transfer to sterile perti-paltes and allow it to solidify. After solidification transfer the preparation and allow it for 48 hours incubation period at a temp $37^0 \pm 2^0$C to find out whether there was a micro- organism growth or not.

HPTLC Method [22-24]

A CAMAG HPTLC system equipped with a sample. Applicator Linomat IV, twin through plate development, chamber, TLC Scanner III and Integration software CATS 4.05.

Preparation of sample solution

About 10 mg of each *Hibiscus sabdariffa* extract, *Cynodon dactylon* extract and *Curcuma longa* extract was dissolved in 1 ml methanol in three separate Eppendorf tubes. It was then mixed in vortex mixture and put to ultra sonication bath till the material completely dissolved in it and then filtered through 0.45µ syringe filter and

kept for further study. The extracts from creams and gel were prepared by mixing 1 g of the formulation in 10 ml of ethanol, stirring until dissolved. The solution was then filtered using 7.0 cm round filter paper into a 10 ml volumetric flask and made up to volume.

Texture Profile Analysis (TPA) [25]

Textural parameters of formulation like hardness, springiness, chewiness and cohesiveness were analyzed using Texture Analyser.

Accelerated Stability Studies

These studies were carried out on cream and gel formulation by using REMI SC-6 plus stability chamber according to ICH guidelines. The accelerated stability conditions were $40^0C \pm 2^0C$ with75±5% RH. The physical stability of cream formulation was observed periodically for the pH, spreadability and viscosity **[26]**.

RESULTS AND DISCUSSION

The plant was authenticated from Botanical Department of Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur and Voucher specimen no:-10094 for *Curcuma longa,* Voucher specimen no:-10095 for *Hibiscus sabdariffa,* Voucher specimen no:-10096 for *Madhuca indica* and Voucher specimen no:-10097 was for *Cynodon dactylon.*

FTIR spectra was recorded for Curcuma *longa, Cynodon dactylon*, *Hibiscus sabdariffa* and physical mixture. All above characteristics peaks of drugs appeared in the spectra of the physical mixture at the same wave number indicating no modification or interaction between the *Curcuma longa, Cynodon dactylon, Hibiscus sabdariffa* and physical mixture.

TLC

The plant extract Rf value was match to the standard value for *Hibiscus sabdariffa*, *Curcuma longa.* The Rf value was found to be 0.62 and 0.46 respectively.

Phytochemical screening

The all plant extract were evaluated for their chemical composition and showed in **Table 1**.

Formulation

The plant extract were formulated in cream and gel formulation on trial and error basis and out of this we selected 9 formulation from cream and 09 formulation from gel for further evaluation parameter.

Evaluation of formulation

As per the above formulations FC1, FC2 and FC3 showed acidic pH Formulations FG6, FG7, FG8 and F9 shows low spreadability. Formulations FG3, FG4 and FG5 show satisfactory results. Thus they were selected for further accelerated stability study.

There were no microbial growth observed in nutrient agar media when placed in incubation for 48 hrs. Thus its shows

satisfactory microbial activity in cream and gel formulations.

Antioxidant activity of both CF and FG formulations showed antioxidant activity compare to marketed formulation and Ascorbic acid. The IC_{50} vale for CF and GF was found to be 301 and 401 which was within the range.

Total flavonoids content of prepared formulation showed considerable of activity when compared with the standard quercetin. The flavonoids are responsible the skin lightening efficacy. So the prepared formulation containing flavonoids is responsible to the skin lightening efficacy.

HPTLC:

The HPTLC study was carried out for all extract and formulation sand showed the desired results

The most abundant constituents of *Hibiscus sabdariffa* extract were found in in final formulations. The solvent system which used Toluene:Ethyl acetate(7:3) *Hibiscus sabdariffa* extract Rf value 0.62. In the physical mixture of extracts, cream formulation and gel formulation found Rf value nearby Hibiscus *sabdariffa* extract Rf value.

The most abundant constituents of *Curcuma longa* extract were found in in final formulations. The solvent system which used Hexane:Ethyl ether (9:1) *Curcuma longa* extract Rf value 0.43. In the physical mixture of extracts, cream formulation and gel formulation found Rf value nearby *Curcuma longa* extract Rf value also the *Cynadon dactylon* and Hibiscus *sabdariffa* containing β-sitesterol and ascorbic acid as well as flavonoids respectively. Hence it showed in formulation.

The most abundant constituents of *Cynodon dactylon* extract were found in in final formulations. The solvent system which used Toluene:Methanol (9:1) *Cynodon dactylon* extract Rf value 0.22. In the physical mixture of extracts, cream formulation and gel formulation found Rf value nearby *Cynodon dactylon* extract Rf value.

Texture analysis

From the analysis Hardness, Springiness, Chewiness of Herbal gel was evaluated and therefore increases in the Cohesiveness of Herbal gel. Hardness (N) of the Herbal Gel lowest ranged from 10126.42 (A3B3) and highest ranged from 10591.18 (A0B1). There were significant differences in the Hardness (N) of the Herbal gel contained herbs. According to the results of this research there were significant differences found in the Springiness, and Chewiness (P<0.05) in different experimental Herbal gel. Herbal gel containing 1% *Curcuma longa Hibiscus sabdariffa*, *Cynodon*

dactylon had significantly higher Springiness (0.632) and Chewiness of Herbal gel was recorded as the higher 655.60 (A1B0), as shown **Table 9**. The addition of Herbs increased the Cohesiveness of Herbal gel with the increase in the level of Herbs added. The lowest Cohesiveness for the control sample was 0.102 (A0B0) and the maximum was herbal gel was 0.151 (A3B3). In vectors represent a graphical display of the loading for the variables used in the PCA. The variables of cohesiveness, hardness and chewiness had similar loads than the variables of protein and fat content, indicating that changes in fat and protein content will affect these texture variables.

On comparing pre stability and post stability data of selected formulations, the formulations F4 and F5 showed satisfactory result while the Formulation F3 showed separation in one month stability. On the basis of evaluation of post stability samples formulation F5 gives an acceptable result.

Table 1: Phytochemical screening of plant extract

Plants	Phytochemical screening							
	Test							
	Carbohydrates	Proteins	Glycosides	Alkaloids	Tannins	Flavonoids	Phenolic compound	Steroids
Hibiscus *Sabdariffa*	++	++	++	++	+	++	++	+
Curcuma *longa*	++	++	++	++	+	++	++	+
Cynodon*dactylon*	++	++	++	++	+	++	++	-
Mahua oil	++	++	++	++	+	++	++	-

++ - Highly Positive test , +- moderately positive ,- negative test

Table 2 Formulation containing herbal extract

Ingredients	Formulation code								
Cream base	FC1	FC2	FC3	FC4	FC5	FC6	FC7	FC8	FC9
Madhuca *indica oil*	5g	7g	5g	5gl	5g	4g	5g	5g	5g
Hibiscus *sabdariffa*	0.5g	0.5g	0.4g	0.4	0.5	0.5g	0.5g	0.5g	0.5g
Curcuma *longa*	0.2g	0.2g	0.1g	0.1	0.1	0.1g	0.1g	0.1g	0.1g
Cynodon*dactylon*	0.2g	0.2g	0.2g	0.2	0.2	0.2g	0.2g	0.2g	0.2g
Stearic acid	1g	1g	2g	2g	2g	1 g	1g	1g	1g
Honey	---	1.5g	2g	4g	4g	4g	4.5g	6g	6g
Deionised water	qs	qs	qs	qs	qs	qs	qs	qs	qs
Total weight	15g	15g	15g	15g	15g	15g	15g	15g	15g
For Gel formulation	FG1	FG2	FG3	FG4	FG5	FG6	FG7	FG8	FG9
Carbopole 934 (1% w/v)	20g	20g	20g	20g	20g	20g	20g	20g	20g
Madhuca*indica oil*	6.5g	6.5g	6.5g	5g	5g	4g	5g	4g	5g
Hibiscus *sabdariffa*	0.5g	0.5g	0.4g	0.4g	0.5g	0.5g	0.5g	0.5g	0.5g
Curcuma *longa*	0.2g	0.2g	0.2g	0.1g	0.1g	0.1g	0.1g	0.1g	0.1g
Cynadon*dactylon*	0.2g	0.2g	0.2g	0.2g	0.2 g	0.2g	0.2g	0.2g	0.2g
Honey	--	2g	2g	4g	4g	4.5g	4g	5g	4g
Triethaloam-ine	qs	Qs	qs	qs	qs	qs	qs	qs	qs
Total weight	28g	30g	30	30g	30g	30g	30g	30g	30g

Table 3: pH, viscosity and spreadability of formulation

Formulation	pH	Viscosity (cp)	Spreadability (%)
FC1	4.83 ± 0.122	64833.3 ± 15.75	11.83 ± 0.476
FC2	4.51 ± 0.025	1133033.3 ± 17.62	11.92 ± 0.62
FC3	5.82 ± 0.052	64993.38 ± 21.07	12.62 ± 0.100
FC4	6.26 ± 0.40	64871 ± 14.81	11.91 ± 0.57
FC5	6.63 ± 0.25	64829 ± 44.136	11.88 ± 0.30
FC6	5.56 ± 0.058	77021± 14.01	10.70±1.016
FC7	6.23 ± 0.021	80273.3±45.44	10.00±1.030
FC8	6.82 ± 0.032	84996.0±32.38	11.90±0.868
FC9	5.14 ± 0.045	76237.2± 34.23	12.88 ± 0.30
FG1	5.83 ± 0.122	740000 ± 15.75	11.83 ± 0.476
FG2	4.51 ± 0.025	758300 ± 17.62	11.92 ± 0.62
FG3	5.82 ± 0.052	67993.38 ± 21.07	12.62 ± 0.100
FG4	6.26 ± 0.40	64871 ± 11.81	13.91 ± 0.57
FG5	6.63 ± 0.25	64829 ± 44.136	13.88 ± 0.30
FG6	5.56 ± 0.058	76021±43.01	16.50±1.026
FG7	6.23 ± 0.021	82273.3±45.44	17.10±1.010
FG8	6.82 ± 0.032	79996.0±34.38	10.85±0.821
FG9	5.14 ± 0.045	747692±45.23	11.83 ± 0.40

N=3

Table 4: Inhibition concentration of formulations

Sr. No.	Sample	% inhibition IC_{50} Value (µg/ml)
1	MF	130 (µg/ml)
2	GF	360 (µg/ml)
3	CF	401 (µg/ml)
4	Std Ascorbic acid	9 (µg/ml)

Table 5: Interpretation of HPTLC plate of *Hibiscus sabdariffa extract*

Peak	Start position	Start hight	Max position	Max hight	Max %	End position	End hight	Area	Area %
1	0.59 Rf	0.0AU	0.60 Rf	12.9AU	4.20	0.62 Rf	3.5AU	78.4 AU	3.15
2	0.58Rf	1.4AU	0.61 Rf	17.7AU	6.54	0.63 Rf	11.6AU	187.5AU	8.00
3	0.62 Rf	5.9AU	0.66 Rf	22.8AU	6.01	0.67 Rf	4.3 AU	226.3AU	4.64
4	0.76 Rf	6.3AU	0.80 Rf	14.7AU	5.07	0.83 Rf	2.0 AU	216 AU	8.43

Table 6: Interpretation of HPTLC plate of *Curcuma longa extact*

Peak	Start position	Start hight	Max position	Max hight	Max %	End position	End hight	Area	Area %
1	0.43Rf	0.5AU	0.44Rf	271.0AU	17.08	0.45Rf	2.7AU	93.7 AU	23.75
2	0.43Rf	107.3AU	0.48Rf	182.8AU	11.54	0.59Rf	70.6AU	408.5 AU	8.00
3	0.40Rf	75.9AU	0.45Rf	14.3AU	4.61	0.29Rf	4.3 AU	226.3 AU	4.64
4	0.43Rf	6.3AU	0.45 Rf	14.7 AU	5.07	0.43 Rf	2.0 AU	216 AU	8.43

Table 7: Interpretation of HPTLC plate of *Cynodon dactylon* extract

Peak	Start position	Start hight	Max position	Max hight	Max %	End position	End hight	Area	Area %
1	0.22Rf	8.1AU	0.25Rf	16.6AU	4.61	0.29Rf	4.3AU	301 AU	3.767
2	0.24Rf	8.9AU	0.26Rf	19.5AU	7.93	0.29Rf	8.6AU	337.8AU	5.84
3	0.11Rf	27.8AU	0.14Rf	46.3AU	21.60	0.19Rf	5.7 AU	101.3 AU	24.64
4	0.16Rf	19.3AU	0.28Rf	274 AU	52.20	0.33 Rf	60.5AU	9524.5 AU	59.91

Final formulation (Post stability)

Table No 8: Final formulation of gel. evaluation

Formulation	pH	Viscosity (cp)	Spreadability (%)
FG3	6.17 ± 0.056	65993.38 ± 212.07	13.12 ± 0.110
FG4	6.36 ± 0.45	66871 ± 124.71	12.91 ± 0.47
FG5	6.83 ± 0.25	64829 ± 44.136	11.88 ± 0.30

Values expressed as Mean ± SD,n=3

FTIR study:

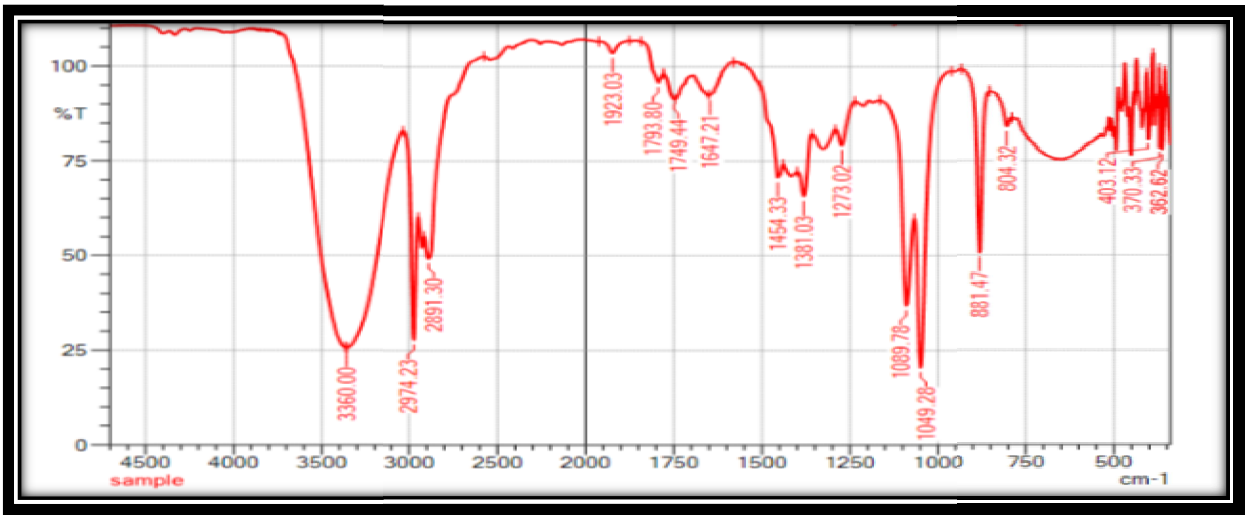

Figure 1: FTIR graph of Physical mixture

Microbial study of formulations

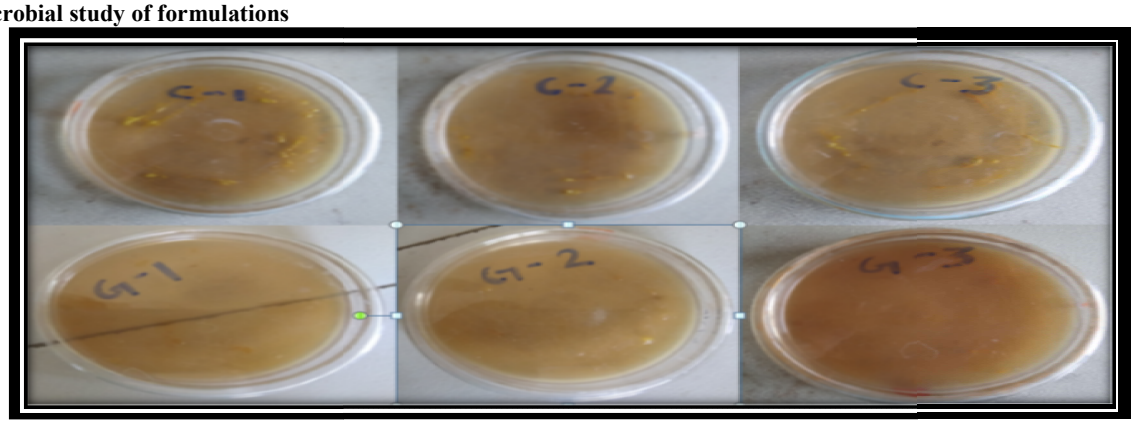

Figure 2: Microbial growth study of cream and gel formulations

***In -vitro* DPPH free radical scavenging activity:**

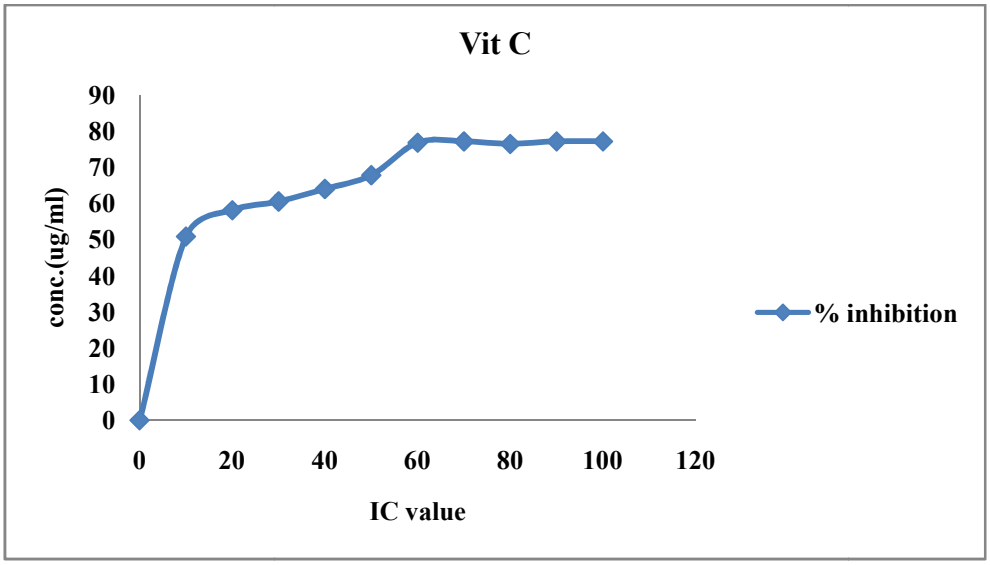

Figure 3: Antioxidant properties of Ascorbic acid

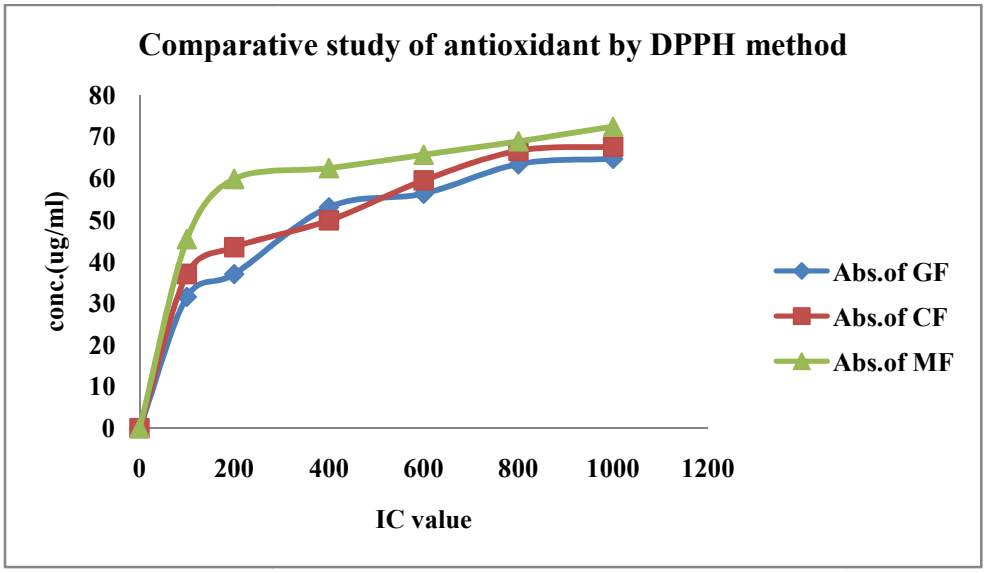

Figure 4: Comparative study of antioxidant marketed formulation, gel formulation and cream formulation

Total flavonoids content

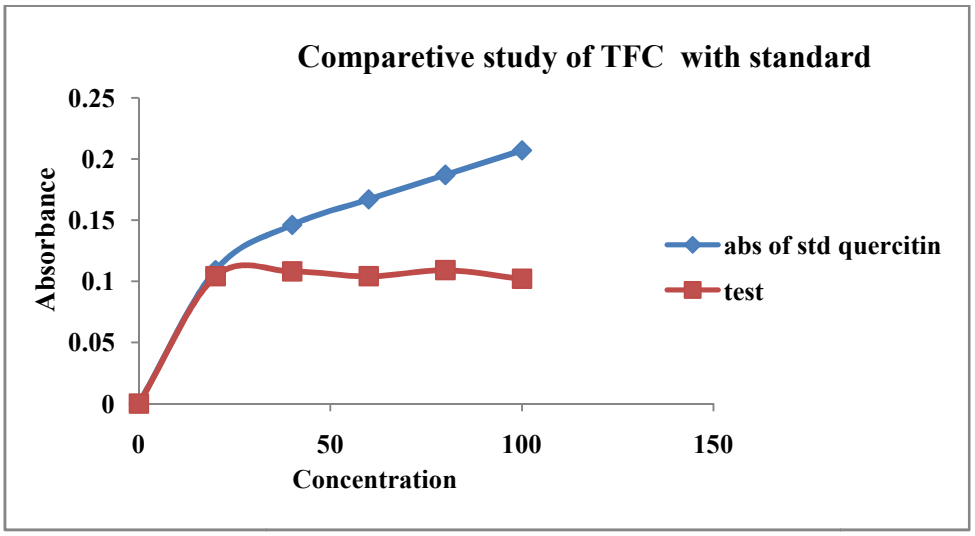

Figure 5: Total flavonoids content of formulation compare with the standard Quercetin

HPTLC chromatogram of Hibiscus *sabdariffa* extract

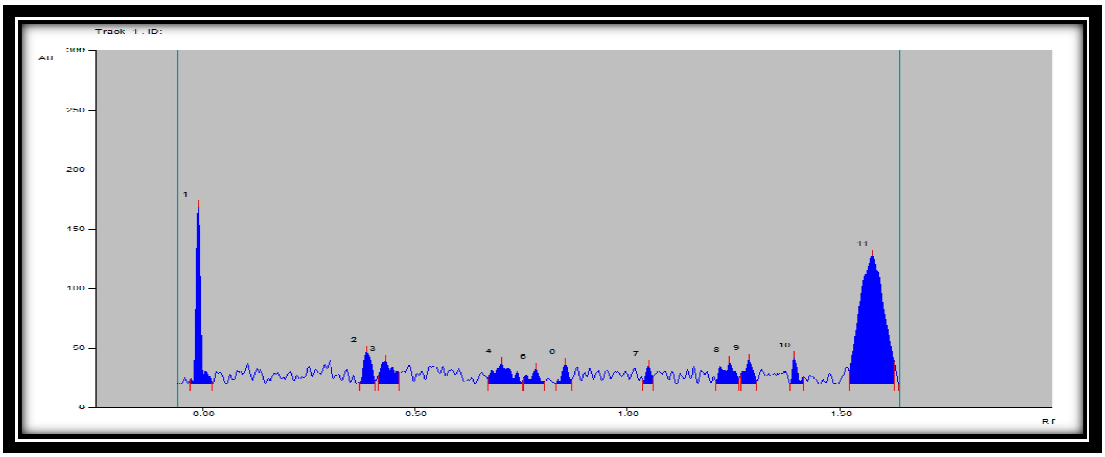

Figure 6: Peak display of *Hibiscus sabdariffa* extract

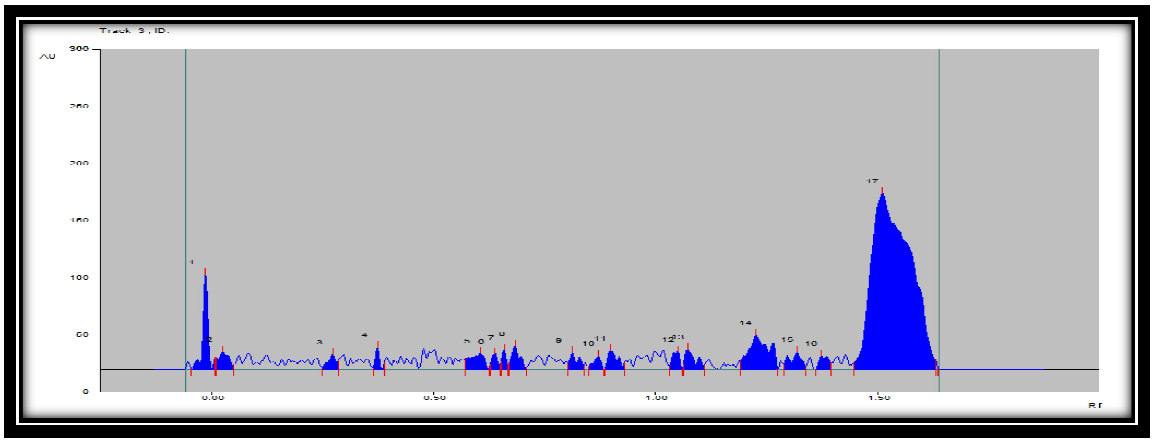

Figure 7: Peak display of *Hibiscus sabdariffa* extract in cream formulation

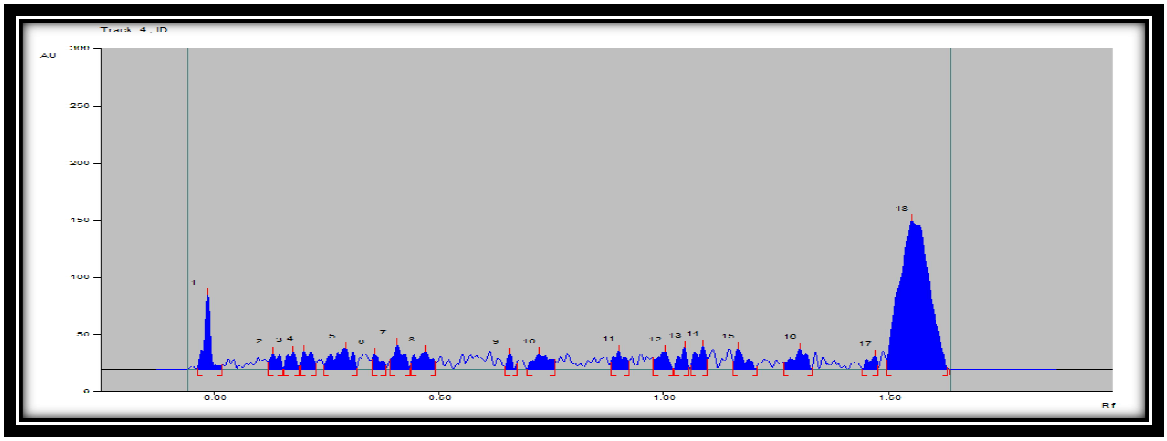

Figure 8: Peak display of *Hibiscus sabdariffa* extract in gel formulation

HPTLC chromatogram of *curcuma longa* extract

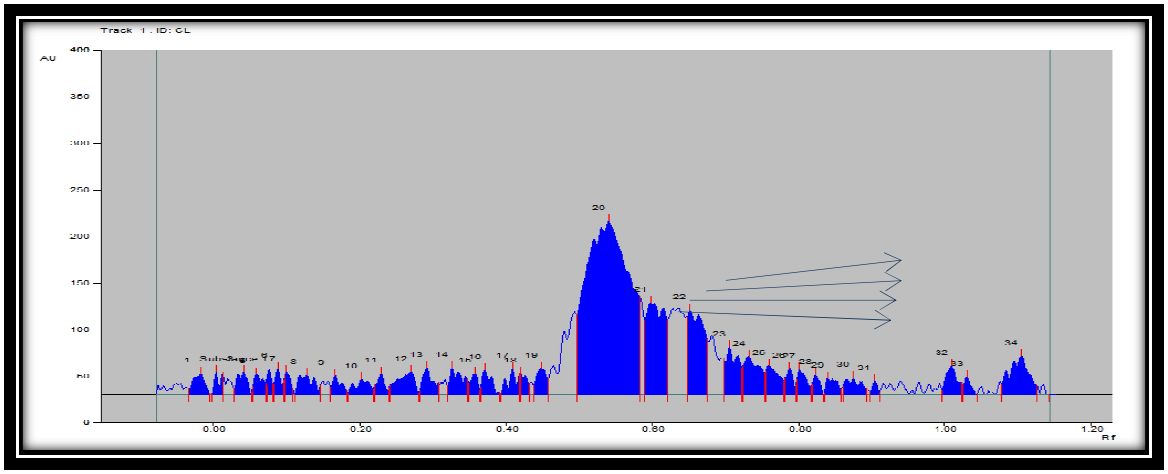

Figure 09: Peak display of *Curcuma longa* extract

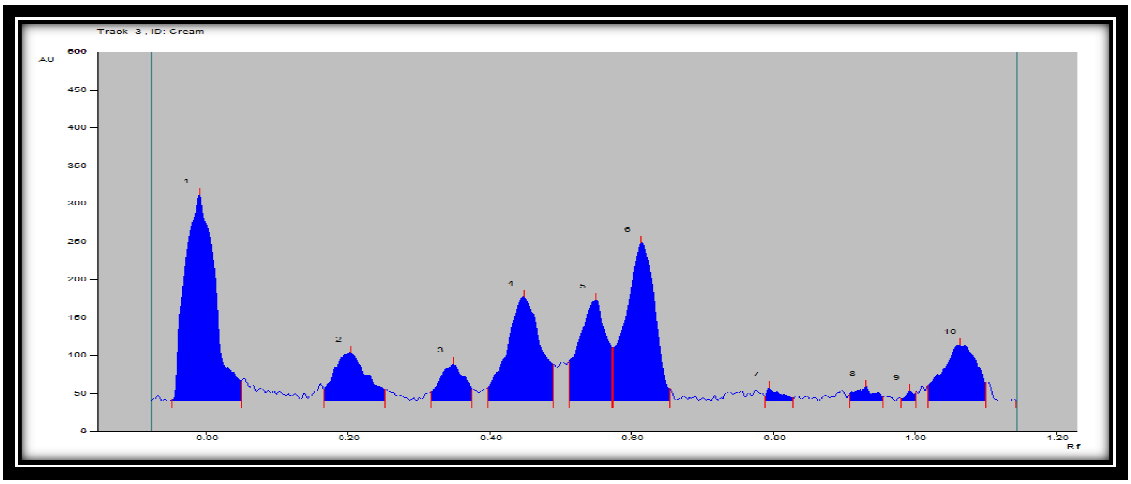

Figure 10: Peak display of *Curcuma longa* extract in cream formulation

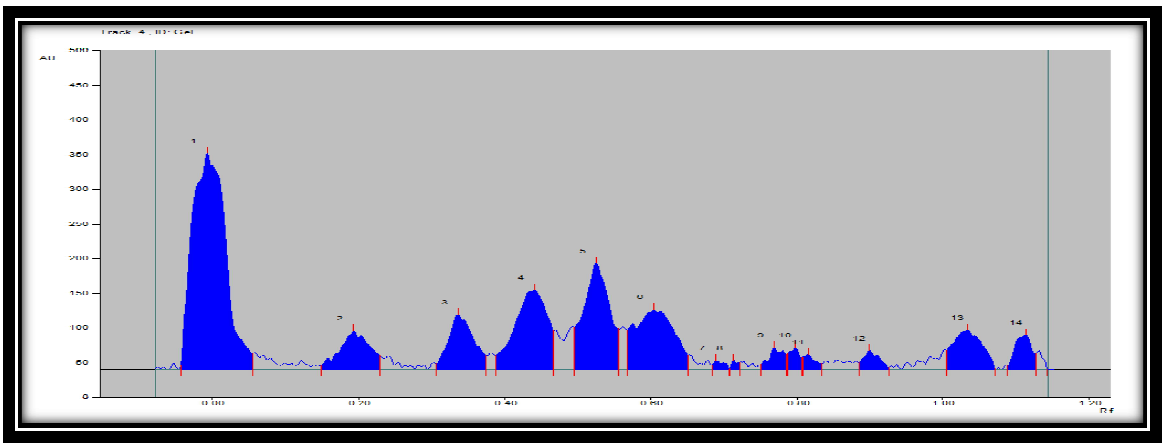

Figure 11: Peak display of *Curcuma longa* extract *in* gel formulation

HPTLC Chromatogram of *Cynadon dactylon* extract

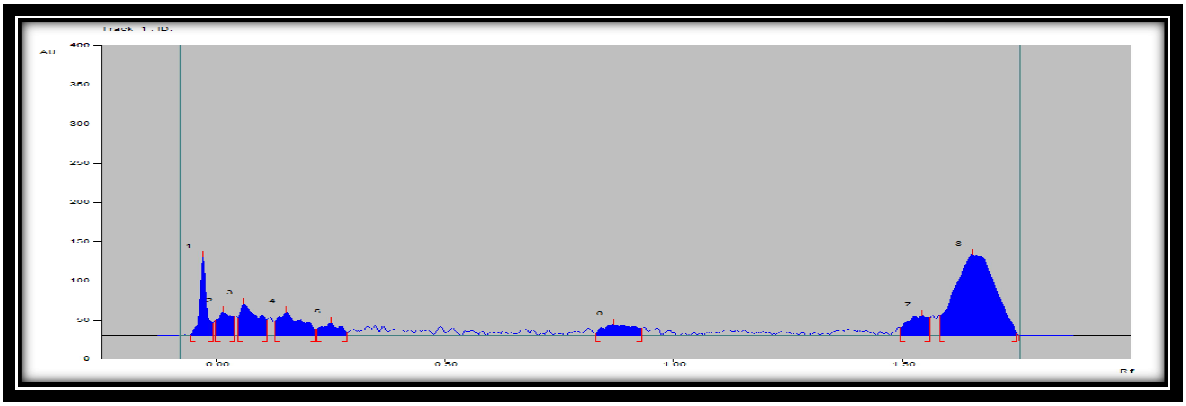

Figure 12: Peak display of *Cynodon dactylon* extract

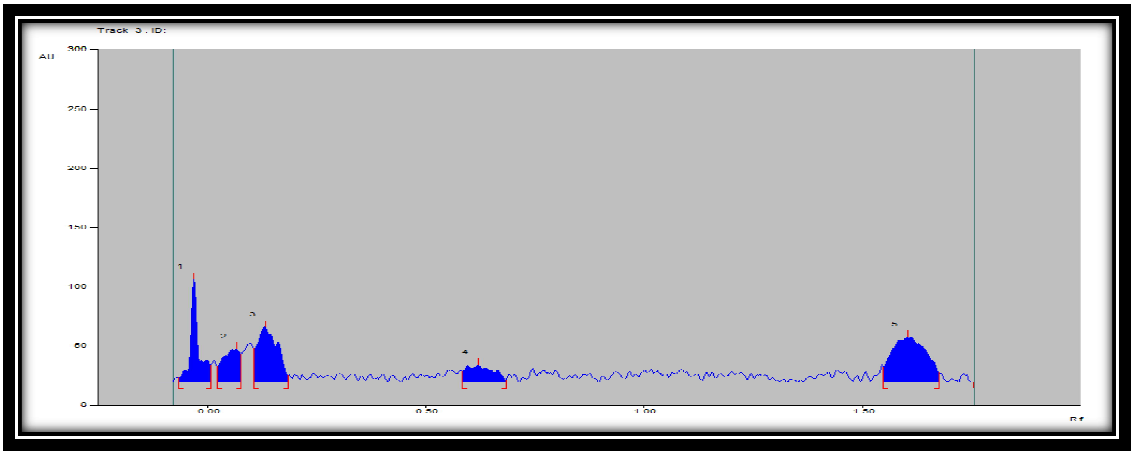

Figure 13: Peak display of *Cynodon dactylon extract in* cream formulation

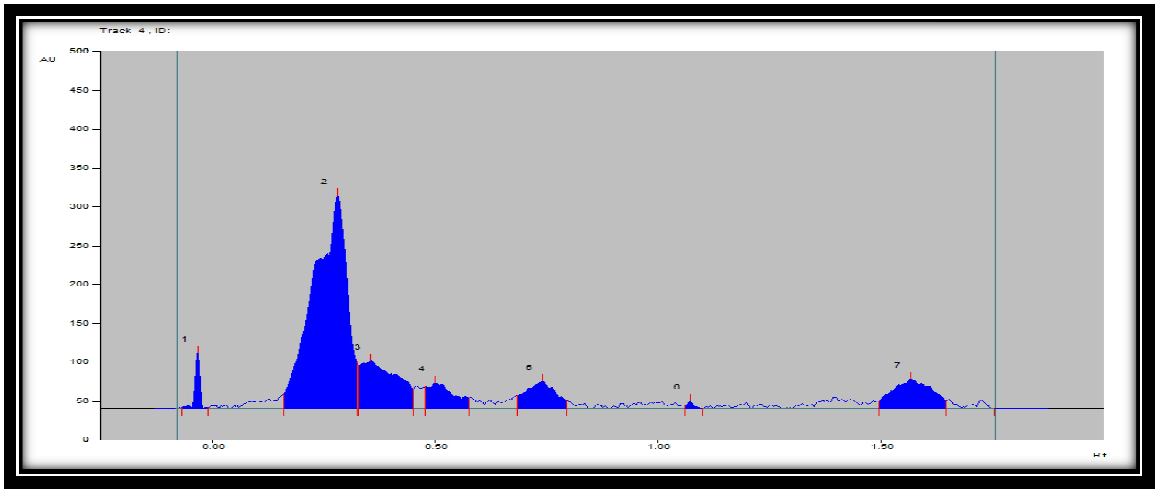

Figure 14: Peak display of *Cynodon dactylon* extract in gel formulation

Rheological Properties of Herbal gel formulation

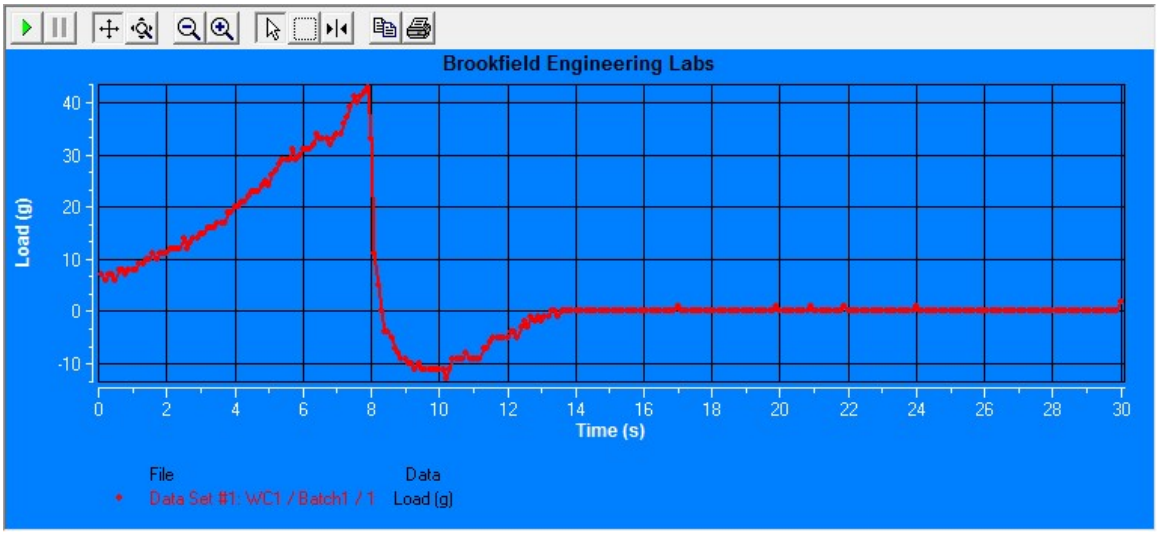

Figure 15: Texture analysis of gel formulation

CONCLUSION

According to phytochemical study of herb extracts and determination of total flavonoids (Curcumin) content study they may have hypo pigmenting capabilities by directly inhibiting tyrosinase activity at distal portions of the melanogenic pathway. The flavonoids are responsible for the skin whitening efficacy. Stability parameters like visual appearance, nature, viscosity and of the formulations showed that there was no significant variation during the study. The prepared formulations showed is approximately pH 6 pH range that is near to skin pH; it confirms the compatibility of the formulations with skin. HPTLC, total flavonoids content and anti oxidants studies showed the flavonoids present in formulation Hence result showed Tyrosinase inhibitors are present in the compositions. Hence our aim of this present study was achieved.

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