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EXTRACTION AND FORMULATION OF TOCOPHEROL CREAM AND ITS ANTIOXIDANT ACTIVITY

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ABSTRACT

Tocopherol is a fat soluble vitamin and potent antioxidant. It is otherwise called as Vitamin-E. It plays an important role in the protection of essential fatty acids against oxidative deterioration of cells. It helps in regulating immune function and balances normal coagulation in human body .Tocopherol is found naturally in many foods such as green leafy vegetables, nuts, seeds and vegetable oil. Even the vegetable waste contains the more amount of Tocopherol. The present study was to ensure the presence of Tocopherol content from the waste parts of *Brassica oleracea var.capitata* (Cabbage), *Brassica oleracea var. botrytis* (Cauliflower) , *Beta vulgaris* (Beetroot), *Brassica rapa subsp.rapa* (Turnip) by using various extraction technique. The components of the extracts were quantified by HPLC technique. And then a comparative study was performed. The result shows that *Brassica oleracea var.capitata* (Cabbage) has the highest Tocopherol content of 306mg/ml when compared to other vegetable waste. Thus, this study confirms the presence of Tocopherol in the waste parts of all these vegetables. Then, extracts of *Brassica oleracea var.capitata* (Cabbage) and *Beta vulgaris* (Beetroot) were taken for formulation of a skin cream because they have vitamin A, D, E in common and then antioxidant assay was performed to know their antioxidant activity

KEYWORDS: Tocopherol, *Brassica oleracea var.capitata* (Cabbage), *Brassica oleracea var. botrytis* (Cauliflower), *Beta vulgaris* (Beetroot), *Brassica rapa subsp.rapa* (Turnip), HPLC technique, antioxidant

1.INTRODUCTION

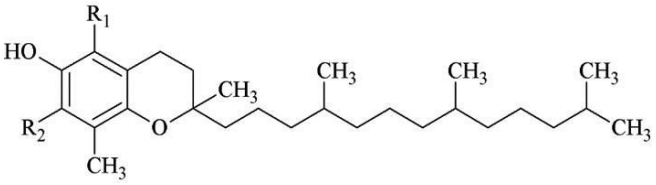
TOCOPHEROL

Tocopherol is a fat soluble vitamin with antioxidant properties that is important for the protection of essential fatty acids in the human body. It has four forms namely α (Alpha), β (Beta),γ (Gamma), δ (Delta), which is determined by the numbers and position of methyl groups on the chromanol ring. Among the four forms of Tocopherol, Alpha Tocopherol is the most abundant one and usually consumed by the humans. Tocopherol acts as an antioxidant, which is important in protecting cells from oxidative stress and cell damage. It also helps in regulating immune functions in the human body. The other properties of Tocopherol include anticancer, anti-inflammatory, immuno-stimulatory and nephroprotective. Naturally, Tocopherol can be found in many foods like Green leafy vegetables, Nuts, Seeds, fruits and Vegetable oil. Based on the nature of sample, various extraction methods can be used such as Soxhlet extraction, Saponification, Direct solvent extraction can be used to release Tocopherol.(DellaPenna D, Pogson BJ.2006,57:711-738)

Fig 1.1: Alpha-Tocopherol

STRUCTURE

Tocopherol structure contains three chiral carbons, one is present at the C_2 in the chromanol ring and the other two present at C_4 and C_8 in the side chains. Naturally occurring α-Tocopherol contains chiral carbons, in the R-conformation in, 2R,4'R and 8'R α- Tocopherol. The chemical synthesis of α-Tocopherol contains eight different stereoisomer's: RRR, SRR, RSR, RRS, RSS, SSR, SRS and SSS. The ester forms of Tocopherol are acetate, succinate and phosphate.(DellaPenna D, Pogson BJ.2006,57:711-738)



Tocopherol	R_1	R_2
α-	CH_3	CH_3
β-	CH_3	H
γ-	H	CH_3
δ-	H	H

Fig 2: TOCOPHEROL STRUCTURE ALONG WITH ITS NUMBER AND POSITION OF METHYL GROUPS ON THE AROMATIC RINGS.

FUNCTIONS OF TOCOPHEROL

Tocopherol plays an important role in regulation of cell growth, proliferation, apotosis, adhesion, angiogenesis and inflammation. It also acts as a free radical scavenger in lipid phase of cell membrane. Generally, Tocopherol helps to prevent oxidative stress and protects cell membrane. It plays a key role in production of prostaglandins, which helps in regulating muscle contraction and controls the blood pressure. It increases the energy level of the body and reduces the fatigue of muscles, after an intense workout. It is key ingredient for the nourishment of hair and prevents hair fall. It prevents ageing and reduces the appearances of scars and also helpful in maintaining the moisture content in the skin. (Anglemeyer A, Horvath HT, Bero L (April 2014)

2.MATERIALS AND METHODS

Sample Collection

The waste parts of vegetables like *Brassica oleracea var.botrytis* (cauliflower), *Brassica oleracea var.capitata* (Cabbage), *Brassica rapa subsp.rapa* (Turnip), *Beta vulgaris* (beetroot) were collected and shade dried for nearly two weeks and then it was made into a coarse powder using a normal mixture grinder

EXTRACTION METHODS:

SOXHLET METHOD OF EXTRACTION:

The powdered samples of all the vegetable wastes were separately subjected to the Soxhlet extraction process. 15g of all the samples were weighed separately and taken in a separate beakers 1mg of NaOH was added in all the beakers and the extraction process was carried out with 200ml of Hexane solution in a Soxhlet apparatus and then the same procedure is repeated for all the other samples. The process of extraction was carried around 3 hours for each sample. The obtained extracts was subjected for evaporation under rotary evaporator. After the complete evaporation, solvents were stored at refrigerator under 4^0C

MACERATION TECHNIQUE

15gm of all the powdered samples were weighed and taken separately in a conical flask and 150ml of ethanol was added in each flask and kept boiling at 100^0c for 15 minutes. The supernatant was decanted and the mixture was poured into separating funnel. Then 40ml of Hexane was added in all the funnels and left undisturbed for 2mins for the layer separation. The separation of two layers such as top layer and bottom layer were achieved. The process has been repeated until the appearance of Yellowish colour. The extracts was then subjected to evaporation after complete evaporation the extracts was stored at 4^0C in refrigerator.

QUANTITATIVE DETERMINATION USING HPLC

HPLC analysis was performed for all four samples under same procedure for all the extracts that was obtained through soxhlet extraction technique. The running volume is 100% methanol in a column and with the following gradient for elution were used. The flow rate was 1.5ml/min in the time of 25 minutes at 36^o C. The injection volume was 100µl. It was controlled by empower software program with the photodiode array detector (PDA).

FORMULATION OF CREAM

PREPARATION PLAN OF CREAM

INGREDIENTS	F1 *Beta vulgaris* (BEETROOT CREAM)
Aloevera gel	1.5 ml
Beet extract	0.5 ml
Bees wax	3 g
Liquid paraffin	5 ml
Borax	0.2 g
Distilled water	0.1 ml
Rose oil	0.1 ml

FIG 3.SHOWS THE INGREDIENT FOR THE PREPARATION OF F1 *Beta vulgaris* (BEERROOT CREAM)

INGREDIENTS	F2 *Brassica oleracea var.capitata*(CABBAGE CREAM)
Aloevera gel	1.5 ml
Beeswax	3 g
Cabbage extract	0.5 ml
Liquid paraffin	5 ml
Borax	0.2 g
Distilled water	0.1 ml
Rose oil	0.1 ml

FIG 4. SHOWS THE INGREDIENT FOR THE PREPARATION OF *Brassica oleracea var.capitata* (CABBAGE CREAM)

The liquid paraffin wax and beeswax was heated at 75^{o} C in a beaker and the temperature was maintained in it. And in another beaker Borax was dissolved in distilled water and heated at 75^{O} C, the aqueous phase was maintained and slowly the aqueous solution was added to the heated oily phase and measured amount of *Aloe barbadensis miller* (aloevera gel), *Glycyrrhiza glabra* (Athimadhuram|) powder and *Rubia cordifolia* (Manjistha) powder were added in it. The prepared sample and the *Beta vulgaris* (Beetroot) extract and *Brassica oleracea var.capitata* (cabbage) extract was added separately and shaken vigorously until it forms a smooth cream and then rose oil was added in drops as a fragrance agent.

PARAMETER ANALYSIS

COLOUR, ODOUR AND CONSISTENCY

Physical parameters like colour and odour and consistency of the cream prepared from Cabbage and Beet Green extracts were examined by visually to know about their physical characteristics.

pH

pH of prepared cream can be measured using the digital pH meter. The cream solution was prepared separately for the creams by dissolving them in 100 ml of distilled water and was left undisturbed for 2 hours, later the pH values were noted.

SPREADABILITY

The spreadability was determined by replacing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time taken to separate the two slides was measured as spreadability, lesser the time taken for separation of two slides results better spreadability. It was calculated by the below given formula,

$$S = (ML) / T$$

S = Spreadability

M =Weight tide to the upper slide

L = Length of glass slide

T= Time taken

SOLUBILITY

The solubility of the prepared creams were analyzed by dissolving the prepared creams in the help of boiling H_2O, and dissolving the 2ml of cream solution in the organic solvents like alcohol, ether or chloroform to know their miscibility level.

ANTIOXIDANT ASSAY

PROCEDURE 1

DPPH (2,2- diphenyl-1-picrylhydrazyl) assay for the cream prepared using *Beta vulgaris* (Beetroot) extract.

At first six test tubes were taken and washed cleanly with washing liquid and left for drying. The test tubes were named as blank, C1, C2, C3, C4 and C5. In blank test tube, 2ml of DPPH solution was taken. Then added concentration of the prepared ***Beta vulgaris*** **(Beetroot)** extract, in C1 test tube 50µl, C2 test tube 100µl, C3 test tube 150µl, C4 test tube 200µl and in C5 test tube 250µl concentration of the prepared Beet extract cream was taken. Then 2ml of DPPH solution was added sin all the test tube from C1 to C5 then all the test tubes were incubated at dark 20-30 minutes. Then UV reading was taken at 517nm to know the antioxidant activity.

PROCEDURE 2

DPPH (2,2- diphenyl-1-picrylhydrazyl) assay for the cream prepared using *Brassica oleracea var.capitata* (CABBAGE) extract.

First six test tubes were taken and washed cleanly with washing liquid and left for drying. The test tubes were named as blank, C1, C2,C3, C4,C5. At the blank test tube 2ml of DPPH solution was taken as blank, in C1 test tube 50µl, C2 test tube 100µl, C3 test tube 150µl, C4 test tube 200µl and in C5 test tube 250µl concentration of the prepared Cabbage extract cream was taken. Then 2ml of DPPH solution was added sin all the test tube from C1 to C5 then all the test tubes were incubated at dark 20-30 minutes. Then UV reading was taken at 517nm to know the antioxidant activity.

3. RESULTS

SAMPLE COLLECTION:

Fig 5. Sample collection of vegetables like *Brassica oleracea var.capitata,Beta vulgaris,Brassica rapa subsp.rapa, Brassica oleracea var. Botrytis*

EXTRACTION PROCESS

Fig 6. Soxhlet extraction process of *Brassica oleracea var.capitata,Beta vulgaris,Brassica rapa subsp.rapa, Brassica oleracea var. Botrytis*

Fig 7. Maceration technique of *Brassica oleracea var.capitata,Beta vulgaris,Brassica rapa subsp.rapa, Brassica oleracea var. Botrytis*

EXTRACTS OF SAMPLES:

Fig 8. OBTAINED EXTRACTS FROM THE SAMPLES

QUANTITATIVE DETERMINATION

HPLC ANALYSIS:

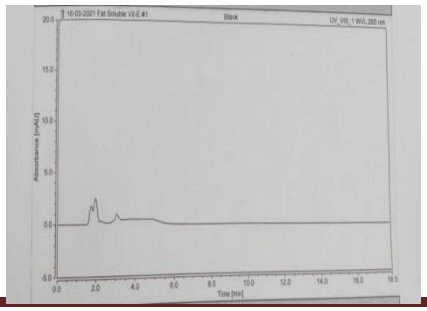


Fig 9. BLANK REPORT OF HPLC

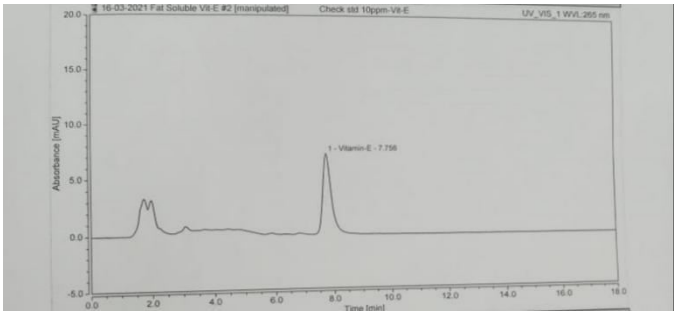


Fig 10. STANDARD HPLC REPORT (ALPHA TOCOPHEROL ACETATE)

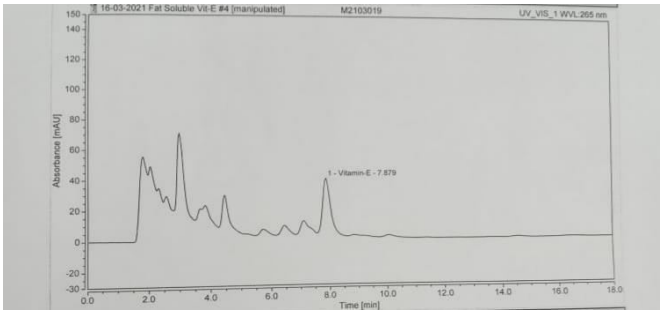


Fig 11. HPLC REPORT OF *Brassica oleracea var. botrytis* (CAULIFOWER)

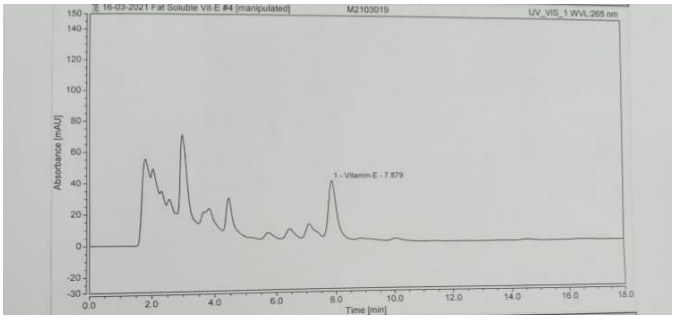


Fig 12. HPLC REPORT OF *Brassica oleracea var.capitata* (CABBAGE)

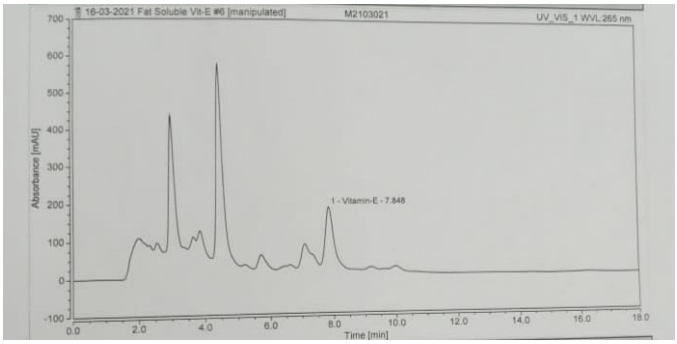


Fig 13. HPLC REPORT OF *Brassica rapa subsp.rapa* (TURNIP)

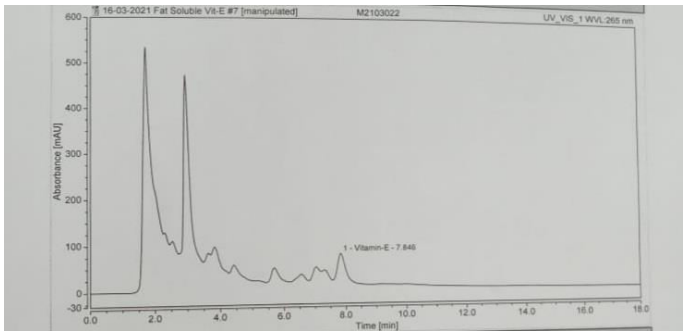


Fig 14. HPLC REPORT OF *Beta vulgaris* (Beetroot)

S.NO	NAME OF THE SAMPLE	PEAK NAME	RETENTION TIME (min)	AREA mAU*SEC
1	Blank	Vitamin-E	n.a.	n.a.
2	Standard	Vitamin-E	7.756	153.619
3	*Brassica oleracea var.capitata* (Cabbage)	Vitamin-E	7.853	4995.361
4	*Beta vulgaris* (Beetroot)	Vitamin-E	7.846	1068.714
5	*Brassica rapa subsp.rapa* (Turnip)	Vitamin-E	7.848	2910.859
6	*Brassica oleracea var. botrytis* (Cauliflower)	Vitamin-E	7.879	703.824

Fig 15.HPLC REPORT OF TOCOPHEROL

FORMULATION OF CREAM

PRODUCT

Fig 16. ***Brassica oleracea var.capitata*** **(Cabbage) cream**

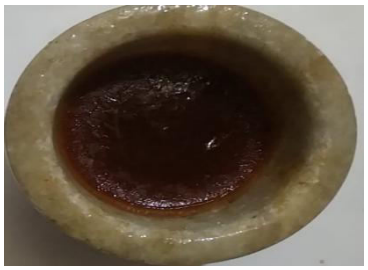


Fig 17.*Beta vulgaris* (Beetroot) cream

PARAMETER ANALYSIS OF PREPARED CREAM

PARAMETERS TO BE ANALYZED DURING THE CREAM FORMULATION	STANDARD VALUES OF A PARAMETERS THAT A CREAM SHOULD HAVE IN GENERAL	VALUES OBTAINED FROM THE FORMULATED BEETROOT (*Beta vulgaris*) CREAM	VALUES OBTAINED FROM THE FORMULATED CABBAGE (*Brassica oleracea var.capitata*) CREAM
COLOUR	Any colour	Red colour	Pale yellow
CONSISTENCY	Thick	Thick	Thick
ODOUR	Mostly pleasant odour	Pleasant odour	Pleasant odour
pH	5.8 - 6.9	6.5	6.3
SPREADABILITY	Lesser the time greater he sreadability	10 Sec	8 Sec
SOLUBILITY	Should be soluble in water or organic solvents	Soluble in both water and organic solvents	Soluble in both water and organic solvents

Fig 18. The tabulated form of results for the creams prepared.

ANTIOXIDANT ASSAY

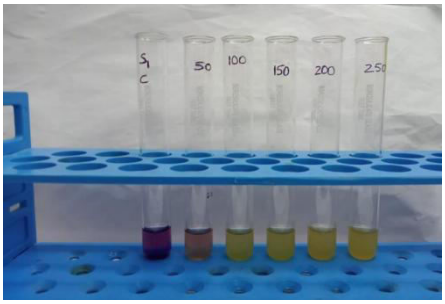


Fig 19. DPPH assay of ***Brassica oleracea var.capitata*** **(Cabbage cream)**

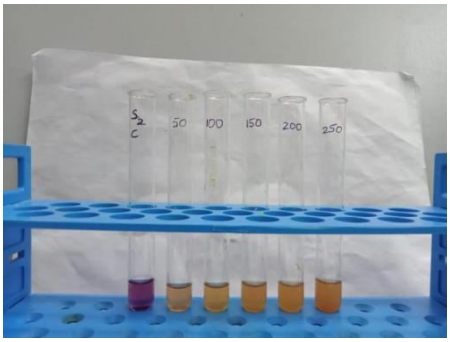
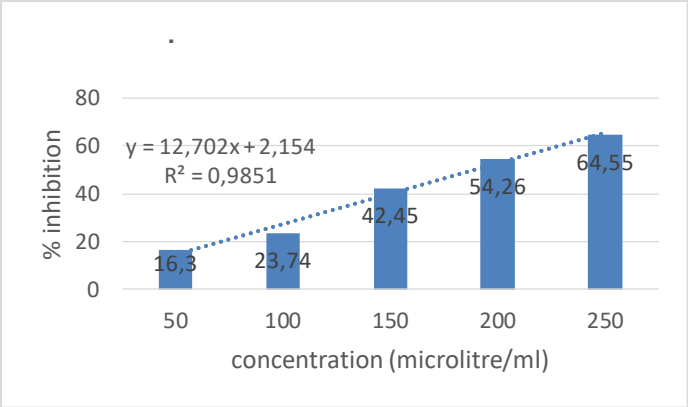


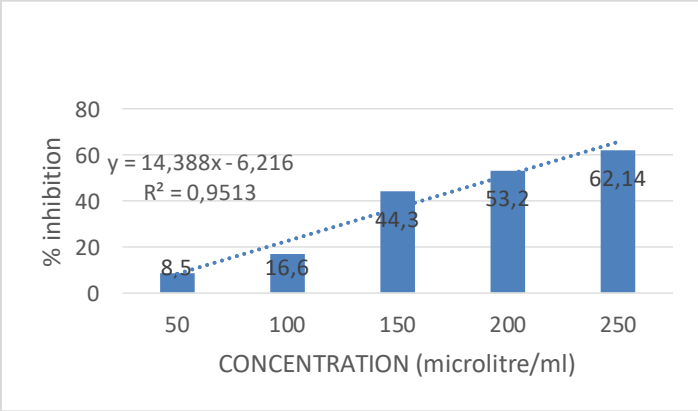
Fig 20. DPPH assay of ***Beta vulgaris*** **(Beet green)**

S.NO	NAME OF THE TEST TUBE	QUANTITY OF THE SAMPLE ADDED	AMOUNT OF THE DPPH SOLUTION	OD AT 517nm	% RADICAL SCAVENGING ACTIVITY	IC50 VALUE
1	Blank	-	2ml	0.914	-	-
2	C1	50µl	2ml	0.765	16.3	1.11
3	C2	100 µl	2ml	0.697	23.74	1.69
4	C3	150 µl	2ml	0.526	42.45	3.17
5	C4	200 µl	2ml	0.418	54.26	4.10
6	C5	250 µl	2ml	0.324	64.55	4.91

Fig 21. Reading of UV-Vis Spectrometer of ***Brassica oleracea var.capitata*** **(cabbage)**



GRAPH-1: DPPH INHIBITION (ANTIOXIDANT ACTIVITY) OF CABBAGE CREAM. ,



GRAPH2: DPPH INHIBITION (ANTIOXIDANT ACTIVITY) OF FORMULATED BEETROOT CREAM

4. DISCUSSION

The studies as of now shows that **Ramesh Kumar Saini *et al.,*** (2016) reviewed the methods of extraction , chromatographic separation, detection of Tocopherols and tocotrienols in plants and their products. Tocols is a well-known for antioxidant, anticancer, anti-inflammatory, immuno- stimulatory and nephroprotective properties, but didn't involve in the cream formulation, with natural ingredients .,

Andreas Schieber *et al.,* (2002) and many other conducted their studies on the identification of Tocopherol , Tocotrienols and other types of vitamin contents in the vegetable samples but they didn't study those contents from the vegetable wastes and didn't involve in cream formulation against the general skin problems such as tan, Sun burns, etc., that occurs on the daily basis

Our study was performed not only to identify the Tocopherol (Vitamin E)content from the vegetable waste of *Brassica oleracea var.capitata*(cabbage),*Brassica oleracea var.botrytis*(cauliflower),*Brassica rapa subsp.rapa*(turnip),*Beta vulgaris*(Beet root). But also to formulate poly herbal skin cream and to know their antioxidant level whether can be used as a skin care cream on the daily basis. Naturally all these vegetables contains vitamins, minerals and natural antioxidants. In addition it also has anti-bacterial, anti-inflammatory, anti- cancer properties. The Comparative study was performed for the above mentioned vegetable waste to ensure the presence of Tocopherol content in their wastes that being usually ignored were the other parts them are being used .

It was performed with the help of Soxhlet and Maceration extraction methods. Then the extracts obtained from soxhlet process was taken for analysis using HPLC (High performance liquid chromatography) technique. The result were shown that all these vegetable waste contains the Tocopherol(Vitamin E),Vitamin A, Vitamin D and other vitamins and minerals .Among them *Brassica oleracea var.capitata* (cabbage) contains the high amount of Tocopherol content of 306mg/kg. Then the formulation of herbal cream is done using the extracts of the *Brassica oleracea var.capitata*(cabbage),*Beta vulgaris*(Beet root).And then the formulated two herbal cream were analyzed for Antioxidant activity using DPPH(2,2-diphenyl-1-picrylhydrazyl) assay. The UV (Ultraviolet-Visible) spectrometer readings were taken at 517nm for the formulated herbal cream sample. The Results were shown that the highest concentration of the formulated herbal cream sample shows the anti-oxidant activity.

5. CONCLUSIONS

Tocopherol (Vitamin E) is one of the important nutrient that must be present in all human beings. The consumption of Tocopherol containing foods are more important because it contains various medicinal properties like Antioxidant activity, anti-bacterial, anti-inflammation etc. It also plays an important role in cancer prevention, balancing the blood coagulation, boosting of the immune system against various micro-organisms. It also aid in prevention of skin related diseases and helpful in prevention of aging problems in humans.

ACKNOWLEDGEMENT

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