THINK INDIA Journal

ISSN:0971-1260
Vol-22-Issue-16-August-2019

Characterization of Synthetic Cream Containing Carvacrol

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Abstract

Ageing is both a biological and sociological process wherein human beings experience and accomplish stages of biological and social maturation. Ageing may be seen as a relatively objective biological process whereby one becomes older and experiences varied biological developments. The causes of ageing may be due to accumulation of damage cells caused by DNA oxidation. One way to slow down aging is by the use of antioxidants.Antioxidants help prevent or stop cell damage caused by oxidants.Antioxidants occur naturally in plants including flavanols, resveratrol and lycopene. Carvacrol one of a flavonoid found in the essential oil of *Oreganum vulgare*having anti-oxidant property used in formulation for cream preparation. The pharmacophore study of formulated cream was done and resulted in high viscosity and spread-ability.

Key Words: Ageing, Flavonoids, Carvacrol and Pharmacophore study

INTRODUCTION

Ageing is an inescapable and traversable process, but it is not necessarily negative. It can't be defined exactly, but largely accepted concept is that it is a crucial part of one's life cycle: which is if a person is born, he/she goes through childhood, followed by adolescence and then adulthood and, at one certain point in time, starts to age. But the ageing process doesn't commence at the same time as everyone, and not even for all the organs for the same person at the same rate [1]. It's challenging to describe ageing; as it involves loss of faculties, but it can

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sometimes prove beneficial. Ageing has a wide-ranging anomaly involving both physical and psychological process. Considering biological perception of ageing, internal organs start to lose their strength with increase in age [2-3]. Chronological age is that appears in the legal documents: the number of years the person survived. Whereas biological age is the age any person desire to survive for instance an eighty-year-olds want to look twenty years younger [4]. These set of people are more active than most people of the same age group. In fact, there are ways of quantifying this (for instance, by checking the testosterone levels they produce). But it's hard to do this methodically, as we might find someone who has likewise renal capacity at the age of 70 as at the age of 40 but is socially inactive or walks slowly. In any case, it's very vital to assume a certain biological age, as people may not be as their arrival advocates. At the age of 30, bone mass stops to increase. It's also said that after a certain age the brain no longer has the competency to learn new things. Then the inquest of hormones comes in: the menopause in females, decreasing levels of testosterone in men [5]. There are certain factors that might increase the risk of diseases: the pancreas ages and ceases to secrete insulin causing diameter bêtes, we put on weight or lose weight, blood vessels narrow down, and blood pressure might increase causing a stroke. The hair turns white, males may go bald, need glasses to see far off or up close, need hearing aids less, and most importantly the skin becomes loose and our face begins to point out wrinkles. Once we observe our skin, it's two-faced normally inherent or written record ageing, with none environmental impacts, it's even and unremarkably untarnished. In oppositeness, extrinsically-aged skin (such that found on our face, hands and chest) it's wrinkled, shallow in colour and has parts of hyper- and physiological state. Skin shows a loss of tone and snap, high fragility, parts of blood disorder because of weak blood vessels and benign lesions like disease of the skin, telangiectasia and skin tags. In microscopic examination, a lot of restrained advancements are visible within the albuminoidal and albuminoidal, that currently thick and fragmented indicate the cross-linking that is allied with wrinkle development [6].

Wrinkles are the depressions on the surface of skin which will be fine or course, it depends on the depth. Wrinkle depth extends from a few of micrometres to a few of millimetres. Skin staining is the alteration in colour, incorporating red, blue, brown and yellow pigmentation [7]. Ageing is differentiated into two types:intrinsic ageing and extrinsic ageing.Intrinsic ageing includes change in the DNA and decline of growth hormone. Extrinsic ageing involves the

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changes in skin due to sunrays, diet and smoke. Thereby, skin starts losing the collagen and elasticity. The color of our skin is defined by melanin content. Melanin gets disrupted and leads to the production of spots in skin in presence of sunlight. Moreover, deregulation of the vasculature respond to inflammatory constituent and cause reddish discoloration in skin. Medical products that have been scientifically proven to generate the collagen regenerate the elastin and rebuilt the hyaluronic acid in skin. De Mouraresearched on antioxidants and found that a grape extract may share some effects similar to those come across in wine and other grape foodstuffs [8]. Wine and grape yields have been exposed to bring many important cardiovascular effects. The effect of wine on vascular even muscle are notorious. Red wine has been shown to convince either vasodilatation that affects the skin texture

Antioxidants play major role in improving smoothness of skin and melatonin content. Apart from the common antioxidant glutathione, secondary metabolites also possess anti-oxidant activity. Aswalin his studies prepared polyherbal cream and found that plant extracts selected for preparing creams did not cause sideeffectson skin [9]. They can be used for whitening or anti wrinkling of skin. Combination of plant's extracts can be used for multi type of results or combined properties. Sahu et al. prepared herbal creams from various plant extracts with oil based or water based emulsions with no side-effects on skin [10].

In this study, carvacrol which is one of the secondary metabolite present in essential oil of *Oreganum vulgare* was selected for preparation of cream. Its anti-oxidant property was studied by Mila Jukicand AChE repressive potential was attenuated within the following order: galanthamine>thymohydroquinone> carvacrol>thymoquinone> total volatile oil >thymol>[11]. Grape seed was extracted and foundrich in polyphenols with 60-70% of quercetin and resveratrol each having an antioxidant effect and reduce the level of chronic inflammation in the body to protectcells from free radical damage [12]. It protects against damage-causing free radicals and enhances the body's ability to fight from forging particle and support the body wound healing process and may inhibit tissue damage. Gelatine film was prepared with the help of carvacrol and they found that carvacrol concentration highly affects the antioxidants and antimicrobial properties [13].

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MATERIALS AND METHOD

Preparation of cream

Samples of cream were prepared using carvacrol as a main component including different components also namely aloevera, starch, stearic acid, grape seed extract and argireline. Chou et al. researched on Tween 20 and explained that tween 20 used for removal of dead cell [14]. Tourmaline is a mineral and having pyro-electric characteristics [15]. E.rieche et al. (2012) It also generate oxygen ion and far-infrared electromagnetic radiation. Neem oil contains a group of active ingredients with different chemical characteristics used for the treatment of different types of disease like malaria, ulcers, cardiovascular disease and skin problem. Steric acid is a saturated long chain of fatty acid with an 18 carbon chain backbone.it is white solid with a mild odour.

The procedure followed by the sterilization of glassware and preparing autoclave water. Simultaneously, chemicals were separated on the basis of powder and oil properties, for example, starch and stearic acid are present in the form of powder whereas argireline and neem oil were having oil property.

The binding agent was prepared using starch slowly and continuously mixed in the autoclaved water at the constant temperature of 60^{o}C till the slurry of mixture is formed. After the slurry is prepared stearic acid and grape seed extract is added to the mixture. In another beaker, aloevera,carvacrol, argireline, tween 20 and neem oil were added and mixed properly. After that, contents of both beakers were merged in one and mixed at constant speed till proper cream formation takes place, maintaining the pH of the mixture at 4.5.

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Figure 1: showing preparation of cream samples

The different formulations were used to prepare five samples of cream as mentioned in the following table number 1.

Chemicals	**Cream 1**	**Cream 2**	**Cream 3**	**Cream 4**	**Cream5**
Grape seed extract	1mg	0.5mg	2mg	0	1.5mg
Carvacrol	2ml	3ml	4ml	3.5ml	4ml
Aloe Vera	20gm	20gm	20gm	20gm	20gm
Tween20	200ul	250ul	300ul	200ul	200ul
Neem oil	20ml	15ml	12ml	10ml	10ml
Starch	10gm	10gm	7gm	10gm	12gm

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Argineline	1ml	1ml	1ml	1ml	1ml
Stearic acid	5gm	4gm	4gm	5gm	4gm

Table 1: showing five samples of cream with different formulations of chemicals.

QC Assays performed for the cream

Rheological properties such as viscosity of semisolids like creams and lotion forms can influence their drug delivery; it will also directly influence the diffusion rate of cream at microstructural level. It will also affect the use of it for different type of application areas so viscosity test is must for creams. Uniformity of cream sample or homogeneous mixing will tell us about uniformity of active agents inside the cream so physical examination of creams is also necessary pH level during formation of creams must be checked because at the time of batch release we can only predict the time and life of cream. Growth of microbes need to be studied in the samples regularly and if required anti-microbial could be added after doing sterility test.

By keeping these important things in mind, the test mentioned below were used to examine the samples.

- PHYSICAL APPEARANCE: Foremost the sample is evaluated on the basis of its physical appearance which could be analysed by observing the following characteristics such as crystal growth, cracking of emulsion, change in viscosity, microbial contamination and lumpy appearance

- SOLUBILITY TEST: In order to perform the assay the sample must be solubilize in nine parts of cold water and 1.7 parts in hot water. Apart from this, the cream should be miscible with alcohol, ether and chloroform.

- VISCOSITY DETERMINATION: In this assay, the viscometer is arranged so that its rotational axis is perpendicular to the horizontal plane. Then, enough quantity of a sample solution is placed in the viscometer and the measuring

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system was allowed to stand until a specified temperature is attained before start operating the rotational viscometer. The indicated value was measured on the scale which was corresponded to the constant rotational frequency value, when the viscous flow has reached a steady state after forced rotation.

- STERILITY TEST: To study the sterility of the samples, microbial growth was observed on nutrient agar medium.Samples of cream were spread on the mediameter after solidification and incubate it for 48 hours.

- DPPH TEST: Each sample was weighed 1mg/ml and dilution of1/10, 1/100, 1/1000, 1/10000, 1/100000 sample in methanol was prepared. Marigold anti-ageing cream was selected as a control sample. DPPH solution was prepared in above diluting solvent [16]. The solutions were prepared according to the following table

Test Tube	**Diluting solvent**	**Methanol**	**Sample**	**DPPH**
Blank	0.1ml	2ml	-	-
Control	0.1ml	-	-	2ml
Test	-	-	0.1ml	2ml

Table 2: Protocol followed for performing DPPH Assay for anti-oxidant activity

Calculate the absorbance of the sample after every 10 minutes using spectrophotometer at 517 nm wavelength.

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Figure 2: showing the test tubes of blank, control and test sample with DPPH solution

- RADICAL SCAVENGING ACTIVITY:%RSA= ((Abs control-abs sample)/abs control) *100 Where RSA is radical scavenging activity. Abs control is the absorbance of DPPH radical +methanol; abs sample is the absorbance of DPPH radical +sample.

- SPREADABILITY TEST: The parallel plate method is the most extensively used method for determining and quantifying the spreadability of semisolid preparations.Positioned the base table on the base of the machine.Lightly tightened the screw of the base table to enable a degree of mobility.Placed the base holder on to the base table and lock into position with screws. 2mg of sample was loaded in the centre of the male plate and put the female plate over it and lock it with a screw so, that there will be no mobility.Put 500gm weight on the female plate for 15 minutes.After 15 min removes the weight and female plate.Measure the diameter from three different sides.Take the average diameter and calculate

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the spreadability of the cream using the formula spreadability of cream: sp%= (average diameter of cream spread/initial diameter of cream)*100

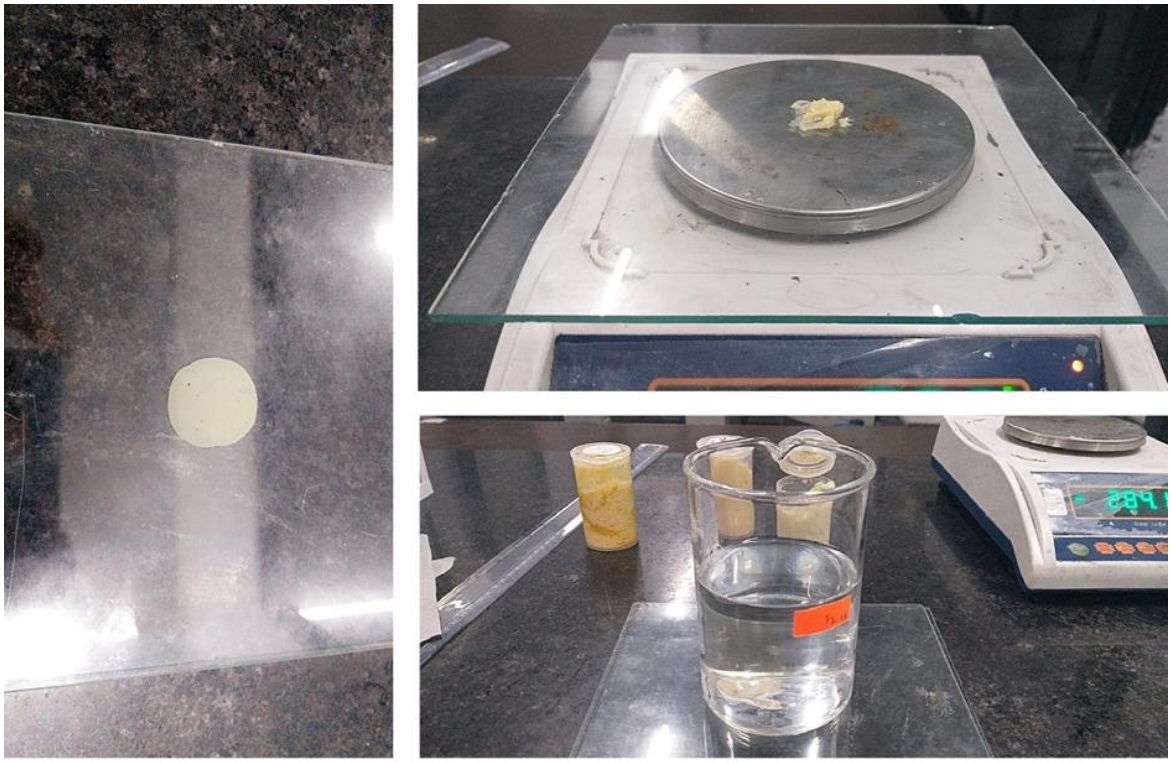

Figure 3: showing the method followed for spreadability assay

RESULT AND DISCUSSION

PHYSICAL APPEARANCE: The creams were analyzed using different parameters for physical appearance and the following table depicts the obtained result.

Cream	Crystal growth	Cracking of emulsion	Contamination	Lumps	Colour	Mixing

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Type 1	proper	no	yes	formed	Light yellow	Proper
Type 2	proper	no	No	Not formed	Dim yellow	Proper
Type 3	proper	no	No	Not formed	Oil colour appear	Improper
Type 4	proper	no	No	formed	white	Proper
Type 5	proper	no	No	small	Light yellow	Improper

Table 3: showing the physical appearance results of five different sample

From the above mentioned result it is concluded that formulation of Cream2 and Cream 4 were found best without contamination and proper mixing.

The only relation between density and viscosity is that they both are affected by temperature. When the fluid is heated, its particle travel apart and also it becomes less viscous. For calculation of weight of cream, first the empty flask weighing was done and then filled flask weight was done and then subtracted from one to another value.

$$\text{Weight of cream} = \text{weight of cream with flask} - \text{weight of empty flask}$$

$$\text{Density of cream} = \frac{\text{Mass of cream}}{\text{Volume of cream}}$$

Sample	**weight of cream with flask − weight of empty flask**	**Weight of cream**
Sample 1	96.5 – 12.87	83.63
Sample 2	79.1 – 12.78	66.23

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Sample 3	68.06 – 12.87	55.19
Sample 4	88.82 – 12.87	75.95
Sample 5	102.11 – 12.87	89.24

Table 4: Weight of sample in grams is depicted for five different samples

VISCOSITY TEST:Viscosity is fluids resistance to flow, it was measured with a viscometer, equipment that measures the force required to move through a liquid. One can say viscosity is equal to fore divided by area generally with an ideal fluid (Newtonian fluid), it is also directly proportional to what is called shear rate. Shear rate is the speed by which the fluid is moving divided by the distance that it moves.

Viscometer function on the same phenomenon discussed above, in the viscometer we put our sample, and a stirrer moves inside the container having cream sample and the sample keep heating as the stirrer shear the cream and produces heat inside cream due to friction formation between particle hence density and viscosity lowers down and stirrer speed increase as the shear force decreases due to heating and when the viscometer shown no change in reading the final reading is to be noted as viscosity of sample.

Sample	**Spindle No**	**RPM**	**Viscosity (Cp)**
Sample 1	64	10	Contaminate
Sample 2	64	10	39360
Sample 3	64	10	29764
Sample 4	64	10	Error
Sample 4	64	6	95206
Sample 5	64	10	36660

Table 5: Viscosity value is depicted for five different samples

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STERILITY TEST:This test was used to confirm that our creams are free from the presence of viable microorganisms. This test is must for medical applications and preparations of samples to insure that our samples are sterile and free from viable microorganisms; hence the product will last long and safe for long run. All samples were examined after 72 hour if they are in unchanged form or got contaminate. It was observed after sterility test that four of our cream samples are free from microorganisms and only one cream sample reported negative results as Cream 2.

Time/cream	**Cream1**	**Cream 2**	**Cream 3**	**Cream 4**	**Cream5**
24hour	No contamination	No contamination	No contamination	No contamination	No contamination
48 hour	No contamination	No contamination	No contamination	No contamination	No contamination
72 hour	Contamination	No contamination	No contamination	No contamination	No contamination
96 hour	Contamination	No contamination	No contamination	No contamination	No contamination

Table 6: Observation of microbial growth in five samples at different interval of time

DPPH ASSAY**:**DPPH(1,1-Diphenyl-2-picrylhydrazyl) is a stable free radiameterl with redcolour(absorbed at 517nm). If free radicals have been scavenged, DPPH will generated it's colour to yellow. This assay used this character to show herbs free radical scavenging activity. The DPPH assay is popular in natural product antioxidant studies. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. The absorbance value of each sample was observed at 517 nm which is mentioned in the following table number 7.

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CREAM/TIME	DILUTIONS	0MIN	15 MIN	25 MIN
	0	1.526	1.698	1.420
Cream 1	1/10	1.501	1.632	1.391
	1/100	1.486	1.528	1.439
	1/1000	1.468	1.506	1.394
	0	0.458	1.756	1.532
Cream 2	1/10	0.025	0	0
	1/100	0.655	1.702	1.528
	1/1000	1.233	1.768	1.438
	0	1.750	1.736	1.546
Cream 3	1/10	1.767	1.733	1.533
	1/100	1.009	1.714	1.526
	1/1000	0.748	1.638	0
	0	1.637	1.746	1.506
Cream 4	1/10	1.622	1.723	1.503
	1/100	1.615	1.700	1.504
	1/1000	1.584	1.638	0
	0	1.107	1.358	0.548
Cream 5	1/10	1.091	1.288	0.438

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	1/100	0.987	1.143	0.468
	1/1000	0.541	0	0

Table 7: Absorbance value of each sample at different intervals of time

SCAVENGING ACTIVITY: It was calculated using the formula %RSA= ((Abs control-abs sample)/abs control) *100 where RSA is radical scavenging activity. Abs control is the absorbance of DPPH radical +methanol; abs sample is the absorbance of DPPH radical +sample.

CREAM/TIME	**DILUTIONS**	**0 MIN**	**15 MIN**	**25 MIN**
	0	13.98	4.23	19.90
Cream 1	1/10	15.34	7.95	21.54
	1/100	16.18	13.81	18.83
	0	74.16	0.95	13.59
Cream 2	1/10	0	0	0
	1/100	63.05	4	13.81
	0	1.29	2.08	12.80
Cream 3	1/10	0.33	0.25	13.53
	1/100	43.09	3.32	13.93
	0	7.67	1.52	15.11
Cream 4	1/10	8.51	2.82	15.22
	1/100	8.9	4.11	15.17

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	0	36.12	21.63	68.37
Cream 5	1/10	3.70	25.67	74.72
	1/100	43.04	33.27	72.97

Table 8: Scavenging activity of each sample at different intervals of time

The above result depicted that the cream formulation of second sample showed the highest scavenging activity out of all other formulations. Radical scavenging activity of second sample at dilution of 1/100ug/ml resulted in 63.05% as compared to the radical scavenging activity of marigold anti-ageing cream at dilution 1/100ug/ml is 70.6%.

SPREADABILITY TEST:In general for semisolid foundations test Spreadability test is must, by which we can know the ability of a cream or gel to evenly spread over the skin or other application area, spreadability perform a vital role in the administration of a standard dose of a medicated formulation to the skin and also used to measure the efficacy of a topical therapy.

$$\frac{After\ dia}{Initial\ dia} * 100 = \%\ spreadability$$

For precise spreadability test, three readings were taken on each type of cream sample and average of three readings was taken to evaluate spereadability of cream.

Cream 1: Contaminate in sterile test.

Cream 2

Initial diameter = 2 Cm

After diameter at test run 1 = 5.5

After diameter at test run 2 = 5.7

After diameter at test run 3 = 5.6

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Average after diameter = (5.5 + 5.7 + 5.6)/3 = 5.6

$$\%\, spreadability = \frac{5.6}{2} * 100 = 280\%$$

Cream 3

Initial diameter = 2 Cm

After diameter at test run 1 = 5

After diameter at test run 2 = 5.4

After diameter at test run 3 = 5.2

Average after diameter = (5 + 5.4 + 5.2)/3 = 5.2

$$\%\, spreadability = \frac{5.2}{2} * 100 = 260\%$$

Cream 4

Initial diameter = 2 Cm

After diameter at test run 1 = 4.4

After diameter at test run 2 = 4.4

After diameter at test run 3 = 4.4

Average after diameter = (4.4 + 4.4 + 4.4)/3 = 4.4

$$\%\, spreadability = \frac{4.4}{2} * 100 = 220\%$$

Cream 5

Initial diameter = 2 Cm

After diameter at test run 1 = 5

After diameter at test run 2 = 5.4

After diameter at test run 3 = 5.4

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Average after diameter = (5 + 5.4 + 5.4)/3 = 5.26

$\%\, spreadability = \frac{5.26}{2} * 100 = 263\%$ *sp*readability= 5.26/2*100=263%

Cream2, Cream 3, Cream 5 are good in spread on skin.

After analysing all the parameters, the results were combined to find the successful formulation out of all the samples prepared.

Samples	**Viscosity**	**Spreadability**	**Weight**	**Sterility**	**Density**
Sample 1	Sample contaminate	0%	83.63	Contaminate	1.03
sample 2	39360	280%	66.23	No physical change	0.82
sample 3	29764	260%	55.19	No physical change	0.68
sample 4	Error	220%	75.95	No physical change	0.94
sample 5	36660	263%	89.24	No physical change	1.11

Table 9: Combined parameter results of five different samples

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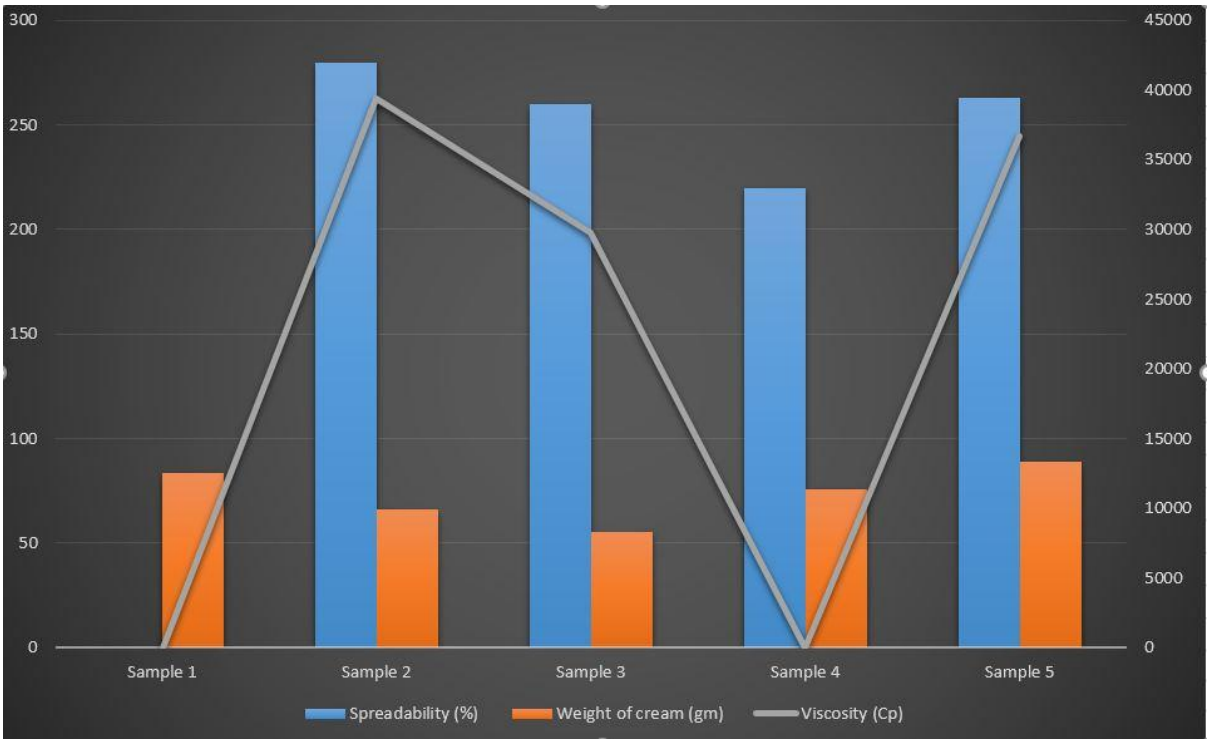

Figure 4: Graphical representation of the combined results of five different samples

After observing the results it is concluded that sample two was found more suitable because it possessed high viscosity value, no contamination and high scavenging activity. Higher viscosity cream, such as lotions and creams will be more helpful for sports massage, swedish massage and trigger point therapy, among others. Whereas the type three cream which is having low viscosity will most likely be used where low friction and more glide required. In addition to allowing hands to easily slide across the skin. But as this was a cream preparation study so we keep in mind that cream cannot be pumped in the way that oils can be that is high viscosity required to be a better cream and less viscosity required to be a good oil.

CONCLUSION

Aswal et al (2013) researched and prepared polyherbal cream and they found that plant extracts which are natural can be a better choice for preparing creams, because no side effects were seen, in comparison to those herbal creams they noticed that cosmetic creams have side- effects on skin. This is the reason different formulations of carvacrol with other components were made to

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develop the five types of cream. Out of five cream, formulations designated as the second showed the best results. The result was based on the appearance of the cream, colour, pearlscene and roughness and graded and determination of homogeneity. The formulations were tested for the homogeneity by visual appearance and by touch. In this study, sample two was having high viscosity value, high scavenging value and no contamination which concluded that it could be use further to study as anti-ageing cream formation possessing great ability to penetrate inside the skin and possessing high anti-oxidant potential.

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