Formulation, In-Vitro Characterization, Antioxidant Potential and vicissitude in rheological parameter of Cosmeceutical Cream Containing *Brassica oleracea* Extract.

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Key words: Antioxidant activity, *Brassica Oleracea*, Emulsion, Formulation, Rheology

Abstract

Study's aim was to incorporate 3% ethanolic extract of *Brassica oleracea* into oil in water emulsion to get a stable cream and to find out antioxidant, tyrosinase inhibition potential along with characterization of stable cream formulation. *Brassica oleracea* ethanolic extract had 74.2% antioxidant activity, determined by using the DPPH method. TPCs were 115.75 mgGAE/g and the TFCs were 5.62 mg QE/g. Cream incorporated liquid Paraffin, Tago® care 450, Cetostearyl alcohol, stearic acid and ethanolic extract of *Brassica oleracea*. Samples of control and formulation were stored at various storage temperatures for 28-days to assess the stability of newly formulated cream. These were evaluated for different organoleptic changes, pH and rheology studies. Both samples of active and test samples did not show phase separation and exhibit agreeable range of pH and rheology. So, both formulations found stable along with antioxidant potential of ethanolic extract of *Brassica oleracea* also presented its antiaging use and applications in the various fields of medicine and cosmetics industries.

1. Introduction

An emulsion comprised of water, oil and emulsifying agent, which stabilizes emulsion (1). Various type of emulsions using as medicines for topical application. Use of emulsion can ease in drug penetration through skin (2). Emulsions are used to deliver drugs and various active agents to the skin, depending on their property (3). Emulsifying agents are used as they form a barrier between the two immiscible liquids and therefore, reduces the interfacial tension. The emulsifying agents employed can be proteins or amphiphilic polymers or they can be ionic or non-ionic surfactants (4). Ability of the emulsion to withstand changes that happened on storing for a long time. Breaking, flocculation and, coalescence can occur (5, 6). Emulsions are considered to be the mostly used platform for the formulation of dermatological as well as cosmetic products. Emulsions may be prepared in a variety of preparation, ranging from lotion to creams, foam, emulgel and emulsion ointments.

Medicinal plants are increasingly used worldwide. There is a great amount of research and evidence available on plants and herbs in health care benefits by humans. (7, 8). More particularly, dietary fruits and parts are shown to play an important part in mitigation of a wide range of diseases (7).

Brassica oleracea (Broccoli) is also known as a wild cabbage in its uncultivated form (9). It is indigenous to the Mediterranean region. It is quite well-known in Italy since the times of Roman empire. It belongs to Brassicaceae family also known as Mustard family or cabbage family. Member of Brassicaceae family as: broccoli, brussels sprouts, cabbage, collards, kale, mustard, rape and many more etc. (10). China is top producer of broccoli while India ranks second and USA third in its production (10). Broccoli, which is an immature flower vegetable of

Brassicaceae family is a well-known health promoter and is largely consumed throughout the world owing to its high amounts of beneficial biologically active compounds (11). The Brassica vegetables all share a common feature. Broccoli varieties vary in the shape and size of the head and time to maturation. It also has early and mid-season varieties. Broccoli is well-adapted to all areas of the state when grown during the coolest months of the year. There are three common varieties of broccoli which are as follows (10, 12). All the cruciferous vegetables including broccoli are the major source of glucosinolates and their bioactive metabolites such as isothiocyanates. Other bioactive compounds are also present in cruciferous vegetables such as polyphenols including flavonoids (e.g. quercetin), minerals (e.g. selenium) and vitamins (e.g. Vitamin C) (13). Fresh broccoli contains 10.27 mg gallic acid per g and 4.16 mg gallic acid per g of total polyphenolic and free polyphenolics respectively, mostly corresponding to flavanols (14). Broccoli florets have two flavanol glycosides namely quercetin and kaempferol (15). Cruciferous seeds are have a reasonable amounts vitamins (16). It has many minerals that are part of nutrition and an important alternative source of Ca among population which have low access to dairy products (17, 18). Broccoli contains proteins having essential amino acids. Quantities of amino acids in florets and stem varies (19). The vegetables of cruciferous family has been used in different health disorders (20). Juice of Broccoli leaves used to mitigate skin diseases such as warts, oxidative stress and kidney's inflammation and the (CV) cardiovascular disorders (21). Purpose of this study was to incorporate 3% ethanolic extract of *Brassica oleracea* into oil in water emulsion to get a stable cream and to find out antioxidant, tyrosinase inhibition potential along with characterization of stable cream formulation. As it effects the collagen biosynthesis thus this product can utilize as an anti-aging and moisturizing product.

2. Material and Methods

2.1. Chemicals and Reagents

Ascorbic acid (Sigma Aldrich, Germany), DPPH (Sigma Aldrich, Germany), Deionized water (Pharmacy department, IUB, BWP), Ethanol (Sigma Aldrich, Germany), Ferric chloride (Merck, Germany), Potassium di- hydrogen phosphate (Merck, Germany), Gallic acid (Sigma Aldrich, Germany), Folin-Ciocalteu reagent (Sigma Aldrich, Germany), Quercetin (Sigma Aldrich, Germany), Liquid paraffin (Sigma Aldrich, Germany), Cetostearyl alcohol (Merck), tagocare® care 450 (Evonik Goldschmidt Germany), Stearic acid (Merck).

2.2. Plant material and identification

Fresh green broccoli (*Brassica oleracea var. italica*) was purchased from the local market; the leaves were removed, and florets were separated from stems and cut into small pieces. It was washed with water to remove dust particles. After drying broccoli was grounded into fine powder and powder was stored in an airtight container at 25°C to avoid any contamination.

2.3. Preparation of botanical extract

80% ethanol solution was used for the extraction of plant materials. It was prepared by taking the ratio of 80:20 of ethanol and water. The powder of *Brassica Oleracea var. italica* was weighed 80g accurately on weighing balance and then transferred to glass beaker. For the purpose of extract preparation, powder was soaked in 800ml of hydro-ethanolic solution for seven days. The beaker was covered with aluminium foil and was shaken on alternate days. Filtered through Whatman filter no.1 to get filtrate free from coarse particles. The filtrate was then evaporated by using the rotary evaporator. For this purpose, the extract was taken in the round bottom flask of the rotary evaporator and was operated at 44°C and at 90rpm.

2.4. Determination of antioxidant activity

To determine antioxidant activity, stock solution having 12mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in 50 mL of methyl alcohol and this solution was carefully kept in the freezer. To maintain the absorbance between 0.9-1.1, take 10 mL of stock solution and dilute it with methanol and set the absorbance in the given range. The antioxidant activity of broccoli was checked on spectrophotometer by taking 2.85 mL of DPPH solution and 0.15 mL of ethanolic extract of *Brassica oleracea* at wavelength of 517nm. Before spectrophotometer determination solution was kept in dark for 30 minutes. Antioxidant capacity was measured by;

%inhibition = (Absorbance of control) -(absorbance of sample) / (absorbance of control) * 100

Where;

Absorbance of sample means absorbance of test solution containing ethanolic extract of *Brassica oleracea*. Same procedure was repeated for vitamin C, which was taken as standard.

2.5. Total Phenolic Contents (TPC) Estimate

TPC determination was carried according to 96-well microplate procedure with some modifications, which is based on Folin–Ciocalteu method. Briefly, 10 µL of 10% FCR was mixed with 100 µL (1mg/mL) of ethanolic extract of *Brassica oleracea*. It was kept for 10 minutes at 37°C to complete reaction, after that 90 µL of 15% Na_2CO_3 solution was added to the above reaction mixture. The solution was incubated at room temperature for 60 minutes and then absorbance was taken at 765 nm using automatic microplate absorbance reader (Labtech Model LT-4500). All measurements of samples were done in triplicate. Calibration curve was generated using various dilutions (10 to 100µg/mL) of Gallic acid. Regression curve was used to calculate the TPC and reported as mgGAE/gram of extract.

2.6. Total Flavonoid Contents (TFC) Estimate

Different concentrations (10-100µg/mL) of quercetin standard were prepared in absolute methanol to generate calibration curve. 100 µL of ethanolic extract of *Brassica oleracea* was mixed with 25 µL of 1.0% sodium nitrite solution in a well plate. Mixture was left for 5 min to complete reaction then 10 µL of $AlCl_3$ solution (10%) was mixed and again left for 5 minutes. After this 35µl of one molar NaOH (4%) was added and diluted with 30µl of methanol. All the reagents were mixed and incubated for 30 minutes in dark at room temperature. The samples absorbance was measured at wavelength of 510 nm. Quercetin was used to draw the standard curve. The results of TFC were reported as Equivalents of mg Quercetin (QE)/g of plant extract. The sample and standard measurements were run in triplicate.

2.7. Preparation of Emulsion

Control emulsion was produced as previously described by Choudhry and N Akhtar (3). Same procedure was used in the preparation of formulation containing 3g of ethanolic extract of *Brassica oleracea*. The ingredients used in the control and formulation were given in **Table 1.**

Table 1. Ingredients of Control and Formulation.

Cosmetic emulsion	Liquid paraffin (%)	Tagocare (%)	Cetostearyl alcohol (%)	Brassica Oleracea extract (%)	stearic acid (%)	Distilled water (%)
Control	8	6	3	0	0.5	q.s. 100
Formulation	8	6	3	3	0.5	q.s. 100

2.8. Characterization of Emulsion

To evaluate the stability and safety of formulation various types of tests were performed in the cosmetic laboratory including organoleptic evaluation, pH measurement, centrifugation test, thermal stability test, electrical Conductivity and rheological analysis.

2.8.1. Organoleptic evaluation

Organoleptic studies performed on both emulsions while kept at 8°c, 25°c and accelerated temperatures for a fixed period like 24, 48, 72 hours and days at 7, 14, 21 and 28.

2.8.2. pH Testing

pH value of all formulations was measured for fresh and emulsion while kept at 8°c, 25°c and accelerated temperatures for a fixed period like 24, 48, 72 hours and days at 7, 14, 21 and 28.

2.8.3. Centrifugation Test

Test performed on freshly prepared emulsion at 5000rpm for 10min. Test was repeated for emulsions while kept at 8°c, 25°c and accelerated temperatures for a fixed period like 24, 48, 72 hours and days at 7, 14, 21 and 28.

2.8.4. Electrical conductivity

Values for electrical conductivity of freshly prepared emulsions were obtained while kept at 8°c, 25°c and accelerated temperatures for a fixed period like 24, 48, 72 hours and days at 7, 14, 21 and 28.by a digital Conductivity-Meter.

2.8.5. Rheological analysis

Rheological studies of formulations stored at different temperature was performed with a programmable Brookfield rheometer while kept emulsions at 8°c, 25°c and accelerated temperatures for a fixed period like 24, 48, 72 hours and days at 7, 14, 21 and 28.

3. Results and Discussion

3.1. Antioxidant activity

Antioxidant activity of *Brassica oleracea* extract was 74.2% determined by using DPPH method. The DPPH assay is used to calculate, the free radical scavenging ability of a large number of natural products (22). Cruciferous vegetables are an excellent source of antioxidant vitamins and minerals such as selenium, potassium and bioactive compounds, for example, isothiocyanates. Brassica vegetables, such as broccoli, brussels sprouts and red cabbage are known to have the highest anti-oxidant capacity (23). In addition, the industrial processing of vegetable, involving, blanching, sterilization, canning and freezing and as far as domestic cooking as well can also influence the content, composition and anti-oxidant activity (24).

3.2. Total phenolic contents

Brassica oleracea extract was evaluated for antioxidant activity and found to be 115.75 mg GAE/g extract. Phenolic compounds are generally known as secondary metabolites, abundantly present in plants. On the basis of their compound structures, they are categorized into different classes (25). TPC of *Brassica oleracea* extract was 115 GAE/g in our study. Phenolics varies from 34.5 to 337.0 mg/100 g in broccoli (24). TPC's variation in leaves and stalk are 99.37–135.64 and 8.13–11.74, respectively (26). According to a study, TPC in broccoli powder were 63 mg GAE/g, comparatively, lower than the findings of my research (27). Furthermore, Phenolic compounds are also shown to possess a number of bioactivities: the most significant one is the antioxidant activity (24).

3.3. Total Flavonoid Contents

Total flavonoid contents (TFC) of ethanolic extract of *Brassica oleracea* expressed as mg of quercetin equivalents/gram were 5.62 mg QE/g extract. Flavonoids are polyphenolic group of natural compounds found in plants. These compounds have well known antioxidant activity (28),(29, 30). According to a reported study, the range of quercetin in broccoli was 0.03–10.85 mg and TFC in ethanolic extract of *Brassica oleracea* was within the standard range (31),(32).

Table 2. Antioxidant, TPC, TFC and tyrosinase inhibition potential of ethanolic extract of *Brassica oleracea*

TPC mg GAE/g	TFC mg QE/g	DPPH (%)
115.75 mg GAE/g	5.62 mg QE/g extract	74.2 %

3.4. Physical Characterization of emulsion

3.4.1. Stability Testing

Emulsions are naturally unstable because they are biphasic systems. Therefore, it is necessary to pre-determine the stability of emulsions (33). The formulation and control evaluated for different parameters such as colour, odour and liquefaction. The data is shown in the **Table 3** for colour, **Table 4** for odour and **Table 5** for liquefaction respectively.

Colour of control was white and that of formulation was light green due to extract. No colour change seen in both control and formulation during the period of 28 days at all storage conditions. Which means control and formulation remains stable during study because change in colour is a sign that emulsion is unstable. In this study, no liquefaction was observed in control and formulation that kept at different temperature. A little liquefaction observed in control and formulation that kept at 25°C at 21st day and 28th day.

Phase separation occurs when the droplets merge with each other. Difference in phase densities, radius of the droplets and the viscosity issues results in creaming (34). By decreasing droplet size and increased viscosity may solve the creaming issue (4).

Table 3. Colour of Control and Formulation.

Parameter.	Time period.	Control.				Formulation.			
		8 C	25°C	40°C	40°C± 75%RH	8°C	25°C	40°C	40°C± 75%RH
Colour.	0hrs	W.	W.	W.	W.	G.	G.	G.	G.
	24hrs	W.	W.	W.	W.	G.	G.	G.	G.
	48hrs	W.	W.	W.	W.	G.	G.	G.	G.
	72hrs	W.	W.	W.	W.	G.	G.	G.	G.
	7th day	W.	W.	W.	W.	G.	G.	G.	G.
	14th day	W.	W.	W.	W.	G.	G.	G.	G.
	21st day	W.	W.	W.	W.	G.	G.	G.	G.
	28th day	W.	W.	W.	W.	G.	G.	G.	G.

* W.= white *G.= green

Table 4. Odour of Control and Formulation.

Parameter.	Time period.	Control.				Formulation.			
		8 °C	25°C	40 °C	40 °C± 75%RH	8°C	25 °C	40°C	40 °C± 75%RH
Odour	0hrs	-	-	-	-	-	-	-	-
	24hrs	-	-	-	-	-	-	-	-
	48hrs	-	-	-	-	-	-	-	-
	72hrs	-	-	-	-	-	-	-	-
	7^{th}day	-	-	-	-	-	-	-	-
	14^{th}day	-	-	-	-	-	-	-	-
	21^{st}day	-	-	-	-	-	-	-	-
	28^{th}day	-	-	-	-	-	-	-	-

* (-) = no change

Table 5. Liquefaction of Control and Formulation.

Parameter.	Time period.	Control.				Formulation.			
		8°C	25°C	40 °C	40 °C± 75%RH	8°C	25 °C	40°C	40 °C± 75%RH
Liquefaction	0hrs	-	-	-	-	-	-	-	-
	24hrs	-	-	-	-	-	-	-	-
	48hrs	-	-	-	-	-	-	-	-
	72hrs	-	-	-	-	-	-	-	-
	7thday	-	-	-	-	-	-	-	-
	14thday	-	-	-	-	-	-	-	-
	21stday	-	-	-	-	-	-	-	-
	28thday	-	-	-	-	-	-	-	-

* (-) = no change

3.4.2. Centrifugation Test

Phase separation occurs when the droplets merge with each other. Difference in the densities of phases, radius of the droplets and the viscosity issues results in creaming (34). By decreasing droplet size and increased viscosity may solve the creaming issue (4). Centrifugation used to assess the physical stability of emulsion. All the samples were checked to see phase separation but oo phase separation was observed as shown in **Table 6.**

Table 6. phase separation of Control and Formulation

Parameter.	Time period.	Control.				Formulation.			
		8 °C	25 °C	40 °C	40 °C± 75%RH	8 °C	25 °C	40 °C	40 °C± 75%RH
Phase separation.	0hrs	-	-	-	-	-	-	-	-
	24hrs	-	-	-	-	-	-	-	-
	48hrs	-	-	-	-	-	-	-	-
	72hrs	-	-	-	-	-	-	-	-
	7^{th}day	-	-	-	-	-	-	-	-
	14^{th}day	-	-	-	-	-	-	-	-
	21^{st}day	-	-	-	-	-	-	-	-
	28^{th} day	-	-	-	-	-	-	-	-

* (-) = no change

3.4.3. Conductivity Test

Each formulation shows electrical conductivity after performing the test at specific time intervals. The values were measured in unit "µS/cm" and shown in the **Table 7 and Table 8.** Electrical conductivity measurement is relatively easy and economical, involving the insertion of electrodes into the formulation (35). Oil in water emulsion will be the conductor of electricity while water in oil emulsion will show little or no conductivity (36). By using two-way ANOVA analysis, change in conductivity values of both were significant at different temperatures. Paired

sample t-test shows that changes were insignificant and shows that no sudden change in conductivity throughout the study period so formulation remain stable for four weeks.

Table 7. Conductivity of control

Parameter	Time period	Temperature			
		8°C	25 °C	40°C	40 °C±75%RH
Conductivity	0hrs	48.5	48.5	48.5	48.5
	24hrs	48.4	48.4	48.7	48.5
	48hrs	48.6	48.3	48.9	48.7
	72hrs	48.4	48.8	48.6	48.4
	7^{th}day	48.5	48.2	48.4	48.1
	14^{th}day	48.3	48	47.9	48.3
	21^{st}day	48	47.5	47.8	48.5
	28^{th} day	48.2	47.9	48.5	48.7

Table 8. Formulation's conductivity at different temperature.

Parameter.	Time period.	Temperature.			
		8°C	25 °C	40°C	40 °C±75%RH
Conductivity	0hrs	48.7	48.7	48.7	48.7
	24hrs	48.5	48.4	48.4	48.8
	48hrs	48.8	48.3	48.5	48.6
	72hrs	48.8	48.1	48.4	48.3
	7th day	47.9	47.5	48.7	48.1
	14th day	47.5	47.4	48.1	47.4
	21st day	47.1	47.8	47.6	47.6
	28th day	46.9	46.7	47.8	47.2

3.4.4. pH determination

The instability of emulsions can be shown through sedimentation, coagulation, coalescence, flocculation and change in viscosity. Furthermore, a change in pH of an emulsion can also be used to indicate an emulsion stability (37). Data results of pH of control and formulation are shown in **Tables 9** and **Table 10.** The pH of control was 5.82 and that of formulation was 5.92. The pH value of control and formulation samples was observed and results shows that pH started to decline continuously with little variation with respect to time. ANOVA results showed that pH of each formulation was significant at different levels of temperature with to time. t-test results showed that each formulation had significant change.

Table 9. pH of Control.

Parameter.	Time period.	Temperature.			
		8 °C	**25 °C**	**40 °C**	**40°C±75%RH**
pH	**0hrs**	5.8	5.8	5.8	5.8
	24hrs	5.79	5.81	5.83	5.81
	48hrs	5.77	5.8	5.81	5.78
	72hrs	5.75	5.77	5.79	5.77
	7^{th}day	5.73	5.73	5.77	5.75
	14^{th}day	5.71	5.72	5.75	5.73
	21^{st}day	5.69	5.7	5.74	5.71
	28^{th} day	5.66	5.67	5.69	5.7

Table 10. pH of Formulation.

Parameter.	Time period.	Temperature.			
		8°C	**25°C**	**40°C**	**40°C±75%RH**
pH	**0hrs**	5.86	5.86	5.86	5.86
	24hrs	5.84	5.82	5.88	5.87
	48hrs	5.83	5.81	5.87	5.88
	72hrs	5.81	5.84	5.85	5.86
	7th day	5.79	5.83	5.86	5.88
	14th day	5.75	5.79	5.83	5.85
	21st day	5.72	5.76	5.8	5.82
	28th day	5.71	5.73	5.76	5.78

3.4.5. Rheology Studies

The rheology analysis was performed on both control and formulation kept at different conditions for 28 days. The Rheogram of both samples fresh, day 14 and day 28 were shown in **figure 1-6**. Rheological studies hold immense amount of significance in cosmetics and pharmaceutical preparations as it helps to ensure the quality of finished products (38). Rheological measurements such as steady state, shear stress and shear rate are used to provide information about the physical stability of various semi-solid formulations like, emulsions, suspensions and gels etc. Moreover, rheology studies are also helpful in determining the instability of formulations like coagulation, coalescence and flocculation which can result in breaking of the formulations, leading to creaming or phase separation. Furthermore, Viscosity is

the key aspect in rheological profile to analyse the behaviour of the formulations (39). The formulation behaves as non-Newtonian, Pseudoplastic behavior, samples showed a decreasing viscosity with increasing shear rate. Shear-thinning products involves those having good suspension ability but can show easy thinning when applied such as hand creams. Two-way ANOVA analysis for viscosity of both control and formulation showed a significant result.

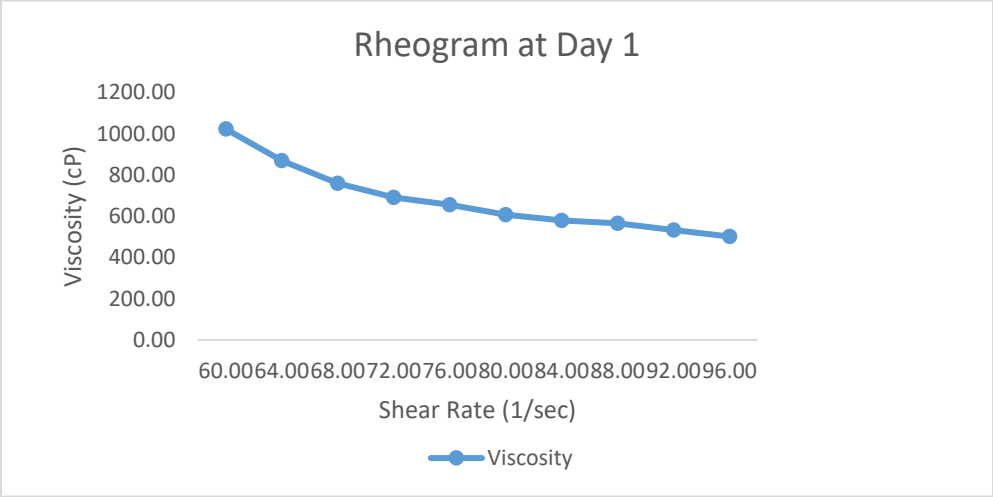

Figure 1. Rheogram of Control at Day 1.

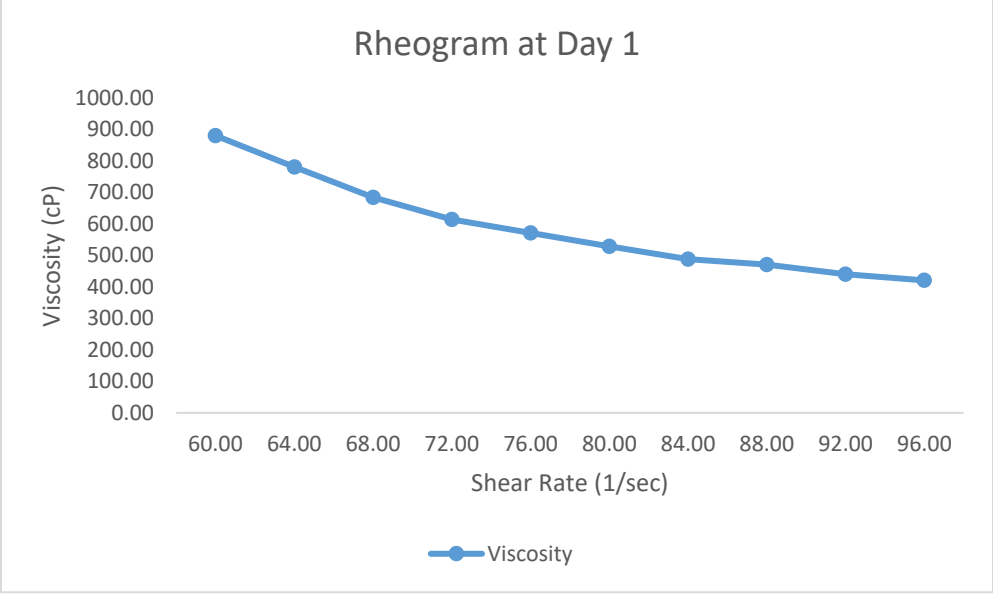

Figure 2. Rheogram of Formulation at Day 1.

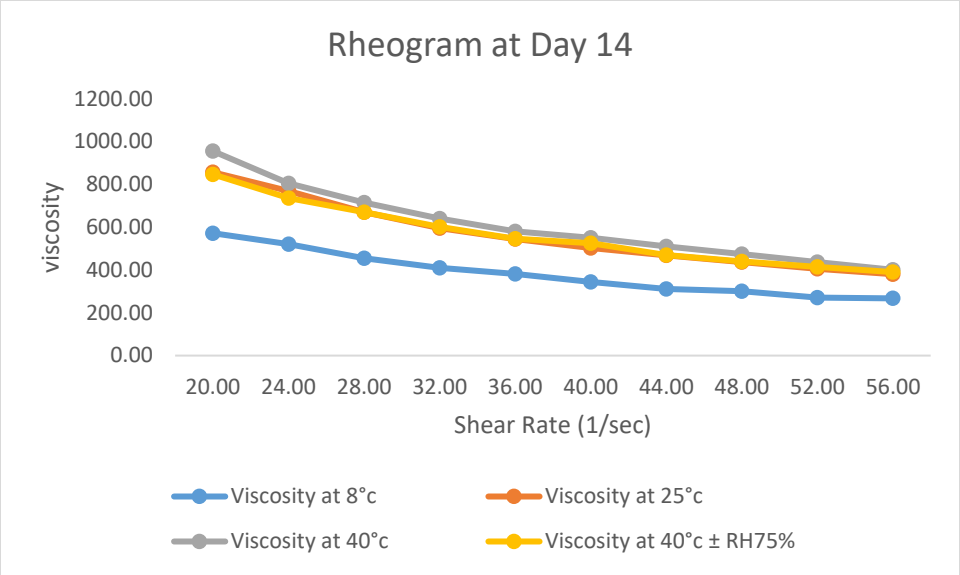

Figure 3. Rheogram of Control at day 14.

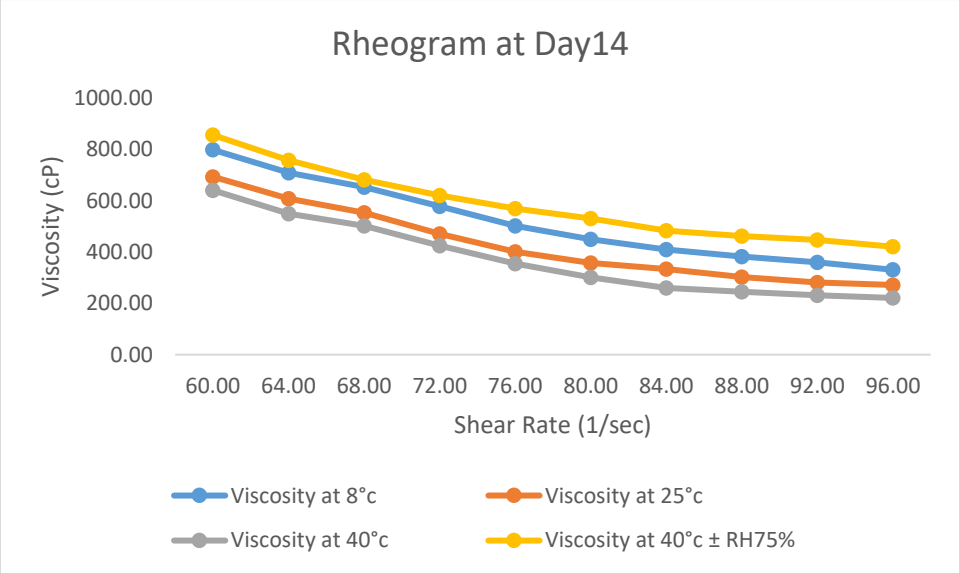

Figure 4. Rheogram of Formulation at day 14.

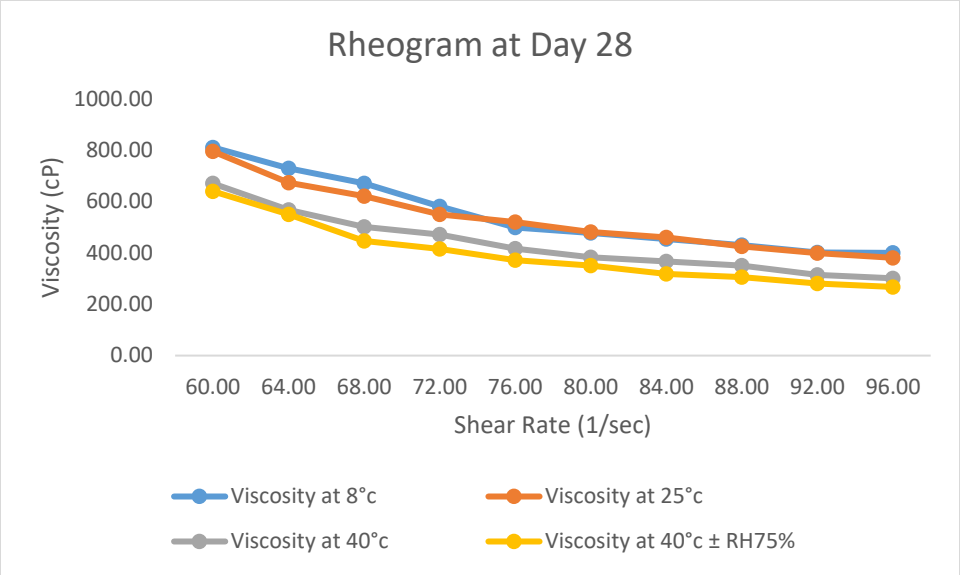

Figure 5. Rheogram of Control at day 28.

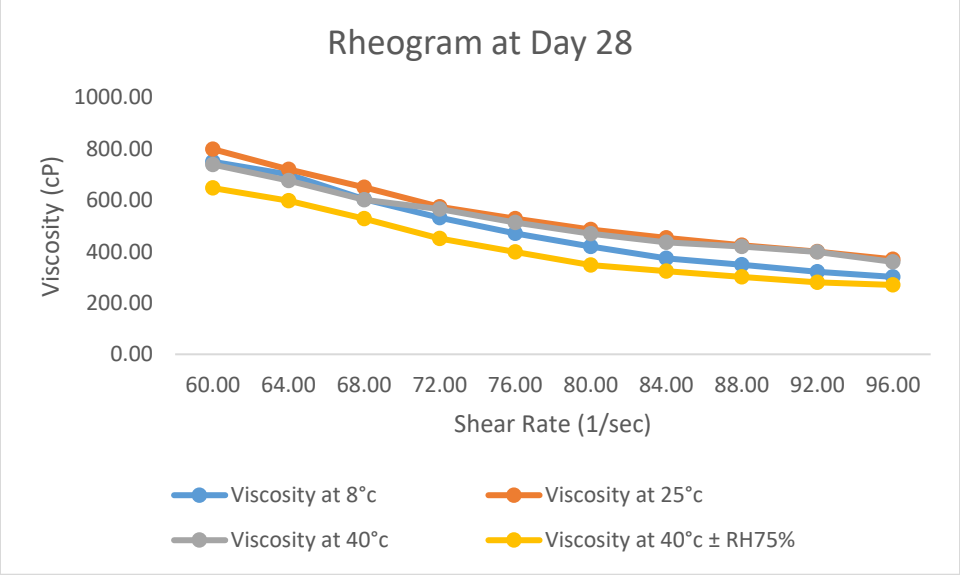

Figure 6. Rheogram of Formulation at day 28.

4. Conclusion

The results of present study showed that a stable oil in water emulsion ethanolic extract having agreeable rheological properties of *Brassica oleracea* was prepared. It had antioxidant activity and polyphenols in the ethanolic extract of *Brassica oleracea*. To sum up, Broccoli is a super

food. And as a result, further research and work is needed to be undertaken to better understand the chemical constituents of broccoli to aid in its use and applications in the various fields of medicine and cosmetics industries.

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