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## Development and Authentication of Skin Care Pharmaceutical Serum Using Herbal Extracts



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### ABSTRACT

The formulated cosmetic serum was successfully developed and evaluated using different standard parameters including skin moisture rising properties. Based on all cosmetic serum formulations studied, all F1, F2, F3 formulation showed significantly different in rising of skin moisture level ( $p < 0.05$ ). On the evaluation of the finished serum's texture, all formulations give a non-greasy, non-oily and homogeneous contents. Without any pH adjustment, all the formulations gave the pH value within the limits of normal skin pH range. Besides that, in the stability study of cosmetic serum, all formulations stable until in the room temperature ( $25^{\circ}\text{C} \pm 1$ ) and cold room ( $2-3^{\circ}\text{C}$ ) but unstable in the oven ( $40^{\circ}\text{C}$ ). Besides that, further studies are warranted to prove safety and efficacy of the formulation.



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## 1.0 INTRODUCTION

Ginkgo biloba L. belonging to the Ginkgoaceae family, extra often regarded as ginkgo or maidenhair tree, is the most historic residing gymnosperm and native to China. It has been broadly used therapeutically in traditional Chinese medicine for centuries. Ginkgo leaves can be used to make tea, herbal extracts, tinctures, and pills. (e essential bioactive compounds of G. biloba are stated to be terpenoids, flavonoids, bioflavonoids, organic acids, polyphenols, and others. Antioxidant endeavor is a very necessary pharmacological property. Many pharmacological functions such as antiaging, antimutagenicity, anticarcinogenicity and pores and skin whitening originated from this property Antioxidants help pores and skin restore itself. Inflammation impedes the skin's renewal process. By reducing inflammation, antioxidants allow skin to restore itself and correct visible damage.

Sugarcane (*Saccharum officinarum* Linn.) is an essential perennial grass of Poaceae family, indigenous to tropical South Asia and Southeast Asia. It is cultivated international due to the cost-efficient and medicinal value of its excessive yielding products. Sugarcane juice is well recognized as an uncooked cloth for the production of refined sugar and its wax is viewed as a workable alternative for the high priced carnauba wax, which is of cosmetic and pharmaceutical interest Sugarcane (*Saccharum officinarum* Linn.) is popular crop of the household Poaceae. India is the 2d biggest producer of sugarcane, after Brazil. *Saccharum* is derived from the Greek phrase 'Sakcharon,' which potential sugar in particular sucrose. *S. officinarum* Linn, is a perennial grass, indigenous to tropical South Asia and Southeast Asia. It has a thick longitudinal stalk, which is commonly three to 5 meters in height, about 5 cm in diameter, and is characterized through its sweet taste due to its excessive sucrose content. It is additionally recognized as chewing and noble cane. Sugarcane (*Saccharum officinarum* L.) juice is widely fed on via human beings of the tropics and subtropics.[11] It has been used to treat jaun-dice and liver-related disorders in Indian structures of medicine. Its feasible mechanism of motion used to be examined in phrases of antioxidant availability. The assays worried exceptional stages of antioxidant action such as oxygen radical absorbance capability antioxidant homes and had been additionally able to protect towards radiation caused DNA harm in pBR322 plasmid DNA and *Escherichia coli* cultures. In conclusion, the study exhibits that the potential of sugarcane juice to scavenge free radicals, decrease iron complex and inhibit lipid peroxidation, can also explain feasible mechanisms by way of which sugarcane juice exhibits its recommended effects with its pronounced health benefits.

Skin Serum is a skincare product you can observe to your pores and skin after cleaning but earlier than moisturizing with the intent of turning in powerful ingredients without delay into the skin. Serums are skin care products that are designed to supply high concentrations of precise lively components to the skin. There are many exceptional kinds of serums on the market that function unique jobs, ranging from hydration to skin brightening. The serum is particularly suitable to this venture because it is made up of smaller molecules that can penetrate deeply into the skin and supply a very excessive awareness of energetic ingredients. This makes them a remarkable device for concentrated on specific skincare concerns, like pigmentation, symptoms of aging. Cosmetic Serum is an incredibly concentrated product based totally on water or oil.[12] Serums, or concentrates, contain approximately ten times greater of biologically active components than creams, therefore faster and greater efficiently coping with beauty problems. Serums act locally upon one-of-a-kind physique parts: face, neck, decollate, eyelids. They can be used irrespective of age.

Anti-aging cosmetics represent a large group of the pores and skin care cosmetics market. Originally, this group included solely merchandise supposed for mature pores and skin to reduce the appearance of wrinkles and different signs and symptoms of skin aging. The function of modern-day anti-aging cosmetics is not solely to improve the appearance of the skin by way of stimulating and regenerating herbal physiological strategies that improve the skin condition, but additionally to shield the pores and skin against factors inflicting pores and skin aging.

Cutaneous ageing is the result of the interplay between endogenous and exogenous factors. However, about 80% of pores and skin aging-associated changes can be attributed to extrinsic elements such as ultraviolet (UV) light, environmental pollution, chemical substances or atmospheric temperature. There are a few theories explaining the ageing process. The oxidative idea (free radicals injury of tissues) is the most popular theory. This is comprehensible if we look at the structure of the skin. Most of the lipids in the stratum corneum are unsaturated and consequently they are sensitive to damage by using free radicals Therefore, anti-aging cosmetics encompass components neutralizing free radicals: vitamins E and C, coenzyme Q10, carotenoids, or the ones contained in plant extracts, like polyphenols and flavonoids (the vegetation that are particularly wealthy in these substances are ginkgo biloba, inexperienced tea, ginseng, medical calendula, chamomile or grape seed extracts). The 2d group of raw substances current in anti-aging cosmetics are materials that protect against

solar radiation (UV filters) the intent of turning in powerful components immediately into the skin.

## Skin

Skin is the outermost and is the most superficial section of the body. It represents about 15 to 20% of the whole body mass. The skin is an ever altering organ that consists of many specialized cells and structures. As we age, modifications occur in the shape of the pores and skin that affect its appearance.

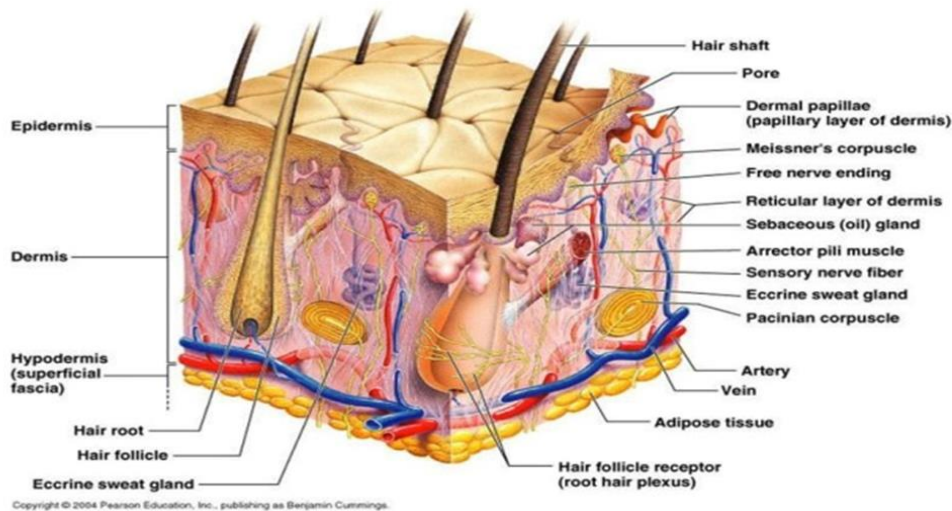


Fig.1 : Structure of Skin

## Epidermis

The epidermis is the most superficial layer of the skin. It is derived from the ectoderm and composed of keratinising stratified squamous epithelium cells. It forms a protective barrier over the body's surface responsible for keeping water in the physique and stopping pathogens from entering. It is thick at soles and palms. The epidermis also helps the skin to regulate physique temperature. The epidermis has four sorts of cells.

- 1) Keratinocytes (skin cells)
- 2) Melanocytes (pigment produce cells)

## **Keratinocytes**

The keratinocytes turn out to be extra mature or differentiated and accumulate keratin as they move.

## **Stratum Corneum**

It is the uppermost layers and is composed of keratinized cells. The thin membrane consisting of dead nucleus, keratinized mobile phone embedded in a lipid matrix.

## **Stratum Granulosum**

The granular cells are so referred to as due to the fact they collect granular structures.

## **Stratum Spinosum**

The stratum spinosum is additionally acknowledged as prickle telephone.

## **Stratum Germinativum**

This layers includes the only cells (keratinocytes) inside the epidermis that endure cell division.



## **Melanocytes**

These are discovered in the basal layer of the epidermis. Melanocytes manufacture the pigment melanin. Melanocytes appear as small cells. They have skinny cytoplasmic strategies which prolong between close by keratinocytes and serve to transfer melanosomes into adjacent keratinocytes and serve to switch melanosomes into adjacent keratinocytes.

## **Langerhans cell**

Langerhans cells are antigen-presenting cells which participate in the surveillance function of the immune system. These cells are smaller than keratinocytes. Dermis The dermis is the primary element of human skin. It is composed of network of connective tissue. Tissue in dermis.

a. collagen

b. Elastin

Layers of the dermis

a) papilla layer

b) Reticular layer

a) Papilla layer – The higher papillary layer carries a thin association of collagen fibers.

b) Reticular layer- The lower reticular layer is thicker and made up of thick collagen fibers that are arranged parallel to the surface of the skin.

### **Hypodermis**

The hypodermis lies below the dermis. The cause is to attached the pores and skin to underlying bone and muscle mass as nicely as presenting it with blood vessels and nerves. It consists of loose connective tissue and elastin. The principal phone type are fibroblasts, macrophages adipocyte. Another identify for hypodermis is subcutaneous tissue.

### **Function of Skin**

Performs the following functions:

Protection: an anatomical barrier from pathogens and damage between the inside and external environment. Sensation: consists of a range of nerve endings that react to warmness and cold, touch, pressure, vibration, and tissue harm. Heat regulation: extend perfusion and heat loss, while constricted vessels notably limit cutaneous blood.

Serum is centred product widely used in cosmetology. The term come itself from professional cosmetology. Cosmetic serum: Cosmetic serum is particularly focused primarily based on water or oil as any other cream. A serum, or different centred product containing ten times extra of biologically active substance than creams, therefore faster and greater efficaciously coping with beauty problems.

Serum effects: When pay attention are used, the pores and skin right away gets the quantity of energetic substance in such from which assimilate simpler. The active substance in excessive concentrations act identically as they moisture, rejuvenate, raise up, etc. The only distinction is that in case concentrate are used successfully and substantive end result will be ricked quicker. According to effect produced all serum are strictly divided in

Following category.

- Lifting up Revitalizing
- Moisturizing
- Nourishing
- Anti-inflammatory
- Something
- Anti stress

Serum act locally upon extraordinary physique parts face, naje, decollate eyelids. Exceptional cases ought to be taken of the skin round the place exceptional preservatives and bases are used, and the doses of energetic constituent precisely calculated. Serum can be used irrespective of age. When the usage of listen you can usually get no longer solely quick cosmetics effects. But also psychological pride after the treatment due to the fact the will be seen virtually immediately. Serum or essence make up for what is lacking in traditional skin care cosmetics. In other words, they are placed price added cosmetics product. There are many transparent, semi-transparent, viscous liquid type in market. Because the serum are used in small quantity and need to fulfill a lot of requirements of body. The position of skin-care cosmetics is to enhance the circumstance and sense of the pores and skin what is a function of its softness and flexibility. The predominant reason of beauty emulsions application is to restore and hold the water-lipid stability in the stratum corneum of the epidermis, therefore they comprise components comparable to these naturally discovered in the SC.

Serum is a phase of skin care hobbies. Importance of pores and skin pursuits are It helps your pores and skin continue to be in true condition: You're shedding pores and skin cells during the day, so it's vital to maintain your pores and skin glowing and in properly condition. Positive movements can assist forestall acne, treat wrinkles, and assist hold your pores and skin searching its best.

Your skin will seem more youthful: As you age, your skin's cells turn over more slowly, make it seem duller and much less radiant. Using a excellent skin care line can help dispose of

dead pores and skin cells so your physique will substitute them with newer, greater youthful cells.

Prevention is easier than correction: Preventing pores and skin troubles is less complicated and less high priced than trying to restore them in the future.

Your self-confidence will get an improve When your pores and skin appears better, you'll feel higher about yourself and have extra self-confidence.

## 2.0 MATERIALS AND EQUIPMENTS

Drug and material used in experiment is listed in Table 2.1.

**Table 2.1: List of drug and materials used in dissertation work**

Sr. No.	Material	Supplier
1	Sugarcane peel	Kolhapur periphery
2	Ginko biloba	Garden center, Nursery, Pune
3	Methanol	LOBA Chemie, India
4	Ethanol	LOBA Chemie, India
5	Glycolic acid	Neostrata , India
6	Sodium nitrite	LOBA Chemie, India
7	Aluminium chloride	LOBA Chemie, India
8	Sodium hydroxide	LOBA Chemie, India
9	Sandlewood oil	Crown Essential oil CO, India
10	Hydrochloric acid	LOBA Chemie, India
11	Aloe vera gel	LOBA Chemie, India
12	Deminralized water	V.Aqua Mech, India
13	Glycerine	LOBA Chemie, India
14	Tween 80	Caesar &Loretz GmbH, Germany



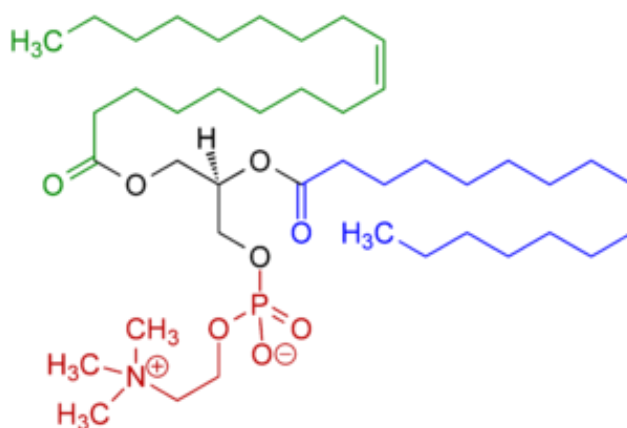
## 2.2 List of Equipments

Sr.No.	Equipments	Manufacturer
1	Digital weighing balance	CONTECH, India
2	Iodine flask	BOROSIL, India
3	Magnetic stirrer	J-SIL, India
4	Beaker	J-SIL, India
5	Funnel	J-SIL, India
6	Hot air oven	COSLAB, India
7	IR. spectrophotometer	Bruker, India
8	P.H paper	Hydrion, India
9	Glass slides	J-SIL, India
10	Soxhlet	Ikon instruments, India

## 2.3 Experimental Work

### 2.3.1. Drug and Excipient profile

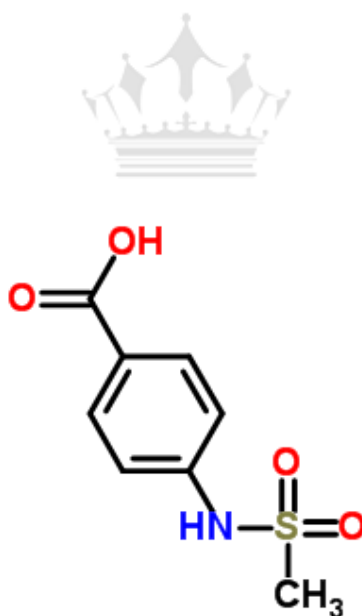
- Structure



- **Molecular formula:**  $C_{35}H_{66}NO_7P$
- **Molecular weight:** 643.9
- **Chemical name:** *Saccharum officinarum*
- **Solubility:** In methanol
- **Category:** Antioxidant
- **Mechanism of action:** potential anti-aging mechanisms of polyphenols, including antioxidant signaling, preventing cellular senescence, targeting microRNA, influencing NO bioavailability, and promoting mitochondrial function. While the trends on utilizing polyphenols in preventing aging-related disorders are getting growing attention, we suggest the exploration of the beneficial effects of the combination of multiple polyphenols or polyphenol-rich foods, as this would be more physiologically relevant to daily life.

### 2.3.2 Ginkgo biloba

- **Structure**



- **Molecular formula:**  $C_8H_9NO_4S$
- **Molecular weight :** 215.226
- **Chemical name:** *Ginkgo biloba*
- **Solubility:** In methanol

- **Category:** Anti-inflammatory, Anti-oxidant
- **Mechanism of action:** Anti-oxidant, scavenger of free radicals, especially nitric oxide induced toxicity, prevention of lipids peroxidation. Anti-inflammatory, antagonizes platelet activating factor (PAF), reduced eosinophil infiltration, reduced neutrophil infiltration.

## 2.4 Experimental Work

### Sugarcane peel extraction:

The 15 g of sugarcane peel powder was immersed in a 300 mL of methanol mixed hydrochloric acid (99:1 v/v) contained in a volumetric flask and then extracted by sonication for 3 hrs. All samples were extracted in triplicate. The extracts were pooled and evaporated the solvent by rotary evaporator. The dried crude extracts were dissolved by methanol and stored in a freezer until analysis.

### Leaf Extract of Ginkgo biloba:

Dried G. biloba leaves were grinded into powder. For extraction, 80% ethanol was used to extract the soluble compounds from the G. biloba L. leaves. (e dried leaf powder (5 g) was mixed with ethanol (1 : 20, w/v) at room temperature for 72 h, and the extraction was repeated three times. (e extracts were filtered and evaporated using a vacuum rotary evaporator. (e extract obtained was then transferred to vials and kept in the dark at – 20°C prior to use.

### Total flavonoid content:

The Total Flavonoid Content (TFC) was determined<sup>29</sup>. The methanolic extracts were mixed with distilled water, 5% NaNO<sub>2</sub> solution and 10% AlCl<sub>3</sub> solution. Finally, 1 M NaOH solution was added into the mixture and left to stand for 15 min. The absorbance at 510 nm was measured and catechin was used as standard.

### DPPH Activity:

The method determines the activity of substances at scavenging free radicals (Brand-Williams, Cuvelier, & Berset, 1995). Analyses were carried out using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method (Brand-Williams et al., 1995; Laskar, Sk, Roy, & Begum, 2010). Solutions of samples in methanol were individually added to 0.1 mM DPPH in methanol. The mixture was incubated in the dark at 25 C for 30 min (Duarte-Almeida, Santos,

Genovese, & Lajolo, 2006). Scavenging capacity was determined by monitoring the absorbance at 517 nm. Lower absorbance indicates higher free radical scavenging activities. The RSA percent was calculated as follows:  $\%RSA = \frac{a \text{ control} - a \text{ sample}}{a \text{ control}} \times 100$  where a control is the absorbance of the control (methanol plus DPPH), and a sample is the absorbance of the sample plus DPPH. A Trolox 80  $\mu$ M methanol solution was used as reference for comparison of relative RSA efficiency of the samples analysed. All assays were run in five replicates.

### **FT-IR spectroscopy of sugarcane and ginkgo biloba extract**

The FTIR spectra of sugarcane and ginkgo biloba extract were recorded over the wave number 4000 to 400  $\text{cm}^{-1}$  using Bruker FTIR spectrophotometer.

### **Preparations:**

#### **Preparation of mobile phase:**

Mobile was prepared by mixing methanol: water (60:40) containing two or three drops of orthophosphoric acid.

#### **Preparation of blank;**

Diluent was used as blank.

#### **Preparation of standard stock solution:**

5 ml each of sugarcane extract and ginkgo biloba extract dissolved in 10 ml of methanol.

#### **Preparation of sample solution:**

Sample solution having concentration of 0.001, 0.01, 0.1, 1.0, 10, 100  $\mu\text{g/ml}$  were prepared by using standard stock solution of concentration 500  $\mu\text{g/ml}$  and diluting it with HPLC grade methanol

### **Emulsion Preparation**

Emulsion (o/w) was prepared as per the formula given below. Oily phase consisting sandalwood oil and emulsifiers tween 20 and alcohol was triturated together till 10 minutes to get a homogenous solution. At the same time aqueous phase was prepared by mixing aloe vera

gel, sugarcane extract, ginkgo biloba extract, glycolic acid, glycerin , and small quantity of demineralized water homogeneously. The oily phase was added to aqueous phase drop by drop under mechanical stirring at 2500 rpm to get an oil in water based biphasic emulsion.

Sr . no	Ingredients	Percentage %
1	Sugarcane peel extract	4.5%
2	Ginkgo biloba extract	4.5%
3	Aloe vera gel	50%
4	Glycerine	25%
5	Glycolic acid	1%
6	Tween 20	1%
7	Preservative	0.01%
8	Sandalwood oil	0.1%
9	Rose water	5%
10	De mineralized water	Q.S upto 100 ml

#### Characterization of serum and stability studies:

##### Physical Appearance:

Observe the color of the serum formulation sample which should be in white milky and glossy appearance. Next, feel some serum formulation sample on the skin to access the texture which should smooth homogeneous texture and non-greasy finish.

##### pH Test:

The pH test will be determined by using Digital pH meter. Dipper of digital pH will be deep into the sample of serum formulation and the pH value will be recorded. The pH of the formulation should having acidic pH as the skin is having an acidic pH of around 4–6.

##### Homogeneity:

This will be confirmed by spread some of the serum formulations on the transparent glass and observe it. The formulation should produce uniform distribution of serum.

### **Rheological Study:**

Viscosity of the formulation is determined by Brookfield® Viscometer at 100rpm, using spindle type model S64.5 ml of the serum. The serum will be placed in a big mouth container with the spindle dipped in it for about 5 minutes before the measurement.

**Spreadability:** Test The product spreads on the skin or affected area and denotes the extent of area to which the serum was applied. Some sizes of filter paper are chosen and each filter paper is measured the total area of filter paper (A1) and weighing of each filter paper (W1). Choose the formulation to be tested and draw several milliliters into the B-D 5mL syringe and drawn onto the center of filter paper for 20 drops. When latest drop hits the filter paper, start a timer or stopwatch to count down for exactly 10 minutes. During the 10-minute test, the liquid will spread in a relatively uniform circular pattern over the filter paper. After 10 minutes, cut exactly on the line between saturated spread and dry filter paper by using scissor. Weigh the remaining dry (unsaturated) filter paper. Record this weight as W2. Measure the diameter of the saturated portion of filter paper. If the spread was not a perfect circle, then take several diameter readings around the spread area and determine an average diameter. Record this measurement as A2. % Spread by Area =  $(A2/A1)100$

### **Skin Moisture Test:**

Skin moisture will be measured by using Scalar moisture checker after applying cosmetic serum on the skin. The scale will show the moisture of the skin after using the cosmetic serum.

### **Stability Test:**

It is to determine physical and chemical stability of the product with accelerated stability analysis which subjects the material to elevated temperatures. Short term accelerated stability study was carried out for 3 months for the formulation. The samples were stored at different storage conditions of temperatures and samples are withdrawn on monthly interval and analyzed.

### 3.0 RESULTS AND DISCUSSION

#### **Physical appearance:**

Physical appearance was evaluated by observation on the texture, color and smell of the formulated cosmetic serum. All formulations gave non-greasy and non-oily properties after 3 weeks observation. Homogeneity of all formulations were uniformly distributed of contents while observing serum on the transparent glass slide. The use of fragrance in cosmetic serum is to cover the unpleasant smell ginkgo biloba extract and sugarcane extract. The diluted fragrance used in formulation made them less smell and unstable to stand in longer time.

#### **pH Evaluation of Cosmetic Serum:**

The result shows on the day 1, pH of three formulations are fall within the range of pH 5 to pH 6. This is because in the outer layers of the stratum corneum, the moisture barrier has a slightly acidic pH (4.5 to 6.5). These slightly acidic layers of the moisture barrier are called the acid mantle. The acidity is due to a combination of secretions from the sebaceous and sweat glands. The acceptance range for the effectiveness of a dermo cosmetic product is lies between pH 4 to pH 6.

#### **Rheological Study of Cosmetic Serum:**

From the test the torque value of all 5 formulations were more than 30% which means the resulted value of viscosity is in the acceptable viscometer range. Viscosity of all formulation 1, 2, 3, are gradually increased after 3 weeks placed in the room temperature (25±2) from 214.546, 276.18, 286.027, to 232.699, 292.079, 310.689, respectively. Viscosity is a measurement of internal fluid friction which is resistance to flow when one layer of fluid is forced to move in over another layer and typically measured with a Brookfield Viscometer. A fluid may be made up of molecules that vary in size, shape, and cohesiveness or a single type of molecule. As these molecules are forced to move or flow past each other, the molecular properties will determine just how much force is required to move them past each other. The force required to cause movement is referred to as shear. Shear force are important in cosmetic product to determine the packaging of pumping product during manufacturing and also the spreading of serum on the skin. According to an article, most of cosmetic products mostly categorize under shear thinning for suspensions and emulsions, where their viscosity

decreases with increasing shear rate. This behavior is also referred to as pseudoplastic and is the result of structural breakdown within the fluid.

#### **DPPH assay:**

The DPPH activity for sugarcane peel extract is 57% of DPPH reduction. The results showed that DPPH activity for ginkgo biloba extract is 64% of DPPH reduction.

#### **Spreadability Study of Cosmetic Serum:**

Spreadability indicates the area on which a semi solid topical formulation spreads on application to the skin. This parameter plays a key role in determining both the efficacy and the consumer acceptance of the product. A poor spreadability may result in an uneven distribution of the formulation on the skin, thus affecting the amount of the dose applied and the efficiency of active ingredient(s) skin permeation. On the other hand, consumers perceive a poor spreadability as a weakness of the product, which could lead to the choice of other products with a better performance, independent on their actual efficacy. From the result of evaluation, it is showed that formulation 1 give the higher percentage of spreadability with 20% compared to other formulations. Secondly, the formulation 2 and 3 also showed more than 15% percent of spreadability. Thus, it was assumed that formulation 1 had a good spreadability and consumer satisfaction when compared to other formulations.

**Skin Moisture Study of Cosmetic Serum:** A total of 10 volunteers, ages ranging from 18 to 40 years old were included in the study. The volunteers are healthy with no preceding skin diseases. Non invasive skin moisture measurements were carried out using Scalar moisture checker at 1 minute, 5 minutes and 10 minutes to determine the short term improvement in skin moisture properties after a From the moisture test of formulation 1, moisture increment in volunteer 1, 2 and 3 are 14.78%, 12.43% and 15.65%. The total percentage increment is 14.36 % ( $\pm 1.61$ ). In the formulation 2, moisture increment in volunteer 1, 2 and 3 are 21.23%, 14.15% and 21.50%.. The formulation 3 shows the highest percentage of moisture increment in all three volunteers which is 19.67 % ( $\pm 6.49$ ). Thus, from the result, formulation 3 show the highest moisture levels raised compared to other formulations.





#### 4.0 CONCLUSION

The study aimed to formulate face serum by using different herbal extract for anti-aging and antioxidant activity on skin. In this serum Ginkgo biloba and Sugarcane peel extract was used which showed combined effect on skin. It showed very good action in acne, pimples, eczema and other skin problems; darkening due to pollution and sun exposure. Ginkgo biloba showed antioxidant property and Sugarcane showed the anti-aging property.

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