EVALUATION OF SOME PROPERTIES OF *CYMBOPOGON CITRATUS* ETHANOL LEAF EXTRACT FORMULATED INTO HERBOSOME CREAM

BY

IBOI OMOYE OBOSE

PHA1908508

SUPERVISED BY

PROF. MATTHEW IKUORIA ARHEWOH

DEPARTMENT OF PHARMACEUTICS AND PHARMACEUTICAL TECHNOLOGY

FACULTY OF PHARMACY

UNIVERSITY OF BENIN

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A PROJECT SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICS AND PHARMACEUTICAL TECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DOCTOR OF PHARMACY (PHARM.D) DEGREE OF THE UNIVERSITY OF BENIN, BENIN CITY, EDOSTATE, NIGERIA

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CERTIFICATION

This is to certify that this work was done by **IBOI OMOYE OBOSE**, in the Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria, in partial fulfillment for the award of the Pharm. D degree from the University.

______________________ ______________________

IBOI OMOYE OBOSE Date

(Student)

______________________ ______________________

PROF. MATTHEW IKUORIA ARHEWOH Date

(Project Supervisor)

______________________ ______________________

PROF. MATTHEW IKUORIA ARHEWOH Date

(Head of Department)

Pharmaceutics and Pharmaceutical Technology

DEDICATION

This project is dedicated to God Almighty whose loving kindness and infinite mercy has seen me through Pharmacy school and to my Mother and Father PHARM DR. Mr and Mrs FRED and LINDA IBOI for supporting and guiding through all my years in pharmacy school.

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First and foremost, I am grateful to God almighty for the strength and inspiration to carry out this project work and for seeing me through my pharmacy school journey, may his name be praised forever.

I extend my sincere appreciation to my supervisor Prof Matthew Arhewoh and for his careful guidance and unwavering support and for assisting me to ensure my project work was done successfully without constraint. God bless you Sir.

To my parents, I am truly grateful for all you have done to support me through pharmacy school and outside pharmacy school, for my mothers endless support and daily prayers and motivational speeches and my fathers moral support and encouragement. To my siblings, PHARM DR Ejemen IBOI and Osemudiamen IBOI for their support and encouragement along the way.

To my friends, Orobosa, Eghosa, Ese-Dan, Osarugue, Fego, Naomi, Raphael and my siblings Ejemen and Osemudiamen Iboi for their love, support, motivation and never ending care they've shown to me throughout my stay in school. I hope we continue to be friends and achieve greater things in the future.

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ABSTRACT

Background and objective: Herbosomes can be defined as modified liposomes that encapsulate botanical extracts and enhance their stability, bioavailability and helps targeted administration in herbal medicine. This study aims to evaluate various properties of *Cymbopogon citratus* extract herbosomal cream and complex *Cymbopogon citratus* extract into a herbosome and incorporate into cream.

Methodology: In this study Ethanolic extraction of *Cymbopogon citratus* was carried out using a maceration method. The resultant dried extract were then complex into herbosomes of ratios 1:1, 1:2, 2:1 using soy lecithin obtained from. Each of these herbosome complexes were then incorporated into a Non-ionic oil in water cream base (SQ1-SQ3) respectively along with creams containing Lecithin, Plain *Cymbopogon citratus* extract and Vitamin E (SQ4-SQ6) respectively. The creams were then weighed and put into their respectively labeled jars. Different tests were then carried out on the formulated creams such as evaluation of physiochemical properties(Viscosity, pH, Spreadability, Homogeneity, Freeze and thaw tests),Evaluation of Antioxidant activity using DPPH radical scavenging test, Evaluation of Antibacterial activity, and biological and safety tests (Ex-vivo studies and Irritancy test).

Results: Creams SQ1-SQ6 showed Viscosity ranges from 26.17 to 36.70, pH ranges from 4.4 to 4.8, Spreadability ranges from 6.03 to 9.65, Homogeneity properties along with the freeze and thaw test were carried out and the cream was seen to be smooth with no coarse particles and, with no physical changes seen after the freeze and thaw test. These creams were seen to have Antioxidant properties with SQ1 showing the highest Antioxidant activity of 56.81%, However, there was no Antibacterial activity seen for the *Cymbopogon citratus* herbosomal creams . For the Ex-vivo studies it was seen that complexing *Cymbopogon citratus* extract into a Herbosome enhanced the permeability of the Cream across the skin membrane of a Sprague-Dawley rat with SQ3 showing a Percentage release of 61.7% after 3 hours. No irritancy was observed with use of the creams.

Conclusion: *Cymbopogon citratus* herbosomal cream exhibited good Physiochemical and Antioxidant Properties. Complexation of *Cymbopogon citratus* in a herbosome enhanced its lipid permeability.

CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

1.1.1 Herbosomes

Herbosomes can be defined as modified liposomes that encapsulate botanical extracts and enhance their stability, bioavailability and helps targeted administration in herbal medicine (Joshi *et al.,* 2024). The difference between herbosomes and liposomes lies in fact that in liposomes there is no chemical bond formed between phosphatidylcholine and the herbal extract, the phytoconstituent is held together by hundred or thousands of phosphatidylcholine molecules, while in herbosomes there is a chemical bond formed between the former constituents (herbal extract and phosphatidylcholine) as seen in Fig 1.1. Herbosomes are formed by a 1:1 or 2:1 mixture of phosphatidylcholine and herbal extract respectively in an aprotic solvent such as acetone. Phosphatidylcholine being a bifunctional molecule comprises a lipophilic phosphatidyl tail and a hydrophilic choline head. Many natural products have been seen to display pharmacological activity. However one of the many drawbacks regarding the use of natural products for therapeutic purposes is their high water solubility which affects their biopharmaceutical properties such as permeability, in vivo solubility and bioavailability of the phytoconstituents. Herbosomes act as vesicular systems that get rid of the concerns linked to the therapeutic efficacy of water-soluble drugs such as those extracts obtained from plants.

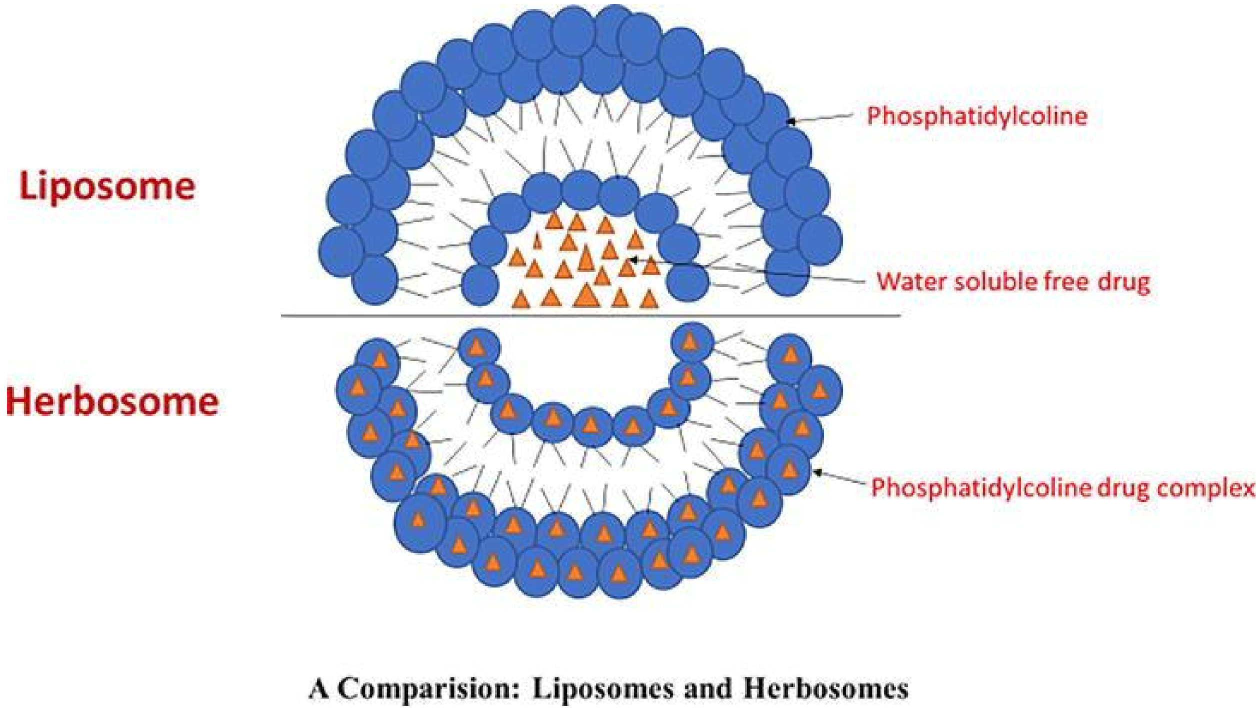

Figure 1.1 Differentiation between herbosomes and liposomes

1.1.2 Mechanism of herbosome as a drug delivery

Development of novel drug delivery systems from natural resources is very much necessary because of the beneficial role of herbal drug in the management of varied diseases (Shakya *et al.,* 2014).

The bioavailability of lipophilic drugs when administered orally or topically as solid dosage forms is low. “Herbosomes” are the combined form of herbal product in combination with the phospholipids having better absorption and utilization profiles in our body and subsequently producing better therapeutic efficacy than the conventional herbal extracts or individual molecule, which can minimize the shortcomings of conventional herbal therapy.

The term "herbo" means plant, while "some" means cell-like. It is also mentioned as a phytosome, planterosome (Shakya *et al.,* 2014).

Herbosomes, also known as Phytosomes, are composed of phytochemicals, plant extracts and phospholipids. They have an increased absorption rate, improved bioavailability compared to the herbal extracts, and a decreased particle size. The polar functional group of the phytochemicals and the polar side of the front of the phospholipid are bound together by hydrogen bonds; this chemical interaction forms a pattern in terms of structural elucidation. The selective targeting of this drug delivery system can be enhanced by modification of the surface of these herbosomes. This could be potentially helpful in the modification of acne agents/extracts as it enhances the selective targeting of skin cells, thus increasing the effectiveness of these extracts.

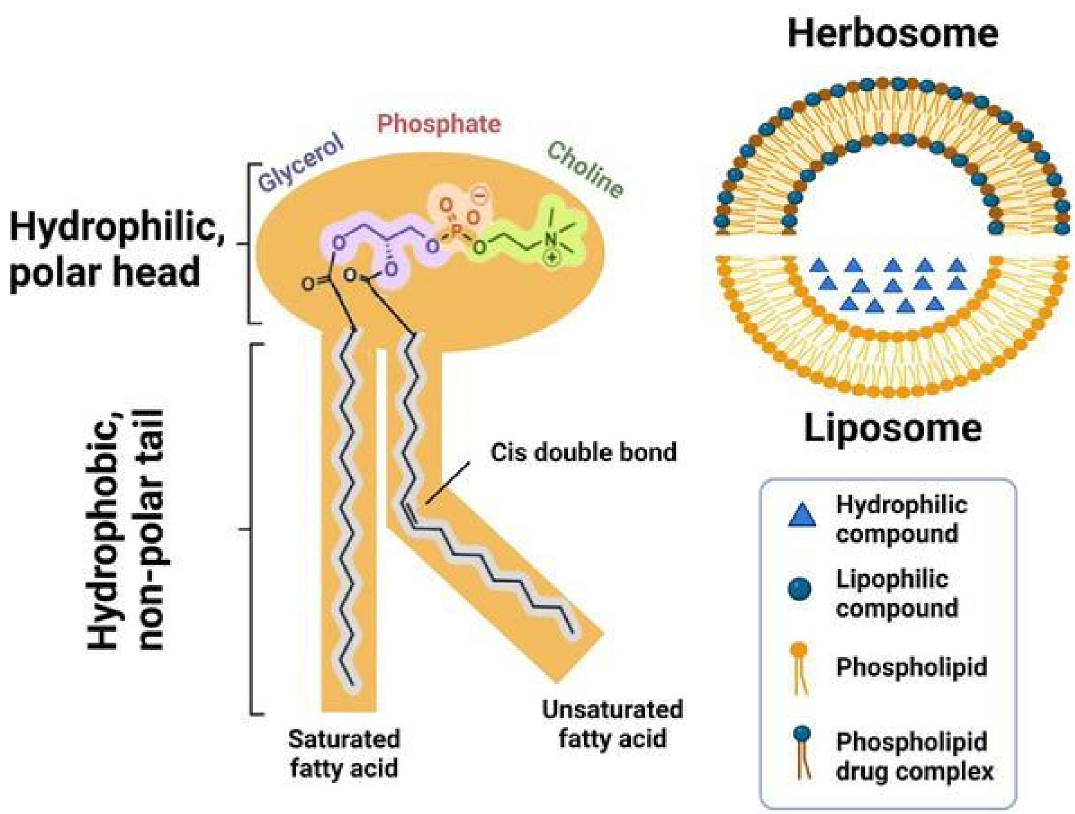

Figure 1.2 Description of the various components of a herbosome structure

1.2 What is Oxidative stress and Antioxidants

Oxidative stress, defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses (Betteridge 2022).

An antioxidant is any substance that when present at low concentrations compared to those of an oxidizable substrate delays or prevents oxidation of that substrate. Mechanisms of antioxidant action can include: removal of O_2, scavenging reactive oxygen/nitrogen species or their inhibiting ROS/RNS formation, binding metal ions needed for catalysis of ROS generation, and up regulation of endogenous antioxidant defenses (Halliwell 1996).

Methanol and methanol/water extracts, infusions and decoctions of Cymbopogon citratus, and particularly the phenolic fractions, have been shown to have the ability to eliminate ROS in several trials of antioxidant activity (Figueirinha *et al.,* 2008).

1.3 Oxidation and Determination of Antioxidant activity

Measurement of antioxidant properties in plant-derived compounds requires appropriate methods that address the mechanism of antioxidant activity and focus on the kinetics of the reactions involving the antioxidants. One of the most common methods used is a spectrophotometric method the DPPH Radical scavenging assay.

Oxidation processes are necessary for cell survival. Aerobic cellular respiration organisms provide energy from organic molecules such as glucose but also cause the formation of free radicals that cause cellular damage during metabolism (Gülçin *et al.,* 2023)A free radical contains an unpaired (free) electron with a quantum-mechanical property called spin. Such an entity typically has high reactivity because of its open shell structure . Free radicals are known to be mostly associated with oxidative stress. Oxidative stress is a comparatively new concept that has been commonly used in the medical sciences recently. It occurs when there is an excess of reactive oxygen species (ROS) produced by a cellular mitochondrion. It is inevitable that free radicals, which are known to cause many degenerative diseases such as carcinogenesis, acute inflammation, high blood pressure, diabetes, preeclampsia, acute renal failure, atherosclerosis, Alzheimer's disease and Parkinson's disorders, mutagenesis, aging, and cardiovascular disorders, are produced in biological systems. There are many factors, including UV radiation and pollutants, that contribute to oxidative stress, which has a daily influence on human health . Cells metabolize oxygen, creating potentially harmful ROS. Under normal conditions, the rate and amplitude of oxidant formation are balanced by the rate at which they are removed]. However, disruption of the balance between antioxidants and pro-oxidants causes oxidative stress (Gülçin *et al.,* 2023).

Anti-oxidants are neutralizing chemicals that minimize oxidative damage to the body by giving free radical electrons and passing them off as harmless.The technique of DPPH testing is associated with the elimination of DPPH, which would be stabilized as a free radical. The free radical DPPH interacts with an odd electron to yield a strong absorbance at 517nm (purple hue). The 1,1-diphenyl-2-picrylhydrazil (DPPH) radical was discovered 100 years ago by Goldschmidt and Renn in 1922 (Goldschmidt *et al.,* 1922).

This assay is based on spectrophotometric measurements of the capacity of antioxidants to scavenge DPPH radicals.The single electron of the nitrogen atom in DPPH is reduced to the corresponding hydrazine by taking a hydrogen atom from the antioxidants. The DPPH· radical has a remarkably stable and intense color. Due to these two properties of the radical, its solution has been used intensively. This radical has been frequently used in polymer chemistry, especially in EPR spectroscopy, and in the evaluation of the antioxidant capacities of chemicals, extracts etc (Bondet *et al.,* 1997)

1.4 Formulation of Herbosomes

When formulating herbosomes there are four main considerations that should be taken account for which are

. The phospholipid of choice

. The solvent of choice

. The Ratio of Phospholipid to Phyto-constituents

. The pH of the system

Herbosomes are complexes formed between natural phytoconstituents and natural phospholipids, most commonly soy-derived phosphatidylcholine. These complexes are created through the reaction of specific stoichiometric ratios of phospholipids and phytoconstituents in an aprotic solvent. In this structure, the active plant compound binds to the polar head of the phospholipid, thus becoming an integral part of the membrane.Herbosomes are an advanced form of herbal drug delivery, they offer improved absorption and utilization within the body, providing more effective therapeutic outcomes compared to conventional herbal formulations. Their enhanced bioavailability has been confirmed through both pharmacokinetic studies and experimental tests conducted on animals and humans (Tufail *et al.,* 2025).

When standardized plant extracts or primarily polar phytoconstituents — such as flavonoids, terpenoids, tannins, and xanthones — are combined with phospholipids like phosphatidylcholine, they form a new drug delivery system known as a herbosome. It improves the compounds' lipid solubility, therefore enhancing their ability to pass through biological membranes and, in turn, increasing bioavailability after oral administration.

Compared to ordinary plant extracts, herbosomes allow a higher concentration of active compounds to reach target organs and cells (such as the liver, brain, heart, or kidneys) even at lower or similar doses leading to a stronger, more noticeable, and longer-lasting therapeutic effect (Tufail *et al.,* 2025).

There are various processes used in the formulation of herbosomes and they include:

. Antisolvent precipitation technique

.Solvent evaporation technique

.Ether injection technique

- Anti Solvent Precipitation Technique

A measured quantity of plant extract and phospholipid is placed in a 100 ml round-bottom flask and refluxed with 20 ml of dichloromethane at a temperature not exceeding 60°C for 2 hours. After refluxing, the mixture was concentrated down to a volume of 5–10 ml. Then, 20 ml of hexane was slowly added to the concentrated mixture while stirring continuously, which led to the formation of a precipitate.The precipitate was then filtered, collected, and stored in a desiccator overnight for drying. Once dried, the precipitate was ground using a mortar and pestle and passed through a #100 mesh sieve to obtain a fine powder (Tufail *et al,*.2025).

- Solvent Evaporation Technique

A specific quantity of plant material and phospholipids was added to a 100 ml round-bottom flask and refluxed with 20 ml of acetone at 50–60°C for 2 hours. The resulting mixture was concentrated to 5–10 ml, allowing the precipitate to form. The precipitate was then filtered, collected, dried, and stored in an amber-colored glass bottle at room temperature (Tufail *et al.,* 2025).

- Ether Injection Technique

In this method, the drug-lipid complex is first dissolved in an organic solvent. This solution is then gradually injected into a heated aqueous medium, which leads to the formation of vesicles. At lower concentrations, amphiphiles exist in a monomeric state, while at higher concentrations, they can form various structures such as round, cylindrical, disc, cubic, or hexagonal shapes (Tufail *et al.,* 2025).

1.5 Evaluation of Herbosomes

The physical size, membrane permeability, percentage of entrapped solutes, drug release rate, chemical composition, and the quantity and purity of the starting materials all play a role in the formation of herbosomes. Herbosomes affect both the physical and biological systems (Tufail *et al.,* 2025).

There are various method to evaluate and characterize already formed herbosomes such as:

- Entrapment Efficiency

  This method evaluates the percentage of the drug present within the phospholipid mesh. This method uses ultracentrifugation to determine the efficiency of drug entrapment within herbosomes. The ultracentrifugation technique may assess the entrapment efficiency of herbal medicine formulation using herbosomes. The herbosomes are centrifuged at 2000rpm for 1 hour at a regulated temperature of 4°C. The supernatant containing unentrapped medication was removed, and UV spectrophotometrically evaluated against phosphate buffered saline PH 67.4 (Joshi *et al.,* 2024) .

- Drug Content

  The drug content can be quantified using a modified High-Performance Liquid Chromatography (HPLC) method or an appropriate spectroscopic technique (Tufail *et al.,* 2025)

- Others

Temperature: The transition temperature of vesicular lipid systems can be determined using a Differential Scanning Calorimeter (DSC).

Surface Tension Activity Measurement: The surface tension of the drug in aqueous solution can be measured using the Du Nouy ring tensiometer ring method (Tufail *et al.,* 2025)

1.6. Applications of Herbosomes

- Herbosomes are/ can also be used as an anti-inflammatory, lipolytic, isokinetic, antioxidant for skin and liver, cardioprotective, anti-wrinkle, and UV protectant
- Phytosomes in grape seeds comprise phospholipid-complexed oligomeric polyphenols with different molecular sizes. Procyanidin flavonoids from grape seeds have several key characteristics, including a significant protective effect against atherosclerosis, a boost to the human body's natural antioxidant defenses, an increase in total antioxidant capacity, and protection against heart damage caused by ischemia and reperfusion. These actions are achieved through complicated mechanisms that go beyond the flavonoids' higher antioxidant potency
- They are useful for safe gene therapy
- They are used to treat hyperlipidemia and vascular and skin problems
- Green tea's long-term health benefits include its anti-oxidant , anti-mutagenic, anti-carcinogenic, antiatherosclerotic, hypo-cholestrolemic, cardioprotective, and antibacterial properties. Green tea polyphenol;s have very low oral bioavailability from standard properties. Green tea polyphenols have low oral bioavailability from standard preparations, notwithstanding their potential activity. Their combinations with phospholipids significantly improves their low oral bioavailability (Joshi *et al.,* 2024)

1.6.1 Method for Storage of Herbosomes

- Refrigeration

  Herbosomes are usually stored in the refrigerator at a temperature of 2-8 degree Celsius (36-46 °F)
- Freeze Drying

In order to reduce water content some herbosomes can be freeze dried using a freeze dryer. This helps preserve them and they can be stored at room temperature or in a refrigerator

- Glass Vials

  Herbosomes can be stored in glass vials, which are then sealed with aluminum caps and rubber stoppers.

- Cryopreservation

  This involves storing herbosomes at extremely low temperatures (typically 196°C) using liquid nitrogen to preserve their structure and function

- Encapsulation

  This involves encapsulation in a protective matrix such as alginate or chitosan to shield the herbosomes from environmental stressors.

1.7 Release Studies

During the last decades, the study of the in vitro dissolution of pharmaceuticals has been strongly encouraged by the FDA in order to determine its relationship with the in vivo bioavailability of a drug (Cascone 2017)

Sprague-Dawley rats are broadly used in preclinical studies for drug development, so a lot of information for the rats can be obtained especially from pharmacokinetic, pharmacological and toxicological studies (Takeuchi *et al.,* 2011).

1.8 Significance of the Study

Several studies have shown the antioxidant activity of *Cymbopogon citratus* extract such as the study by (Unuigbe *et al.,* 2019) which show the antioxidant activity of different solvent extracts of *Cymbopogon citratus* such as methal, ethyl acetate etc. In this study the antioxidant activity of Ethanolic extracts of *Cymbopogon citratus* were determined. In addition it was formulated into a herbosomal structure so as to enhance its lipid solubility so as to aid in reducing the presence of reactive oxidative species (ROS) on the skin which act as a contributor and enhancer of acne. The formation of this extract into a herbosomal structure enhances the lipid permeability of the extract thus enhancing its ability to permeate through the cells and exert their effect. The herbosomal extract was then formulated into a cream and its properties were evaluated.

1.8.1 Failings of Herbosomes

- Complicated manufacturing process: The intricate manufacturing procedure needed to create herbosomes is one of their disadvantages. Active plant constituents are extracted and bound to phospholipids. This tedious process is a major drawback of herbosomes.
- The phytoconstituent may leach off this reducing the concentration of the medication, thus suggesting the unstable nature of herbosomes - Plant constituents are rapidly removed and have a short half life (Joshi *et al.,* 2024)

1.9 Aims and Objectives

. This study aims to evaluate some properties of *Cymbopogon citratus* extract herbosomal cream i.e physiochemical, Antioxidant, Antimicrobial properties.

. Formation of *Cymbopogon citratus* extract into herbosome and incorporation into cream

CHAPTER 2

MATERIALS AND METHOD

2.1 Sample Preparation

Dried leaves of *Cymbopogon citratus* were collected from the Pharmacognosy Laboratory garden in University of Benin fresh and were air dried at room temperature for a few days. It was then pulverized using a British milling machine then weighed. The weight was actualized to be 250 g. It was then macerated with 2.5 litres of ethanol for 72 hours with constant checking and stirring. Filtration was then carried out to separate the residue from the filtrate using filter paper, funnel and conical flask. The filtrate was then concentrated under a hot water bath for 3 days then the crude extract was then reserved in a sample bottle inside a refrigerator. The final extract was seen to weigh 16.32 g with a percentage yield of 6.528%.

2.2 Anti-solvent Precipitation Technique

The phytosome termed *Cymbopogon citratus* was prepared by the Maceration method. *Cymbopogon citratus* extract was accurately weighed in a round bottom flask and dissolved in 100 mL of absolute ethanol at 60 °C under reflux and magnetic stirring until a homogeneous suspension was obtained. Separately, soy phosphatidylcholine (PC) was solubilized in 25 mL of absolute ethanol and slowly added under reflux and stirring to the solubilized that reacted for *Cymbopogon citratus* 2 h at 60 °C. Ethanol was then evaporated in a porcelain dish under a low heat below 45 °C in a hot water bath to remove any traces of the solvent. The resulting sticky/oily, translucent, orange-colored compound was dried and weighed.(Deleanu *et al.,* 2023)

Table 2.1 Formula Table for the Preparation of Batches of *Cymbopogon citratus* of

BATCH	PHOSPHO LIPID	EXTRACT	CHLOROF ORM	ETHANOL	MOLAR RATIO
SQ1	1	1	100	25	1:1
SQ2	2	4	100	25	1:2
SQ3	4	2	100	25	2:1

Herbosomal Structure

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Figure 2.1 Preparation of *Cymbopogon citratus* extract herbosome

2.3 Preparation of Herbosome Cream

The ingredients for the oily phase (Liquid paraffin, Stearic Acid ,Methyl and Propyl paraben, Cetyl alcohol and Emulsifying wax) were weighed into a clean dry porcelain dish and placed under the hot water bath to heat up,at the same time the aqueous phase ingredients (Glycerin, Disodium EDTA, Propylene Glycol) were also heated under a hot water bath to heat to the same temperature as the oily phase. The oily phase was then transferred into the aqueous phase in a clean dry mortar and placed in a bowl of cold water and stirrer until a thick clicking cream had been formed. The herbosome formulation (SQ1,SQ2,SQ3) were dissolved in the oily phase as it is not soluble in the aqueous phase. This method was repeated using SQ1, SQ2, SQ3, Lecithin (SQ4), Plain extract (SQ5),Vitamin E (SQ6) samples.

Table 2.2 Formulation Table for Preparation of Cream

	Cream 1	Cream 2	Cream 3	Cream 4	Cream 5	Cream 6
Emulsifying wax (g)	7.00	7.00	7.00	7.00	7.00	7.00
Cetyl alcohol(g)	3.00	3.00	3.00	3.00	3.00	3.00
Liquid paraffin(ml)	11.50	12.00	12.00	12.00	12.00	12.00
Glycerin(ml)	3.88	3.88	3.88	3.88	3.88	3.88
Propylene glycol(ml)	2.88	2.88	2.88	2.88	2.88	2.88
Methyl/Propyl paraben(g)	0.40	0.40	0.40	0.40	0.40	0.40
Disodium EDTA(g)	0.1	0.1	0.1	0.1	0.1	0.1
Stearic acid(g)	2.00	2.00	2.00	2.00	2.00	2.00
SQ1(g)	1.25	–	–	–	–	–
SQ2(g)	–	5.25	–	–	–	–
SQ3(g)	–	–	2.27	–	–	–
SQ4(g)	–	–	–	2.00	–	–
SQ5(g)	–	–	–	–	1	–
SQ6(ml)	–	–	–	–	–	0.5
Water						

Keywords: SQ1- 1:1 Herbosome cream
SQ2- 1:2 Herbosome cream
SQ3- 2:1 Herbosome cream
SQ4- Lecithin cream
SQ5-Plain Extract cream
SQ6-Vitamin E cream

Figure 2.2 Preparation of *Cymbopogon citratus* Extract Herbosomal Cream

2.4 Evaluation of Physiochemical Properties of Herbosomal Cream

Various Physiochemical test were conducted on the formulated creams such as Viscosity, PH, Spreadibility, Homogeneity, Irritancy tests

2.4.1 Evaluation of Herbosomal Cream Viscosity

The Digital viscometer was turned on and the spindle attached and the machine set at 6 rpm. The spindle was then placed in the cream and the start button was pressed. The viscosity was displayed and recorded. This procedure was carried out for all sample creams.

2.4.2 Evaluation of Herbosomal Cream pH

The pH meter was first placed in the buffer solution to zero the pH meter after which it was placed in the sample cream. This procedure was carried out for all sample creams ensuring to clean the reader after each reading.

2.4.3 Evaluation of Herbosomal Cream Spreadability

1 g of cream was placed on one of the microscope glasses and the other was placed on top. A jar weighing 111.3 g was placed on top and the timer was set for one minute. After the time had elapsed the diameter was measured and recorded. This test was repeated for all sample creams.

2.4.4 Homogeneity Test

Homogeneity test is carried out to ensure even distribution of the active ingredient and evaluate the smoothness of the cream. Here visual and tactile assessments were carried out. A small amount of cream was rubbed between the thumb and index finger and assessed for smoothness, grit, or the presence of coarse particles.

2.4.5 Freeze and Thaw Test

This test evaluates the ability of the formulated creams to withstand temperature fluctuations. Temperature fluctuations can cause degradation, physical changes or phase separations of creams. The cream is kept in the freezer at -20 °C for 48 hours and then removed and kept at room temperature 25 °C and evaluated for changes in physical properties of the cream.

2.5 Evaluation of Antioxidant Activity of Formulated Creams

1 g of was titrated with 10ml of distilled water in a 100ml beaker and made up to volume with 40 ml. A 1% dilution was then carried out on the mixture using distilled water in a test tube. To further dilute the sample a 0.1% dilution was carried out again using distilled water. Separately, in a dark room, 0.00394 g of DPPH obtained from the pharmaceutical chemistry Laboratory in

University of Benin was dissolved in 100 ml of 0.1 mM methanol obtained from the Pharmaceutical Chemistry Laboratory in University of Benin in a conical flask wrapped in aluminum foil and kept in a dark cupboard for 30 minutes. 0.2 ml of the DPPH solution was then added to the test tube containing 10 ml of the 0.1% dilution and kept in a dark cupboard for 30 minutes. After the time had elapsed, the solution was introduced into the UV-vis spectrophotometer and the results read at a wavelength of 517 nm

2.6 Evaluation of Anti-microbial Activity

Mullein Hinton agar medium was prepared and inoculated with *Cutibacterium Acnes* and *Staphylococcus Aureus* as test organisms obtained from the ripe pimples of a consenting volunteer and allowed to solidify in two separate petri dishes. A 10 mM stem borer was then used to bore a cavity into each plate and it was then filled with the *Cymbopogon citratus* extract , another hole was then bored adjacent to the first hole and filled with a plain cream base to compare for activity. The petri dishes were then incubated at 37°C for 24 hours. After which the Inhibition Zone diameter was measured and recorded.

2.7 Biological and Safety Tests

Biological and safety tests such as Ex-vivo/Release studies and Irritancy tests were carried out

2.7.1 Ex-vivo Studies

1 g of extract was dissolved in 100 ml of distilled water to create a 1000 mg/dl concentration. 10 ml of this solution was then added into a test tube with 10 ml of distilled water making up to 20 ml. 10 ml of that solution was then added to another test tube with 10ml of distilled water. This was repeated 8 times and the final 10ml was discarded.

Using the UV-VIS Spectrophotometer , the different concentrations were scanned to determine a marker in the extract that is sensitive at a particular wavelength. After the wavelength was determined (297nm) quantitation was then carried out to determine the absorbance of the various concentrations and a calibration curve was then plotted.

Six dead rats were collected from the pharmacology lab and the skin was harvested and cleaned with acetone to remove the fat and NaCl (Sodium Hydroxide) to remove the hair on the rat skin. The rat skin was then stored in a phosphate buffer in the refrigerator. The basket dissolution method, which involves the attachment of a cylindrical basket constructed from non reactive mesh; to prevent any unwanted chemical reactions that may alter the final result, is attached to the rotating shaft. 1 g of the sample cream SQ3 was placed in the center of a cut out piece of the rat skin, and tied closed ensuring no leakage of the sample cream and placed in the dissolution mesh which is attached to the rotating shaft; 250 ml of phosphate buffer obtained from Pharmaceutics laboratory in University of Benin was then put in the dissolution basket. The machine was then started and allowed to run for 3hours, with 10ml samples being collected every 1 hour. For every 10ml sample that was collected 10 ml of fresh phosphate buffer was reintroduced back into the dissolution basket. Each sample was then read in the UV-VIS spectrophotometer to determine the absorbance of each sample at the specified wavelength (297nm). This method was repeated for sample creams SQ4, SQ5.

2.7.2 Irritancy Test on Volunteers Skin

A 3 consenting individuals were used for this test as ethical approval was not given. A small amount of cream was placed on a small area of the individual's skin and was left for 24 hours. After 24 hours the area of the skin where the cream was applied was examined for edema, redness, inflammation and irritation.

CHAPTER 3

RESULTS AND DISCUSSION

This chapter presents the results obtained from the evaluation of the formulated *Cymbopogon citratus* (lemongrass) creams. The analysis includes; Evaluation of Physiochemical properties (Viscosity, Ph, Spreadability, Homogeneity, Freeze and thaw test), Evaluation antioxidant activity, Evaluation of Antibacterial activity, Biological and Safety Tests Ex-vivo Studies) which serve as indicators of the likely skin compatibility of the creams. The samples: SQ0, SQ1, SQ2, lecithin, vitamin E, and the plain extract, were analysed to assess how formulation composition and storage duration affected their overall quality. Release studies were also carried out to determine the amount of *Cymbopogon citratus* (lemongrass) extract was able to diffuse across a membrane(rat skin).

3.1 Results for Physiochemical Properties of Formulated Cream

Evaluation of the Physiochemical Properties of the formulated creams were determined

3.1.1 Viscosity Test Results

Viscosity measurements were performed using a Brookfield viscometer fitted with a spindle at a rotational speed of 6 rpm and a temperature of 24.6°C on day 1 and 29.4 °C on day 21. These conditions were maintained throughout the analysis to ensure consistency and reproducibility of results. The viscosity of the creams varied both with sample type and storage duration. SQ1 exhibited a steady increase from 25.6 mPa·s on day 1 to 37.1 mPa·s on day 21. Initially there was a high variation of the PH results on Day 1, however, over time the cream viscosity stabilized, which indicates improved consistency and possible polymer network formation over time (Gupta *et al.*, 2014) . SQ2 showed mild to moderate fluctuations in PH values and SQ3 showed high fluctuations, suggesting that interactions between the active extract and the cream base may have occurred thus creating this variability in cream viscosity. Plants extracts are known to contain numerous constituents that may interact with the intermolecular forces in the cream such as van der wall force thus reducing their attraction to each other and minimize the formation of closely linked intermolecular bonds. SQ3 containing plain *Cymbopogon citratus* extract was seen to show moderate fluctuations.

The standard deviation indicates the dispersion of the data from the mean(Barde and Barde). It shows how much the individual Viscosity measurements vary from the average value within a single group. A small Standard deviation indicates data points clustered closely around the mean, indicating high precision in measurements.While a large SD means your data is more spread out from the mean, hence indicating higher variability

Vitamin E(SQ6) and Soy lecithin(SQ4) maintained relatively stable viscosity values, consistent with their established role as stabilising agents in emulsions. It is seen that SQ6 and SQ4 had higher viscosity values ,this can be attributed to the fact that Vitamin E oil is a naturally thick and highly viscous liquid and Soy lecithin which is a common emulsifier can increase the intermolecular bond network within the cream thus increasing its viscosity. In cosmetic formulations, optimal viscosity ensures good spreadability, adherence, and consumer acceptability (Swarbrick, 2019). Therefore, all creams demonstrated acceptable rheological behaviour suitable for topical application. The observed stability over the 21-day period indicates that the formulations were physically stable with no significant phase separation or structural breakdown.

Table 3.1 Results of Viscosity test for Cream

	DAY 1	DAY 21
SQ1	29.77 ± 3.67	36.70 ± 0.37
SQ2	26.17 ± 0.57	27.90 ± 2.21
SQ3	25.10 ± 3.36	26.30 ± 2.36
SQ4	29.00 ± 0.55	29.77 ± 1.36
SQ5	28.90 ± 2.25	27.73 ± 1.50
SQ6	31.90 ± 3.07	32.77 ± 1.14

3.1.2 PH Test Results on Formulated Cream

The pH values of all formulations ranged between 4.4 and 4.8, remaining relatively stable over the 21-day storage period. This range is within the acceptable pH for topical creams (4.0–6.0), ensuring skin compatibility and reduced risk of irritation (Chaudhary *et al.,* 2018).

SQ1 maintained a near-constant pH (4.67–4.66), suggesting good formulation stability and minimal degradation of active compounds. In contrast, SQ2 showed a minor drop in pH by day 14 (4.54), which may reflect mild oxidation or hydrolysis of constituents. The stability of the lecithin and vitamin E controls further supports that the system resisted significant pH drift during storage. Ageing of lecithin samples on storage was noted to result in degradation and a decrease in pH (Haidar *et al.,* 2017) From the results in Table 3.2 it can be seen that SQ4 containing lecithin exhibited a slight decrease in pH over the course of 21 days , however there was minimal variation in the daily PH results.

The standard deviation indicates the dispersion of the data from the mean(Barde and Barde, 2012). It shows how much the individual pH measurements vary from the average value within a single group. A small SD indicates data points clustered closely around the mean, indicating high precision in measurements.While a large SD means your data is more spread out from the mean, hence indicating higher variability. From the results obtained the results were seen to be more clustered to the mean thus indicating high precision and hence stability as the results exhibit low variability.

Overall, the data suggest that incorporation of *Cymbopogon citratus* in a herbosomal base does not negatively affect the pH stability of the cream. The slight acidic nature of the formulations aligns well with the natural acid mantle of the skin, aiding in barrier function and antimicrobial defence (Lambers *et al.,* 2006).

Table 3.2 Results for pH of Formulated Cream

SAMPLES	DAY 1	DAY 7	DAY 14	DAY
SQ1	4.425 ± 0.017	4.312 ± 0.023	4.418 ± 0.014	4.437 ± 0.016
SQ2	4.662 ± 0.010	4.443 ± 0.014	4.348 ± 0.023	4.834 ± 0.031
SQ3	4.620 ± 0.058	4.357 ± 0.041	4.412 ± 0.067	4.629 ± 0.078
SQ4	4.517 ± 0.026	4.412 ± 0.012	4.295 ± 0.031	4.214 ± 0.023
SQ5	4.735 ± 0.048	4.522 ± 0.025	4.234 ± 0.021	4.615 ± 0.014
SQ6	4.807 ± 0.017	4.611 ± 0.023	4.719 ± 0.015	4.564 ± 0.082

3.1.3 Results for Spreadability Test on Formulated Creams

Cream base should spread easily without too much drag and should not produce greater friction in the rubbing process (Sabale *et al* . 2011).A good range of spreadability for creams is 5 cm to 7 cm, as determined by standardized spread tests where the cream is applied to a surface and the resulting diameter is measured after a set time and under a specified load. A greater spread distance indicates better spreadability and potentially better penetration, impacting ease of application, user experience, and effectiveness. From the results obtained in Table 3.3 it was seen that the creams exhibited good spreadability properties.

The spreadability is calculated with the formula

S = m * l/t

m – weight tied on upper slide

l – length of cream on glass slide

t – time in sec(Sabale *et al* . 2011)

Table 3.3 Results for Spreadability Test

SAMPLE	DAY 1	DAY 21
SQ1	8.35 ± 0.990	6.32 ± 0.231
SQ2	7.88 ± 0.495	7.55 ± 0.145
SQ3	7.98 ± 0.566	9.21 ± 0.851
SQ4	9.65 ± 0.707	8.26 ±0.712
SQ5	6.03 ± 0.071	8.32 ±0.371
SQ6	6.68 ± 0.141	7.43 ±0.312

3.1.4 Homogeneity Test Results for Formulated

It was seen that Creams SQ1-SQ6 displayed good homogeneity as it was evaluated to be smooth and there were no presence of grits or coarse particles within the cream

3.1.5 Freeze and Thaw Test Results

After the specified time had elapsed and the creams SQ1-SQ6 were thawed and assessed, it was seen that there was no change in physical properties or phase separation of the creams

3.2 Results for Antioxidant Activity Determination on Formulated Creams

As shown in Table 3.4, the percentage inhibition for the absorbance values for the antioxidant activity varied among the formulations. Lower absorbance; Higher percentage inhibition values generally indicate higher antioxidant potential because of stronger free-radical scavenging ability (Ebrahimzadeh *et al.,* 2010). SQ1 recorded the highest percentage inhibition (56.81%), this stoichiometric ratio of herbosome (1:1) is known to be the most stable, hence this may suggest why it possessed the highest antioxidant activity among the formulated herbosome cream samples (SQ1-SQ3).SQ1 exceeds thresholds for "promising" antioxidants (>50%), supporting efficacy in anti-inflammatory topicals.(Brand-Williams *et al.,* 1995)

When compared to the SQ5 (plain extract) it can be seen that the herbosome cream displayed higher antioxidant activity, suggesting the effect of lecithin which was complexed with *Cymbopogon citratus* ;in enhancing the antioxidant effect of the *Cymbopogon citratus* extract. While as seen in Table 3.4 SQ3 (35.80%) and SQ2 (25.59%) offer fair protection, suitable for supportive Antioxidant roles. SQ4 (0.00%) exhibits no activity, possibly due to absent/inactive antioxidants or formulation incompatibility. SQ5 (11.36%) and SQ6 (5.11%) are weak, suggesting dilution effects or degradation during prep.

To determine the antioxidant activity of these samples the DPPH radical scavenging assay was used. To determine the percentage of DPPH radical scavenging activity the formula below is used

$$(Ao-A1)/Ao \times 100$$

Where, Ao is the absorbance of the control (DPPH solution without the sample)

A1 is the absorbance of the sample (DPPH solution with the test sample)

After calculations were made the following percentage results were calculated

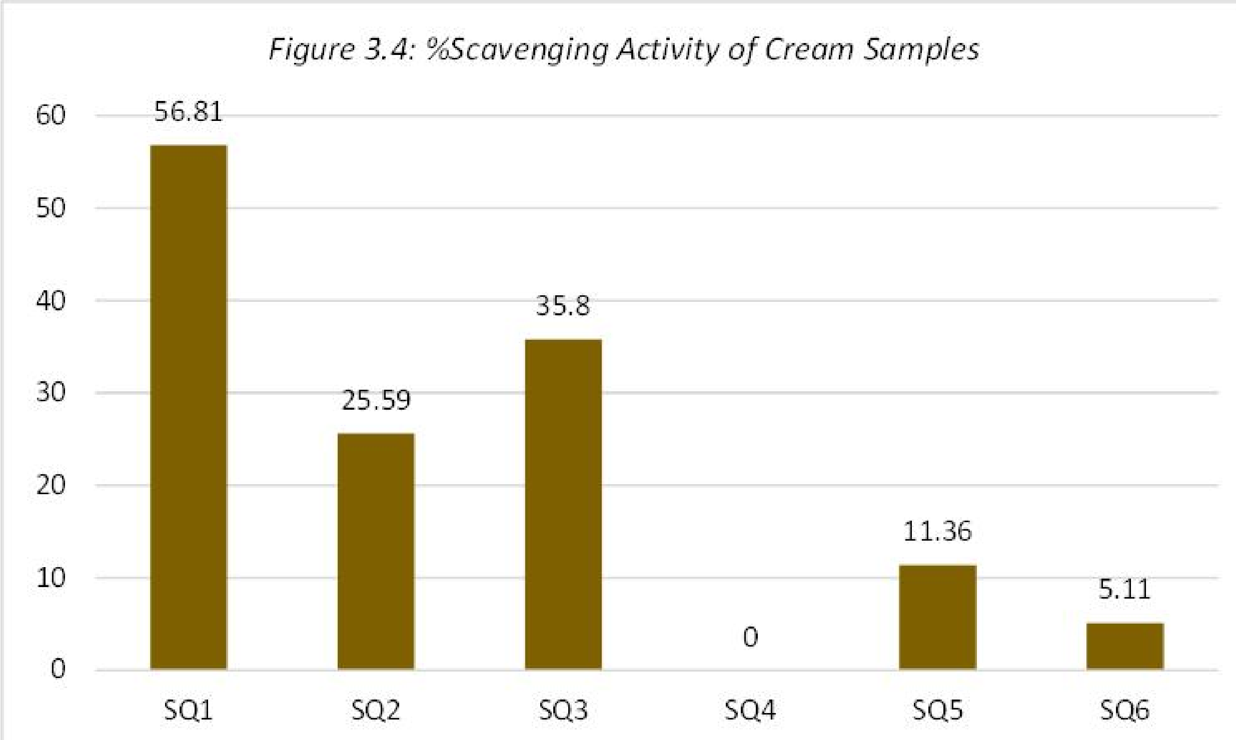

Figure 3.1 Bar Chart for the Scavenging Activity of Cymbopogon citratus cream Samples

DPPH Radical scavenging ability is based on the ability of DPPH to scavenge the Antioxidant constituent in the plant extract in the cream. Hence the lower the absorbance ,the higher the Antioxidant activity; the higher the percentage inhibition the greater the Antioxidant activity. SQ6 which contains Vitamin E was used as the control for this study. From the results of this test it can be seen that SQ1 has the highest antioxidant activity.

3.3 Results for Antibacterial Testing of Formulated Cream

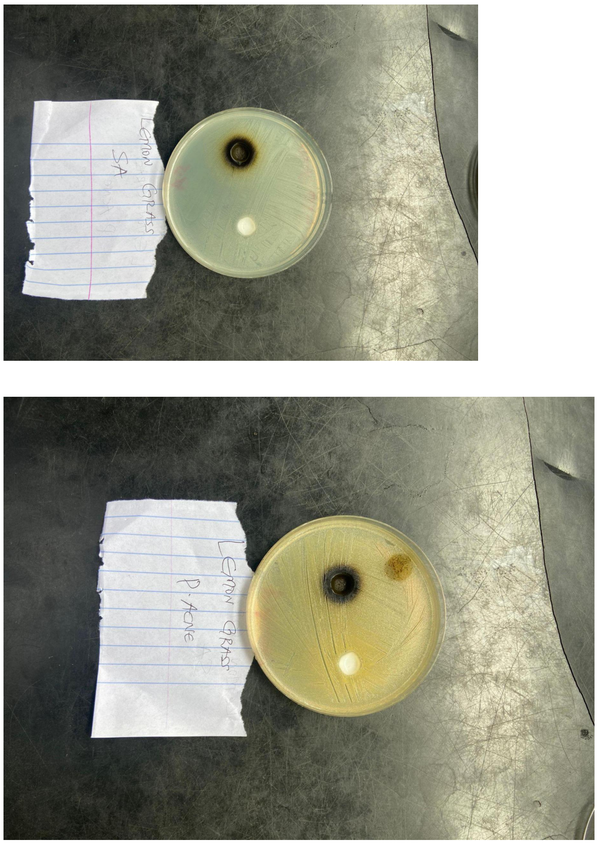

Figure 3.2 Results for Antimicrobial testing

The results of this test showed that *Cymbopogon citratus* extract had no activity against *Cutibacterium Acnes* and *Staphylococcus Aureus* test organisms. Hence, it has no Bacteriostatic or Bactericidal activity.

3.4 Results for Biological and Safety Tests

Ex-vivo studies and irritancy tests were conducted

3.4.1 Ex-vivo Studies Results

From the methodology described in 2.7.1 the calibration curve as seen in Fig 3.3 was drawn. In calibration curve r2 is the coefficient of the determination, a statistical measure that represents the proportion of the variation in the dependent variable (e.g., instrument response/absorbance) that can be explained by the independent variable (e.g., analyte concentration) (Turney, 2022) . It also shows how well the data points fit the regression line. Optimal The r2 value ranges from 0 to 1 (or 0% to 100%). An r2 value of 1 indicates a perfect fit, meaning the model explains the variability of the data and data points, a r2 value close to 1 i.e 0.9995 indicates a strong linear relationship and reliable curve that can predict data within the calibrated range. As seen in Fig 3.3 the r2 value of the data set concentrations is 0.9912 thus indicating a reliable curve and a strong linear relationship.

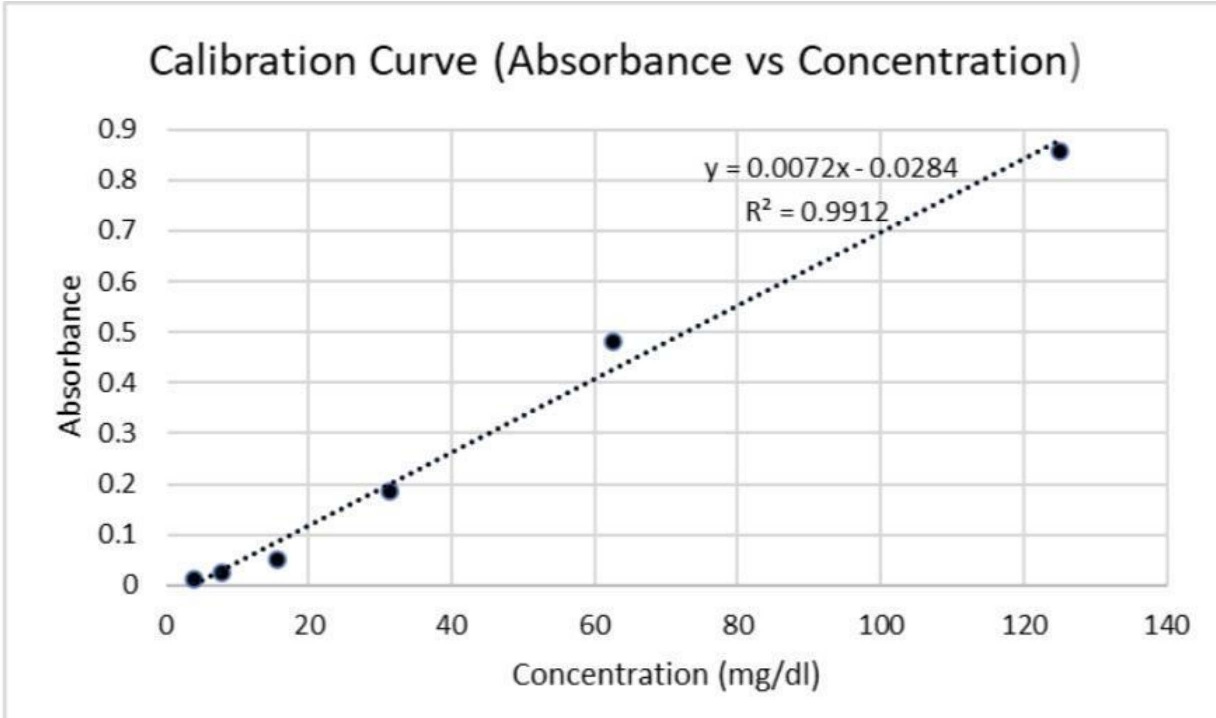

Figure 3.3 Calibration curve for the ex vivo release assay of Cymbopogon citratus cream.

The amount of Active ingredients contained in 1g of the formulated creams undergoiing Ex-vivo studies (SQ3, SQ4,SQ5) were then calculated and seen to be 7.82 mg/dl, 7.84 mg/dl and 7.92 mg/dl respectively. In order to determine the absorbance at 100% release these values were multiplied by the equation y(0.0072) and were seen to be 0.056, 0.056 and 0.057 respectively.

The percentage release of the Active ingredients contained in SQ3, SQ4,SQ5 were then calculated using the formula

A_t / A_{100} x 100

where A_t is the absorbance at Time t

A_{100} is the absorbance at 100% release

From the results in Table 3.5 below it can be seen that the complexation of *Cymbopogon citratus* into a Herbosome (SQ3) enhanced its lipid permeability with a percentage release of 61.7% after 3 hours, compared to the SQ5 which contains Plain *Cymbopogon citratus* extract; with a Percentage release of 21.7%. At 0 hours, no release was detected from any of the formulations, indicating baseline equilibrium prior to diffusion. After 1 hour, SQ3 demonstrated the highest release (26.14%), followed by SQ4 (12.50%) and SQ5 (12.14%).At the 2-hour mark, SQ3 had a release of 30.20%, while SQ4 and SQ5 had a release of 21.42% and m 21.70%respectively.By the third hour, SQ3 exhibited the highest cumulative release 61.70%, showing a consistent and progressive increase over time. SQ4 also recorded a noticeable rise 39.28%, as well as SQ5 which reached 37.50%, indicating and increase in the release of the medication

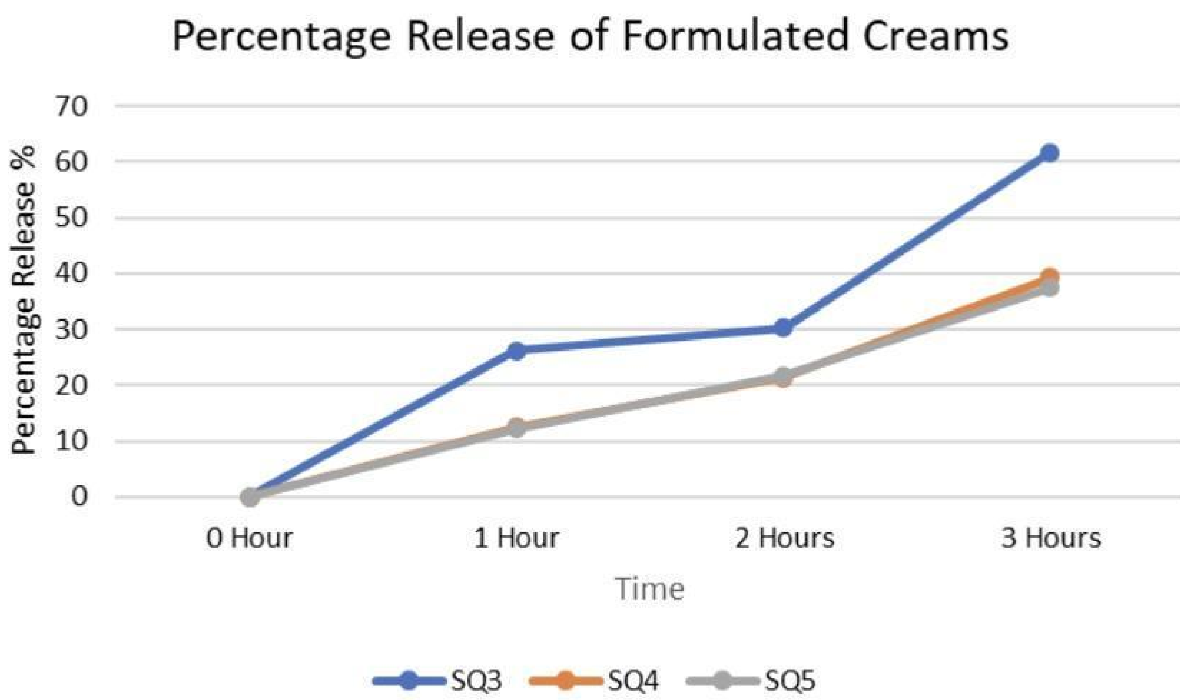

Figure 3.4 Graphical Representation of Percentage Release of creams

3.4.2 Irritancy Test Results

After 24 hours, the segment of the skin that the cream was applied on was evaluated for edema, redness, inflammation and irritation and none were observed on any of the individual's skin.

CHAPTER 4

CONCLUSION

The various ratios of *Cymbopogon citratus* extract were successfully complexed into a herbosome and incorporated into an oil in water cream.

From the results of the tests carried out it can be seen that *Cymbopogon citratus* herbosomal creams SQ1-SQ6 showed Viscosity values ranging from 26.17 to 36.70, pH values ranging from 4.4 to 4.8, Spreadability values ranging from 6.03 to 9.65. They were also seen to have Antioxidant properties with SQ1 exhibiting the highest Antioxidant activity among the herbosomal creams formed with a percentage inhibition of 56.81%, However, there was no Antibacterial activity seen for the *Cymbopogon citratus* herbosomal creams. Good homogeneity was seen in the cream with no coarse particles. No phase separation or physical changes were observed after the freeze and thaw test. For the Ex-vivo studies it was seen that the complexation of *Cymbopogon citratus* extract into a herbosome enhances its lipid permeability across a membrane as SQ3 showed the high rest percentage release of 61.70% .No irritancy was observed with use of the creams.

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APPENDIX

Ex-vivo Calibration curve values

	Concentration(mg/dl)	Absorbance
A	125.00	0.856
B	62.50	0.481
C	31.25	0.187
D	15.63	0.052
E	7.813	0.025
F	3.91	0.012

Ex-vivo/ Release studies absorbance values

	0 Hour	1 Hour	2 Hours	3 Hours
SQ3	0	0.012	0.007	0.022
SQ4	0	0.210	0.068	0.122
SQ5	0	0.172	0.149	0.352

Results for Percentage Inhibition(%)

SAMPLE	**PERCENTAGE DPPH RADICAL SCAVENGING ACTIVITY (%)**
SQ1	56.81
SQ2	25.59
SQ3	35.80
SQ4	00.00
SQ5	11.36
SQ6	5.11

Results for wavelength scanning (Spectrum activity)

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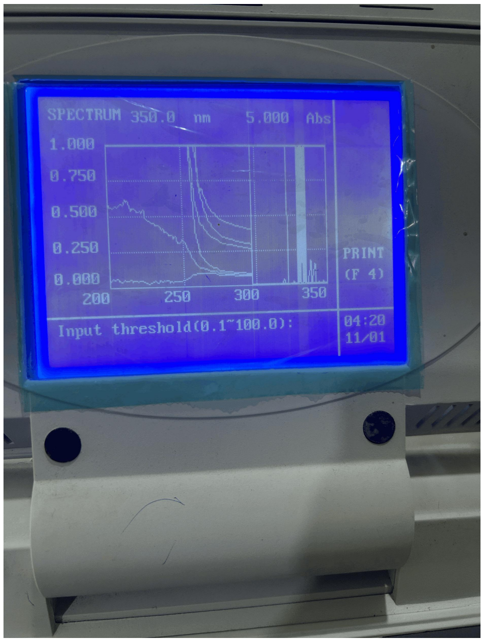

Results for Percentage Release of Formulated Creams

	0 Hour	1 Hour	2 Hours	3 Hours
SQ3	0	26.14	30.30	61.73
SQ4	0	12.50	21.42	39.28
SQ5	0	12.14	21.70	37.50