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Formulation Evaluation and Development of Natural Oils Containing Multipurpose Skin Care Serum

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ABSTRACT

There is urgent need for Serum production and from local raw materials in order to supplement the existing ones. I recommend more research to be carried out on extraction of essential oil and its formulation from vast variety of oil bearing plants in our ecosystem. Further work should be carried out to analysis the Natural oil as this could not be done due to time constraint. Characterization of All oils components should be made in order to determine which is responsible for the characteristics of Pungent and Aromatic odor. Furthermore, large scale extraction of Natural oil through enzymatic process should be explored; feasibility studies on the economic viability of the process should be conducted.Skin is the vital barrier against abrasion, chemicals, and pathogens. Proper skin hygiene is the best way to keep your skin healthy. Removing dead cells, dirt and microbes on the surface is key to good hygiene. Herbs are generally defined as non-woody plants, which die after blooming. This definition has been expanded to any of the plants of which parts or whole can be used in medicinal treatments, culinary preparations, nutritional supplementation, or used as a colouring or cosmetic agents.Today's there are number of popular active ingredients that claim to cure such infections, still new active is commonly being identified, studied and promoted, because there is always a room to experiment with new ideas.The present project is also a humble attempt in the same direction, where incredible benefit of herbs is its ability to promote healthy clear skin. It is very mild but still has a powerful effect on the skin. Due to its antiseptic properties it prevents acne and rashes, heals itching and inflammation, cools and soothes sunburns, gently exfoliates

Keywords: Natural Oils, Hydro Distillation,Serum.

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INTRODUCTION

The benefits of face serum include more than just that. It basically gives you flawless skin with minimum effort. You don't want to miss out on effortlessly healthy and impeccable skin, do you? Nobody does. Using a face serum is an important step in your skincare regimen and here are all the reasons why. Face serums do what most skincare products can't. They give hydration boost to your skin, reduce blemishes and fight ageing signs, all at once. If this piqued your interest, then read on to know 6 ways face serum benefits your skin and why you need this wonder elixir in your skincare regimen now.[1]

Serum is a skincare product you can apply to your skin after cleansing but before moisturizing with the intent of delivering powerful ingredients directly into the skin. Serum is particularly suited to this task because it is made up of smaller molecules that can penetrate deeply into the skin and deliver a very high concentration of active ingredients. This makes them a great tool for targeting specific skincare concerns, like wrinkles. Goodbye, signs of aging![2]

So, what exactly is a serum? It is a concentrate of active ingredients, which targets specific skincare concerns, and the ingredients are powerful, and made up of smaller molecules. The level of active ingredients is higher than in a usual face cream, since the heavier oils and ingredients have been done away with. So while the latter could have around ten per cent of active ingredients, the former has a whopping seventy per cent or more! Serum is a skincare product you can apply to your skin after cleansing but before moisturizing with the intent of delivering powerful ingredients directly into the skin. Serum is particularly suited to this task because it is made up of smaller molecules that can penetrate deeply into the skin and deliver a very high concentration of active ingredients. This makes them a great tool for targeting specific skincare concerns, like wrinkles. Goodbye, signs of aging!.

MATERIALS AND METHODS

A proper method has to be carried out while formulating the Skin Care Serum from Naturals Oils are as,

Selection of active: The analysis of Essential oils are generally derived from one or more plant parts, such as flowers (e.g. rose, jasmine, carnation, clove, mimosa, rosemary, lavander), leaves (e.g. mint, Ocimum spp., lemongrass, jamrosa), leaves and stems (e.g. geranium, patchouli, petitgrain, verbena, cinnamon), bark (e.g. cinnamon, cassia, canella), wood (e.g. cedar, sandal, pine), roots (e.g. angelica, sassafras, vetiver, saussurea, valerian), seeds (e.g fennel, coriander, caraway, dill, nutmeg), fruits (bergamot, orange, lemon, juniper), oils

contains some useful phytochemical constituent like vitamins, Tannins, Gums, Resins Antioxidants, Pinaceae or Cupressaceae, Vitamin C, Vitamin E,

Collection and Authentication: Natural oils were purchased from the Herbal Drug Supplier and authenticated in botanical department by botanist.

Selection of base: The main objective of the present study was to prepare a Serum from Naturals Essential Oils base are used.

Formulation of base: For the preparation of Serum some constituents are used including drug, which are:

Vehicle: Vehicle should follow the ideal characters given in the Pharmacopeias.

Aqueous material: The aqueous phases used are water, alcohol, etc.

Distilled Water- Solvent

distilled water is used as a solvent in cosmetic and personal care products as it dissolves many of the ingredients that impart skin benefits like cleansing and conditioning agents. It is universal solvent acts as hydrating agent.

Glycerine- Humectant

acts as hydrating agent. This medication is used as a moisturizer to treat or prevent dry, rough, scaly, itchy skin and minor skin irritations.It can increase skin hydration, relieve dryness, and refresh the skin's surface. It's also an emollient, which means it can soften skin. This is great if eczema or psoriasis leave you with rough or dry patches. Glycerin also has antimicrobial properties, which means it can protect the skin from harmful microorganisms.

Propylene glycol- Humectant

which means that it is an ingredient that is added to cosmetics to increase moisture retention in skin and hair. Propylene glycol is well tolerated by the skin and shouldn't cause redness or irritation.

Xanthum gum- film-forming agent

The easiest and most effective option however is to blend Xanthan Gum into Glycerin, where it will make a paste that can easily be mixed into your formulation.

Sodium EDTA- as a preservative/ chelating agent

It also helps to maintain clarity, protect fragrance compounds, and prevent rancidity. EDTA is included in many hair and skincare products as a preservative, helping to prevent the growth of bacteria, yeasts, and molds in your skincare product.

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Allantion- It is a keratolytic agent/ Healing agent

Allantoin is commonly applied in a variety of topical vehicles or applications such as cosmetic creams, toothpastes, mouthwashes, shampoos, lipsticks, and lotions for the purpose of moisturizing skin, enhancing the smoothness of skin, stimulating the healing of wounds, and soothing irritated skin

DMDM Hydentoin- Antimicrobial agent

1,2-Dimethylol-5,6-dimethylhydantoine Glydant DMDM hydantoin is a preservative and antimicrobial agent found in a wide range of cosmetics and skin-care and hair-care products.

Procurement of Raw Material

Table 1: Procurement of Raw Material

Sr. No.	Ingredients	Role
1.	Glycerine	Humectant
2.	Propylene glycol	Humectant
3.	Xanthum gum	Gelling agent
4.	DMDM Hydentoin	preservative
5.	Sodium EDTA	Chelating agent
6.	Allantoin	Healing agent

Formulation of Active Herb

Table 2: Formulation Of Active Herbs

Ingredients	Parts Used	Category	Qty%
Grape seed oil	Seed	Antioxidants Anti-inflammatory	3
Rose oil	Petals	Anti-bacterial Anti- Microbial Anti-Oxidant	3
Sandalwood oil	Wood	Anti- bacterial	4

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		Aromatheraphy	
Lemon oil	Peel	Anti-oxidant Anti-Microbial	
Pomegranate Seed oil	Seed	Anti-inflammatory Anti-aging	

EXPERIMENTAL WORK

Optimization of Serum Base

Table 3: Optimization Of Serum Base

Sr. No.	Parameter	M1	M2
1	Appearance	++	+++
2	Colour	+	+++
3	Consistency	++	+++
4	Spreadability	+	+++
5	Feel	+	++

From the above observation formula M2 was stable and it shows consistency, spreadablity, and feel therefore it was selected and extract was added with different concentration and forward for in vitro study as in vivo study with human volunteers Here, + = good, ++ = Better, +++ = Best

Extraction of Grape seed oil, Pomegranate seed oil, and Lemon oil using Soxhletion Methods

Soxhlet extraction

Soxhlet extraction has traditionally been used for a solid sample with limited solubility in a solvent in the presence of insoluble impurities. A porous thimble loaded with a solid sample is placed inside the main chamber of the Soxhlet extractor. By refluxing the solvent through the thimble using a condenser and a siphon side arm, the extraction cycle is typically repeated many times. Soxhlet extraction is a rugged, well-established technique and permits unattended extraction. However, it requires a long extraction time and the consumption of a large amount of solvent. Soxhlet extraction is a very useful tool for preparative purposes in

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which the analyte is concentrated from the matrix as a whole or separated from particular interfering substances. Sample preparation of environmental samples has been developed for decades using a wide variety of techniques. Solvent extraction of solid samples, which is commonly known as solid–liquid extraction (also referred to as leaching or Lixiviation in a more correct use of the physicochemical terminology), is one of the oldest methods for solid sample pretreatment.

Procedure

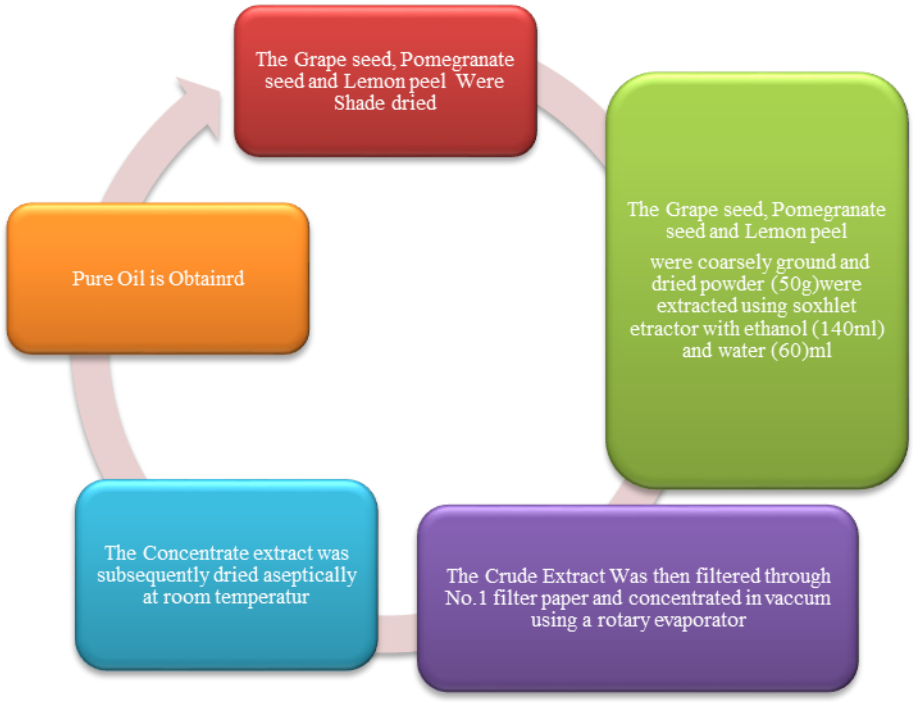

Fig. 1: Extraction Of Natural Oil By Soxhletion

Extraction of Rose oil using Vaccume Distillation

Vaccume Distillation

Vacuum distillation is distillation performed under reduced pressure, which allows the purification of compounds not readily distilled at ambient pressures or simply to save time or energy. This technique separates compounds based on differences in their boiling points. This technique is used when the boiling point of the desired compound is difficult to achieve or will cause the compound to decompose.Reduced pressures decrease the boiling point of compounds. The reduction in boiling point can be calculated using a temperature-pressure Industrial-scale vacuum distillation [10] has several advantages. Close boiling mixtures may require many equilibrium stages to separate the key components. One tool to reduce the number of stages needed is to utilize vacuum distillation.Vacuum distillation c typically used in oil refineries have

diameters ranging up to about 14 meters (46 feet), heights ranging up to about 50 meters (164 feet), and feed rates ranging up to about 25,400 cubic meters per day (160,000 barrels per day).

Fig. 2: Extraction Of Natural Oil By Vaccume Distillation

Preparation of Multipurpose Serum

Apparatus and reagents

- Pipette
- Funnel
- 50ml and 120ml beakers
- Distilled water
- Chemicals

Procedure

All the ingredients were weighed according to the different percentage listed (Table). The net weight of all formulated serum was 100g. Add water and EDTA into a disinfected glass beaker and stir, until EDTA has

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dissolved. Add Xanthum gum and mix thoroughly with a stick blender or homogenizer until phase A is free of lumps. Add phase B to phase A, stir well after each ingredient has been added. Mix with stick blender. Add phase C to phase A/B, again, stirring well after each ingredient. Especially after sodium acrylate uses the stick blender. Serum should be free of any lumps. Viscosity can be adjusted by adding, 2.5% of the Allantoin [11-15]

Evaluation of extract

Preliminary phytochemical screening

Flavonoids: To test solution add few drops of NaoH solution formation of dilute acid indicates presence of flavonoids.

Glycosides: A small amount of alcoholic extract of samples is dissolved in 1ml water and then aqueous sodium hydroxide is added. Formulation of yellow colour indicates the presence of glycosides.

Alkaloids (Mayer's test):1.36gm of mercuric chloride is dissolved in 60ml and 5gm of potassium iodide is dissolved in 10ml of distilled water respectively. These two solvents are mixed and dilute to 100ml using distilled water. To 1ml of acidic aqeous solution of samples few drops of reagent is added. Formation of blue or green colour indicates the presence of alkaloids.

Phenols (ferric chloride test): To 1ml of alcoholic solution of sample. 2ml of distilled water followed by a few drops of 10% aqueous free chloride solution is added. Formation of blue or green colour indicates the presence of phenols.

Tannins (lead acetate test): In a test tube containing about 5ml of an aqueous extract a few drops of 1% solution of lead acetate was added. Formation of a yellow or red precipitate indicate the presence of tannin.

Lipids: In a test tube 5 drops of the sample was taken and a pinch of sodium hydrogen sulphate was added. Pungent odour emantes from the tube which indicates that glycerin is present which is produced by hydrolysis in fixedoil which shows the presence of lipids. [15-20]

Test for Antioxidant Activity of Extracts

1. Reducing Power method

Principle: This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity. In this, the antioxidant compound forms a coloured complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, which is measured at 700nm. Increase in absorbance of reaction mixture indicates the reducing power of the samples.

Procedure: The reducing power was assayed by taking different concentration of extract (1ml) from each

other were mixed in different test tubes with 2.5 ml of phosphate buffer (pH-7) and 2.5 ml of 1% potassium ferric cyanide. The mixture was then incubated at 50®C for 20 minutes. Then 2.5 ml of trichloroacetic acid (10%) solution was added to the mixture, which was mixed for 15 minutes. Finally 1.25 ml of distilled water was mixed with 0.50 ml of Feclȝ solution (0.1 w/v). The absorbance was measured at 700nm.

2. In Vitro Studies

Determination of pH

Apparatus:pH meter, preferably equipped with glass electrode.

Principle: The formulation of serum are meant for topical application. So their pH should be similar to that of skin. The skin has an acidic range and the pH of the skin serum should be in the range of 5 – 9 To ensure the required shelf life of skin serum, chemical inertness is essential i.e. it should neither be too acidic nor too alkaline. Based on above point it was through that the standard pH of skin should be in the range of 6-6.5

Procedure: Take 5gm of sample in a beaker and add 45 ml of distilled water in it. Mix it properly until the whole gel is dissolved in water, then note the pH of the sample mixture by using pH meter.

3. Determination of Viscosity

Appartus : Brook Field Viscometer.

Principle : The viscosity is the most important parameter in the evaluation of cosmetic product. Viscosity governs the many properties such as spreadablity, pourablity of the product from the container. As viscosity is affected by many factors such as change in temperature, change in manufacturing condition, quality of the raw material. Hence it is very important to measure the viscosity of product.

Procedure : The viscosity of serum was determined by using spindle no. 4 using brook field viscometer then all the operating conditions was set up. Then five readings were taken at different rpm and average of there will be the final reading. Viscosity was measured at 6 rpm in cps.

4. Determination of Spreadablity Time

Principle: It is very important for any cosmetic product that after application the product must be easily spread over the skin. Spreadability is affected by many factors such as viscosity, temperature etc. The spreading time must be very less.

The apparatus consist of a wodden block, with a movable glass slide with one end tied to weighted pan rolledon pulley.

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Procedure: 2 Gm of serum sample was placed on a surface. A slide was attached to a pan to which 20 gm weigth was added. The time (seconds) required to seprate the upper slide from surface was taken as a measureof spreadability.

5. Microbial Examination of the Product

Cosmetics do not need to be sterile, but they must be adequately preserved. When consumers use cosmetics they repeatedly challenge the cosmetics with micro organism in salivaon dirty hands, in tap water. Microbial growth may occur in cosmetics and toiletry product like cream, lotion and gel and many more intended to be use as skin care preparation. Hence it is very important that the cosmetics product must be free from microbial contamination,so that it will ensure safety to product to the client. The cosmetic product must be safe and adequately preserved.

Procedure : Sterilize the work area with disinfectant. Wash and dry thoroughly all the apparatus required. Prepare the dilution of the product take 1gm/ml of product and add to first test tube with pipette and shake it thoroughly then take 1ml from it in second test tube and prepare further dilution in same way.

6. Total bacteria count

Weigh accurately required quantity of nutrient agar and add 50 ml of water in an autoclave conical flask. Autoclave it at 121®C for 15 min. When the temperature reduces to 45®C add 1ml of dilution of the product toautoclave petridish and add 20ml of nutrient agar medium and mix by rotating in the clock wise and anticlockwise direction Allow the plate to solidify.Incubate this plate for 48 hours at 37^0C.

7. Total Fungal Count

Pipette out in duplicate 1ml of pretreated sample aseptically into 5 sterile petridishes. Pour 15 to 20 ml ofmolten sabouraud's chloranphenicol agar (SCA) maintained at about 450 C mix the content of the plate by swirling. Allowing the plates to solidify, invert and incubated at 23+ 2 0 C for three days. Count the number of colonies in each plate.

8. Stability study of serum

The sample of serum was kept at 5®C, room temperature 40®C. The changes in physical appearance, colour, feel etc were studied. [21-30]

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Table 4: Stability Studies Of Serum

Sr. No.	**Parameters**	M1	**M2**
1	Appearance	Opaque	Opaque
2	Colour	White	White
3	Spreadability	Good	Very good

Accelerated Stability Studies

Cyclical Temperature Tests

These tests are not carried out at fixed temperature and humidity. In this test, temperature was changed cyclically every day e.g. low-high-low-high to stimulate the changes in temperature daily.

Table 5: Cyclic Temperature Test

Sr. No.	**Parameter**	M1	**M2**
1	Freeze temperature	Stable	Stable
2	Room temperature	Unstable	Stable
3	High temperature	Unstable	Stable

In vivo studies

Patch test: Patch test was performed on sensitive part of skin, e.g. blend of elbow, popliteal space of skin behind ears. The cosmetic was tested by applying to an area of 1 sq.cm of the skin. Central patches were also applied. The site of the patch was inspected after 24 hours. There was no reactions and then test was repeated

once more on the same side. Since there was no reaction as the person was considered as not hyper sensitive and product pass the test.

Patch test for Serum

Table 6: Patch Test of Serum

Sr. No.	Parameter	M1	M2
1	Immediately after removal of product	N.R.	N.R.
2	After 24 hrs	N.R.	N.R.
3	After 48 hrs	N.R.	N.R

N.R = No Reaction

Analysis of moisturizing property by using corneometer

Corneometer is device which is equipped with a moisture sensitive probe which is used to determine the accurate moisture content of stratum corneum. Hence it plays important role in determining the moisturizing activity of product on stratum corneum after its application on skin.

Apparatus : Corneometer equipped with a probe.

Procedure : First clean the hand with a soap and then dry it completely, then touch the probe on hand in order to note the initial reading of moisture of skin, then apply product on skin and wash it. Then again note the reading by touching the probe on the part of application of analysis of moisturizing property of serum.

Test for antioxidant activity of serum

1) Reducing Power method

Principle: This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity. In this, the antioxidant compound forms a coloured complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, which is measured at 700nm. Increase in

absorbance of reaction mixture indicates the reducing power of the samples.

Requirement: UV Spectrophotometer, Incubator.

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Procedure: The reducing power was assayed by taking different concentration of sample (1ml) from each other were mixed in different test tubes with 2.5 ml of phosphate buffer (pH-7) and 2.5 ml of 1% potassium ferric cyanide. The mixture was then incubated at 50®C for 20 minutes. Then 2.5 ml of trichloroacetic acid (10%) solution was added to the mixture, which was mixed for 15 minutes. Finally 1.25 ml of distilled water was mixed with 0.50 ml of Fecl₹ solution (0.1 w/v). The absorbance was measured at 700nm.

Table 7: Antioxidant Activity Of Serum

Sr. No.	Product	Absorbance	
		Without actives	With actives
1	Serum	0.13	0.93

EVALUATION PARAMETERS

Table 8: Evaluation Parameter of Serum

Sr.No.	Parameters	Observation
1	Color	Colourless
2	Odor	Aromatic
3	Consistency	Good
4	pH	pH 5.2
5	Viscosity	293.15-343.15*
6	Irritability	Non-irritant

RESULTS AND DISCUSSION

Evaluation of Extract

Preliminary Phytochemical Screening

Table 9: Preliminary Phytochemical Screening

Sr. No.	Alkaloids	Flavonoids	Phenols	glycosides	Tannins	Lipids
1	Natural oils	+	+	+	+	+

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Determination of pH

a)Determination of pH of serum

Table 10: Determination of Ph of Serum Incorporated

Sr. No.	Time Interval	M1	M2
1	Initial	5.5	6.5
2	8th	5.5	6.5
3	16th	5	6.2
4	24th	5	6.2
5	30th	4.8	5.8

Determination of Viscosity

Table 11: Determination of Viscosity For Serum

Sr. No.	No. of days	M1	M2
1	1st day	14360	13730
2	15th day	14375	13759
3	30th day	14375	13759

Determination of Spreadability

Table 12: Determination of Spreadability

Sr. No.	Days of interval	Initial area	Weight	Time
1	Initial day	8cm	20gm	2.1 sec
2	15^{th} day	8.7cm	20gm	2.1 sec
3	30^{th} day	8.9cm	20gm	2.1 sec

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Table 13: Determination of Viscosity For Serum

Sr. No.	Test	Result	Specification	Unit
1.	Total microbial count	20 CFU/gm	NMT/100 CFU/gm	CFUgm

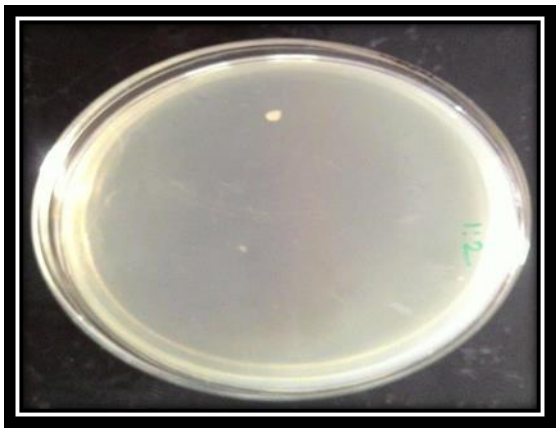

Fig. 3: Total Microbial Count

RESULT

The total microbial count of serum was found to be 20cfu/gm that is <100 cfu/gm. Therefore the serum passes the test**.**

Determination of Moisturizing Activity

Table 14: Determination Of Moisturizing Activity

Product	Days	% of Moisture content
Serum	Before App	101
	1st day	102
	2nd day	102.2
	3rd day	103

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	4th day	103.5
	5th day	103.6
	6th day	104.2
	7th day	105.2

Soxhletion Method

Result obtained by Soxhlet extraction is shown in Table below

Table 15: Weight of Oil With Respect To Time

Weight of oil (g)	Time (mins)
0.4	**250**
0.5	**500**
0.6	**750**
0.80	**1000**
0.90	**1200**

The amount of pure Natural oil obtained by extraction method was 3.2g of essential oil per 100g of dry seed sample. This gave 3.02% yield of essential oil per 100g of dry Grape seed, Pomegranate seed and Lemon peel the temperature used was 780C i.e. the boiling point of ethanol. The volume of oil was measured at every 4hr interval to determine the oil yield at varying time. As the time increases the Ethanol solvent reduces thereby leaving the oil in the mixture.

Vaccume Distillation

Result obtained by is shown in Table below

Table 16: Weight of Oil With Respect To Time

Weight (g)	Time (mins)
0.35	250
0.40	500
0.50	750

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0.55	100
0.65	1200

The oil produced by Vaccume Distillation Method is 2.45g weight of oil per 100g of dry Rose Petals sample thereby producing 2.45% oil yield at 780C.

Table 17: Result of Essential Oil Extraction

Method of extraction	**% yield**	**Method of extraction**
Soxhletion Method	3.02	Soxhletion Method
Vaccume Distillation	2.45	Vaccume Distillation

CONCLUSION

From above discussion it is concluded that Natural oil Extract had antimicrobial property. From the above experimental work, the Natural oil Extract showing good activity against Propionic bacterium acnes.

Finally it was concluded that extract of Natural oil shows antibacterial activity against selected microorganism with increase in concentration the activity is increase therefore it can be incorporated in cosmetics products.

Soxhletion and Vaccume distillation methods are effective and efficient means of extracting Serum. Extraction is the most common and most economically technique for extracting Natural oil in modern Herbal industry because of its simplicity.

There is high demand for essential oils for various purposes such as medicinal, perfumery, aromatherapy, cosmetic, soap making, insectides to mention but a few. Imported essential oils are very expensive to meet the demand of our local consumer industries, therefore it becomes necessary to source and synthesis these oils from local sources, in particular..

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CONFLICTS OF INTEREST

Authors have no conflicts of interest to declare.

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