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OPTIMIZATION FORMULATION AND EVALUATION OF HERBAL LOTION FOR ANTIMICROBIAL ACTIVITY

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ABSTRACT: Herbal drugs constitute traditional medicines which primarily use medicinal plant preparations for therapy. Allopathic medicine system and modern medicine system has gained popularity due to more and more scientific discoveries and advancement in modern scientific research .Herbal medicine was also an effective healing method and more therapeutic. The maceration is a method of solid–liquid extraction. Bioactive compounds in plants include alkaloids, terpenoids, coumarins, flavonoids, nitrogen-containing compounds, organosulfur compounds, phenolics, etc. A wide spectrum of bioactivities is exhibited by these compounds such as anti-inflammatory, immunostimulatory, anticancer, antioxidant, antimicrobial, etc. Curcuma longa is a perennial herband family is Zingiberaceae. Ayurvedic systems of medicine, particularly as an anti-inflammatory agent, and for the treatment of flatulence, jaundice, menstrual difficulties, hematuria, hemorrhage, and colic. Emblica, Indian goose berry (amla) the fruit that contains considerably higher concentration of most minerals and amino acids. The pulpy portion of fruit, dried and freed from the nuts contains: gallic acid 1.32%, tannin, sugar 36.10%; gum 13.75%; albumin 13.08%; crude cellulose 17.08%; mineral matter 4.12%; and moisture 3.83%. Tannins are the mixture of gallic acid, ellagic acid, and phyllembin. Lotions are defined as monophasic or biphasic solution, emulsion or suspension design to apply on unbroken and also broken or inflamed skin without friction. Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, emergence and dissemination of multidrug resistant (MDR) strain in pathogenic bacteria have become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for the infection caused by pathogenic bacteria. Many plants have been used because of their antimicrobial traits, which are due to phytochemicals synthesized in the secondary metabolism of the plant . Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been found in vitro to have antimicrobial properties.

KEYWORDS: Herbal medicine, Akaloids, Microbial Activity, Anti-inflammatory.

I. INTRODUCTION

1. **Herbal and Conventional Drugs:** Herbal drugs constitute traditional medicines which primarily use medicinal plant preparations for therapy. General belief is that herbal drugs are without any side effects besides being cheap and locally available. The WHO has recently defined traditional medicine including herbal drug. Traditional preparations comprise medicinal plants, minerals and organic matter etc. The classical Indian texts in Ayurveda from Atharvaveda, includes Sushruta Samhita and Charak Samhita have mentioned use of herbal medicines in various ailments and were part of teachings in Gurukuls. Herbal Medicines are considerably a difference exist between herbal medicine and conventional pharmacotherapy which broadely can be grouped in three categories:

i. **Whole Plants:** It is claimed that these can work together synergistically so that the effect of the whole herb is greater than the summed effects of its components. It is also claimed that toxicity is reduced when whole herbs are used instead of isolated active ingredients buffering.

ii. **Combining Herb:** Often several different herbs are used together. Practitioners say that the principles of synergy and buffering apply to combinations of plants and claim that combining herbs improves efficacy and reduces adverse effect. This contrasts with conventional practice, where polypharmacy is generally avoided whenever possible.

iii. **Diagnosis Purposes:** Herbal practitioners use different diagnostic principles from conventional practitioners. For example, when treating arthritis, they might observe, “under functioning of a patient’s symptoms of elimination” and decide that the arthritis results from “an accumulation of metabolic waste products”. A diuretic, choleretic or laxative combination of herbs might then be prescribed alongside herbs with anti-inflammatory properties.(1)

1.2 Extraction: Extraction is a mandatory process for most of the industries, especially in the food processing industry to obtain the targeted compounds from various sources such as plant or seed samples.

1.2.1 Maceration: Maceration is a method used for medicinal preparation. The maceration is a method of solid–liquid extraction. In this process, the powdered solid materials are placed in a closed vessel and the solvent is added. It is allowed to stand for a long time with occasional shaking. Sufficient time is allowed for the solvent to diffuse through the cell wall to solubilize the constituent present in plant. After the desired time, the liquid is strained off; the solid residue is pressed to recover as much solvent as possible. When the solvent is water and the period of maceration is long, a small quantity of alcohol may be added to prevent microbial growth.(2)

1.2.2 Extraction of Bioactive Compounds from Medicinal Plants and Herbs: Natural products are currently of considerable significance due to their unique attributes as a significant source of therapeutic phytochemicals and their efficacy, safety, and minimal side effects. Bioactive compounds in plants include alkaloids, terpenoids, coumarins, flavonoids, nitrogen-containing compounds, organosulfur compounds,

phenolics, etc. A wide spectrum of bioactivities is exhibited by these compounds such as anti-inflammatory, immunostimulatory, anticancer, antioxidant, antimicrobial, etc.

1.2.3 Primary and secondary metabolites: Primary metabolites are known vital or essential compounds and are directly involved in the average growth, development, and reproduction of plants. Primary metabolites include cell constituents e.g. carbohydrates, polysaccharides, amino acids, sugars, proteins, and lipids.

Secondary metabolites are not directly involved in those processes and usually have a function but are not that important for the organism e.g., phenolic, steroids, lignans, etc. They are found only in specific organisms or groups of organisms, and express of the individuality of species. Secondary metabolites are produced after the growing stage and are used to increase the ability of plants to survive and overcome their local challenges. Bioactive compounds are classified as terpenoids, alkaloids, nitrogen-containing compounds, organosulfur compounds, and phenolic compounds.(3,4)

1.2.4 Extraction techniques of actives compounds from plants and herbs: Extraction is separating the medicinally active mixture of many naturally active compounds usually contained inside plant materials (tissues) using selective solvents through the standard procedure. There are three common types of extraction: liquid/solid, liquid/ liquid and acid/base. The extraction of these active compounds needs appropriate extraction methods that consider the plant parts used as starting material, the solvent used, extraction time, particle size and the stirring during extraction. Extraction methods include solvent extraction, distillation method, pressing, and sublimation according to the extraction principle. Solvent extraction is the most widely used method.(5)

1.3 Alkaloids: The alkaloids are low molecular weight nitrogen-containing compounds found mainly in plants and a lesser extent in microorganisms and animals. They contain one or more nitrogen atoms, typically as primary, secondary, or tertiary amines, which usually confers basicity on the alkaloids. If the free electron pair on the nitrogen atom is not involve in mesomerism, the salt formation can occur mineral acids. This fundamental property of alkaloids is used in their extraction and further clan-up. According to the nature of the nitrogen-containing structure, alkaloids are classified as pyrrolidine, piperidine, quinoline, isoquinoline, indole, etc.(7)

1.3.1 CURCUMA LONGA (Haldi): Curcuma longa a perennial herb, is a member of the Zingiberaceae (ginger) family. The plant grows to a height of three to five feet, and is cultivated extensively in Asia, India, China, and other countries with a tropical climate. It has oblong, pointed leaves and bears funnel-shaped yellow flowers. The rhizome is the portion of the plant used medicinally; it is usually boiled, cleaned, and dried, yielding a yellow powder. Dried Curcuma longa is the source of the spice turmeric, the ingredient that gives curry powder its characteristic yellow color.

Figure 1: Curcuma Longa

Turmeric is used extensively in foods for both its flavor and color. Turmeric has a long tradition of use in the Chinese and Ayurvedic systems of medicine, particularly as an anti-inflammatory agent, and for the treatment of flatulence, jaundice, menstrual difficulties, hematuria, hemorrhage, and colic. Turmeric can also be applied topically in poultices to relieve pain and inflammation. Current research has focused on turmeric's antioxidant, hepatoprotective, anti-inflammatory, anticarcinogenic, and antimicrobial properties, in addition to its use in cardiovascular disease and gastrointestinal disorders.

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1.3.2 AMLA: Amla is known as Emblica, *officinalis* and belonging to family Euphorbiaceae. This consists of dried, as well as fresh fruits of the plant *Emblica officinalis*. It is a small- or medium-sized tree found in all deciduous forests of India. It is also found in Sri Lanka and Myanmar. It is grown by seed germination. It can also be propagated by budding or cutting. It does not tolerate the frost or drought. It is normally found up to an altitude of 1500 m. Commercially, it is collected from wild-grown plants.

Morphology:

Table-1: Morphology of Amla

Colour	Green changing to light yellow or brick red when matured
Odour	None
Taste	Sore and astringent
Shape	Globose
Size	1.5 to 2.5 cm in diameter
Extra Features	Fruits are flesly obscurely four lobed with 6-trygous seeds.

Microscopy: Fruit shows an epicarp consisting of epidermis with a thick cuticle and two to four layers of hypodermis; the cells in hypodermis is tangentially elongated, thick-walled, smaller in dimension than epidermal cells; mesocarp consists of thin-walled isodiametric parenchymatous cells; several collateral fibrovascular bundles scattered throughout mesocarp; xylem composed of tracheal elements, fibre tracheids and xylem fibres; tracheal elements, show reticulate, scalari form, and spiral thickenings; mesocarp also contains large aggregates of numerous irregular silica crystals.

Chemical Constituents: It is highly nutritious and is an important dietary source of vitamin C, minerals, and amino acids. The edible fruit tissue contains protein concentration 3-fold and ascorbic acid concentration 160-fold compared to that of the apple. The fruit also contains considerably higher concentration of most minerals and amino acids than apples. The pulpy portion of fruit, dried and freed from the nuts contains: gallic acid 1.32%, tannin, sugar 36.10%; gum 13.75%; albumin 13.08%; crude cellulose 17.08%; mineral matter 4.12%; and moisture 3.83%. Tannins are the mixture of gallic acid, ellagic acid, and phyllembin. The alkaloidal constituents such as phyllantidine and phyllantine have also been reported in the fruits. An immature fruit contains indolacetic acid and four other auxins—a1, a3, a4, and a5 and two growth inhibitors R1 and R2.

Uses: Fruits are diuretic, acrid, cooling, refrigerant, and laxative. Dried fruit is useful in haemorrhage, diarrhoea, diabetes, and dysentery. They are useful in the disorders associated with the digestive system and are also prescribed in the treatment of jaundice and coughs. It has antioxidant, antibacterial, antifungal, and. antiviral activities. Amla is one of the three ingredients of the famous ayurvedic preparation, triphala, which is given to treat chronic dysentery, bilousness, and other disorders, and it is also an ingredient in chyavanprash.(9)

1.4 LOTION: Lotions are defined as monophasic or biphasic solution, emulsion or suspension design to apply on unbroken and also broken or inflamed skin without friction.

Types of lotions: Lotions are classified in following classes as.

a) Simple Lotion
b) Therapeutic Lotion
c) Suspension Type Lotion

d) Emulsion Type Lotion

a) Simple Lotion: This kind of lotions generally used. These are used form cooling and soothing effect for smooth skin. It helps to retain moisture in body also provide humectant effect. e.g., Simple lotions.

b) Therapeutic Lotion: Therapeutic lotions contains different kind of therapeutic agent depending on desired effect required. e.g., calamine lotion as protectant and astringent and salicylic acid lotion as keratolytic bacteriostatic and fungi static.

c) Suspension Type of Lotion: Some lotions contain insoluble solids called suspension type of lotion. Here, bentonite, sodium carboxy methyl cellulose uses as suspending agent. e.g., calamine, Sulphur, zinc oxide.

d) Emulsion Type of Lotion: These are diluted lotions with o/w emulsion stabilized by emulsifying agents like emulsifying wax. e.g., Benzoyl benzoate lotion.(10,11)

1.5 Antimicrobial Activity of Some Medicinal Plants

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, emergence and dissemination of multidrug resistant (MDR) strain in pathogenic bacteria have become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for the infection caused by pathogenic bacteria.

Many plants have been used because of their antimicrobial traits, which are due to phytochemicals synthesized in the secondary metabolism of the plant . Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been found in vitro to have antimicrobial properties. A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases as urinary tract infections, gastrointestinal disorders, respiratory disease, and cutaneous infection.

Mechanisms of action of antibacterial agents

- Cell-wall biosynthesis
- Destruction of bacterial membrane
- Inhibiting nucleic acid synthesis

Antimicrobial activity: Antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome this review, we focused on the use of antimicrobial testing methods for the in vitro investigation of extracts and pure drugs as potential antimicrobial agents.

After the revolution in the “golden era”, when almost all groups of important antibiotics (tetracyclines, cephalosporins, aminoglycosides and macrolides) were discovered and the main problems of chemotherapy were solved in the 1960s, the history repeats itself nowadays and these exciting compounds are in danger of losing their efficacy because of the increase in microbial resistance . Currently, its impact is considerable with treatment failures associated with multidrug-resistant bacteria and it has become a global concern to public health.

For this reason, discovery of new antibiotics is an exclusively important objective. Natural products are still one of the major sources of new drug molecules today. They are derived from prokaryotic bacteria, eukaryotic microorganisms, plants and various animal organisms. Microbial and plant products occupy the major part of the antimicrobial compounds discovered until now.

The fact that a plant extract exhibits antimicrobial activity is of interest, but this preliminary part of data should be trustworthy and allow researchers to compare results, avoiding work in which researchers use the antimicrobial activity investigation only as a complement to a phytochemical study.(12,13)

1.5.1 Diffusion methods

Antimicrobial gradient method (E-tests): The antimicrobial gradient method combines the principle of dilution methods with that of diffusion methods in order to determine the MIC value. It is based on the possibility of creating a concentration gradient of the antimicrobial agent tested in the agar medium. The Etests (BioMérieux) is a commercial version of this technique. In the procedure, a strip impregnated with an increasing concentration gradient of the antimicrobial agent from one end to the other is deposited on the agar surface, previously inoculated with the microorganism tested.

This method is used for the MIC determination of antibiotics, antifungals and anti-mycobacterial. MIC value is determined at the intersection of the strip and the growth inhibition ellipse.

1.5.2 Agar disk-diffusion method: Agar disk-diffusion testing developed in 1940, is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and yeasts testing. Although not all fastidious bacteria can be tested accurately.

In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface. The Petri dishes are incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured.

Moreover, the agar disk-diffusion method is not appropriate to determine the minimum inhibitory concentration (MIC), as it is impossible to quantify the amount of the antimicrobial agent diffused into the agar medium. Nevertheless, an approximate MIC can be calculated for some microorganisms and antibiotics by comparing the inhibition zones with stored algorithms.(14,15)

II. MATERIAL AND METHODS

Material used in the formulations are Glycerin, Propyl Paraben, Methyl Paraben, Bentonite, Petroleum Ether, Triethanolamine, Cetyl Alcohol, Steric Acid, Mineral Oil and Ethanol.

2. **Preparation of Neem Extract-** In this process Leaves & Fruits of the plant will be collected and washed thoroughly with distilled water and shade dry for 10 days. Dried leaves & Fruits were grinded into powder form and make extraction with alcoholic solution ethanol with the help of extraction process according to reviewed articles. These methods could be Maceration**,** Decoction, Soxhlet Extraction and Hot Extraction.

III. EXPERIMENTAL METHODS

The ground work starts from plant collection and authentication. After this process moves up and goes to extraction then chemical analysis according to my objectives of this research work. Then, formulation development and evaluation of the preparation.

3. **Plant materials:** Azadirachta Indica leaves & Phyllanthus Emblica were collected from a plantation.

Figure 2: Plant of Neem

Figure 3: Plant of Amla

3.1 Preparation of extracts : In which the 500 g of dried powder of Neem leaf and Amla fruits was extracted in ethanol by Soxhlet extraction method. The extraction process was carried, filtrates and collected in a beaker.

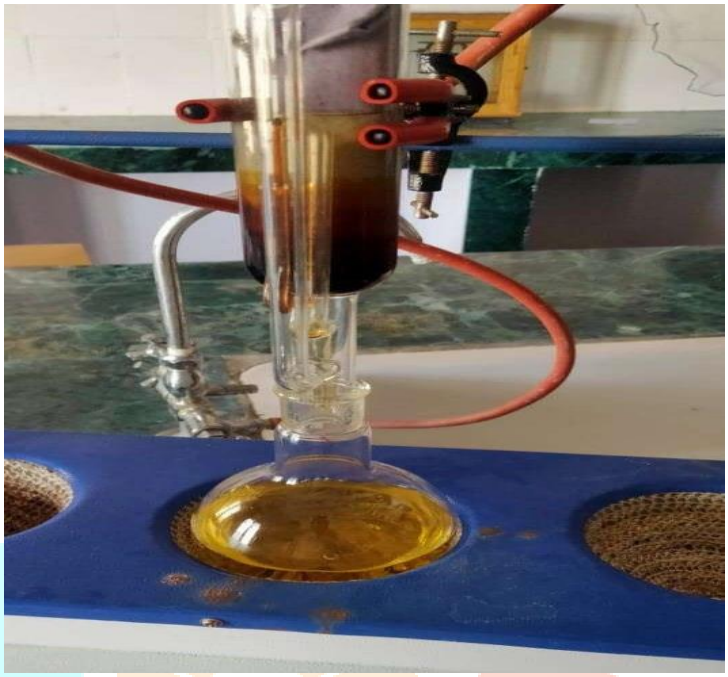

Figure 4: Soxhlet extraction process for powder drug

3.2 Pre-Formulation: Preformulation study was perform in powder of drug.

Table 2: Parameters of Powder Extract

S/N	Parameter	Observation
1.	Particle size	400-500µm
2.	Angle of repose	26^0
3.	Bulk density	1.4gm/cc
4.	Ph	6.6-7.2

3.3 Formulation Development: The herbal lotion were formulated

Method: Prepared by W/O emulsion method.

Table 3: List of Ingredients used in the formulation

S/N	**Ingredients**	**Category**	**F1**	**F2**
1.	Herbal Drug	Herb extract	Neem extract	Polyherb
2.	Glycerine	Humactant	1.5 ml	1.5 ml
3.	Bentonite	Thickening agent	1.25 gm	1.25 gm

4.	Methyl paraben	Preservatives	0.05gm	0.05gm
5.	Methyl paraben	Preservatives	0.05gm	0.05gm
6.	Triethanolamine	Neutralizer	0.45gm	0.45gm
7.	Cetyl alcohol	Co-emulsion	1gm	1gm
8.	Water	Diluent	40ml	40ml
10.	Mineral oil	Occulsive	2.5ml	2.5ml

Figure 5: Formulation of Lotion

IV. EVALUATION STUDY OF LOTION:

The lotion was evaluated for organoleptic properties, homogeneity, irritation test, viscosity, pH, stability and microbial test.

4. **Determination of organoleptic properties:** The appearance of the lotion was judged by its colour, odour texture, roughness, pearlscence and washing from skin.

4.1 Determination of pH: The pH meter was calibrated and measured the pH by digital pH meter placing in the beaker containing 100mg of the at a temperature room temperature.

4.2 Wash ability Test: The removal of the lotion applied on skin was done by washing under tap water with minimal force to remove the lotion.

4.3 Irritancy test: The lotion was applied on left hand dorsal side surface of 2sq.cm and observed in equal intervals up to 24hrs for irritancy, sensitivity and edema.(18,19)

4.4 Determination of homogeneity: The formulations were tested for the homogeneity by visual appearance and by touch.(20)

4.5 Spreadability test: 500mg of the lotion was sandwiched between 2 slides. A weight of 200gm was placed on upper slide. The weight was removed and extra formulation was scrapped off. The lower slide was fixed on board of apparatus and upper slide was fixed with non-flexible string on which 100gm load was applied. Time taken by upper slide to slip off was noted down.(21)

$$S = m \times \frac{l}{t}$$

Where,

S – Spread ability

m- Weight tied to upper glass slide.

l- Length moved on a glass slide

t- Time taken.

The determinations were carried out in three times and the average are readings was recorded and calculate.

4.6 Determination of viscosity: The viscosity determinations were carried out using a Brookfield Viscometer (DV II+ Pro model) using spindle number NDJ-8S at a 20 rpm at a temperature of 25oC. The determinations were carried out in triplicate and the average of three times readings was recorded.(22)

4.7 Stability test: The stability test of final optimized lotion was measured out and it was found that the lotion was stable in room temperature for at least three months. The value of pH, viscosity and spreadability all lay within the required range. In which no major changes in values of pH, viscosity and spreadability as compared to the initial value of formulation.(23)

V. RESULT AND DISCUSSION

Table 4: Evaluation Parameter of herbal lotion

S/No.	Parameter	Observation		
		F1	F2	F3
1.	Appearance	Yellowish-white	Yellowish-white	Light brown
2.	Odour	Characteristics	Characteristics	Characteristics
3.	pH	pH 5.5	5.3	5.8-6
4.	Irritancy test	No redness and edema	No redness and edema	No redness and edema

5.	Washability	Easily Washable by tap water	Easily Washable by tap water	Easily Washable
6.	Homogeneity • By visual • By Touch	Smooth and Consistent	Smooth and Consistent	Smooth and Consistent
7.	Spreadability	Uniform with a value of 35-42 g.cm/sec Easily spreadable	Uniform with a value of 35-42 g.cm/sec Easily spreadable	Uniform with a value of 40-42 g.cm/sec Easily spreadable
8.	Viscosity	Viscosity 18000-19000cps.	Viscosity 18000-19000cps.	Viscosity 16000-17000cps.

5.1 Stability test: To assess the formulation stability, was performed in the lab. Each formulation was stored at 4°C room temperature and 40°C temperature for 2-3 month and observed for physical stability.

Table 5: Stability test Parameter of herbal lotion

Admissibility Conditions (Initial)		Admissibility Conditions (30 Days)
Appearance	Homogeneous Cream	Concordant
Odour	Characteristic odor, Perfumed	Concordant
Color	White Beige	Concordant
pH	5.5-6.0	5.5-6.0
Viscosity	35.000 mPas	45.000 mPas

Table 6: Stability test Parameter of herbal lotion

Ideal Properties of Semisolid Dosage Forms	
1 Physical Properties	
A	Smooth Texture
B	Elegant in Appearance
C	Non Greasy & Non Staining
D	Non Gritty
E	Non Dehydrating
F	Non Hygroscopic
2 Physiological Properties	
A	Non Irritating
B	Do not Alter Membrane/Skin Functioning
C	Miscible with skin Secretion
D	Have low sensitization effect
3 Application Properties	
A	Easily Applicable with Efficient Drug Release
B	Easily Washable with Water

5.2 Microbiological tests: Microbiological testing is a key aspect of cosmetic product safety and quality. In order to meet the regulatory requirements, such as those specified by the Cosmetics Regulation (EC) 1223/2009, the microbiological quality of raw materials must be well understood and furthermore, batches of formulated cosmetic products must be tested for microbiological quality before released.

Our centers of excellence for cosmetics microbiology testing in the US and Europe provide expertise that can support you from product include preservative efficacy testing (also known as challenge testing), quality control testing and research and development support, helping our clients to ensure product quality and safety.

5.3 Preservative Efficacy Testing: We conduct challenge testing of cosmetics preservative systems to help you to evaluate the antimicrobial protection of a cosmetic product during the product shelf-life according to relevant international standards including the International Standard ISO 11930, European Pharmacopoeia (EP), French Standard NF T 75-611 and the United States Pharmacopeia (USP) Chapter <51> Antimicrobial Effectiveness Testing. Microorganisms typically used for challenge testing include Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans and Aspergillus brasiliensis however other microorganisms can be added if required.

5.4 Total Viable Count Tests: To determine the microbiological specification of the finished cosmetic product, measurement of the total count of aerobic mesophilic microorganisms as well as yeasts and fungi is required with comparison against industry established allowable limits. Our microbiology teams deliver robust testing services to help you understand the microbiological cleanliness of your products and support the release of your batches in accordance with the standards ISO 16212 (Enumeration of yeast and mould), ISO 21149 (Enumeration and detection of aerobic mesophilic bacteria).

Figure 6: Culture media preparation

VI. CONCLUSION: Lotion was prepared for microbiological tests total viable count and gram-negative pathogens known as Escherchia coli and antimicrobial activity is noted.

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