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Formulation And Evaluation of antimicrobial dusting powder

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ABSTRACT :

Dusting powders are powder in fine state of subdivision that can be used for external application to areas where skin is intact. Dusting powder should have: Free flowability, easy spreadability, non – irritability, non – grittiness, good absorption and adsorption, compatible with skin secretions. An antimicrobial is an agent that kills microorganisms or inhibit their growth antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome. In this research, we focused on the use of antimicrobial testing method for the in vitro investigation of extracts as potential antimicrobial agent. In the present study Calotropis procera, Aegle marmelos and Annona squomosa plant leaves were selected to prepare poly herbal antimicrobial dusting powder. The plants selected for complete study was based on its easy availability, degree of research work which is not done and folkore claiming its therapeutic activity as antimicrobial. Hydro-alcoholic extract of leaves were subjected to preliminary phytochemical screening for possible presence of bioactive antimicrobial compounds. Dusting powder formulations were prepared using leaves fine powder. Evaluation was carried out using parameters like Flow Property, Determination of pH, Moisture content, Ash values, Extractive values and Irritancy test. Antimicrobial susceptibility testing was done by Agar diffusion method (Cup plate method) using 'Staphylococcus aureus' a gram positive bacteria and standard as Neomycin powder. Antimicrobial activity of sample was less as compared to standards. the leaves of three plants contain the tannins, alkaloids and as per results, the formulation shows the antimicrobial activity. Thus, the formulation can be used as antimicrobial dusting powder.

KEYWORDS: Calotropic procera, Aegle marmelos, Annona squamasa, Spradabilty, Anti- microbial.

INTRODUCTION :

Dusting powders are powder in fine state of subdivision that can be used for external application to areas where skin is intact. Dusting powders should be homogenous and in a very fine state of sub division to enhance the effectiveness of the medicament and minimize the local irritation. Hence dusting powder should be passed through sieve no. 120 or 180, to get very fine powder. Dusting powder should have: Free flowability, easy spreadability, non – irritability, non– grittiness, good absorption and adsorption, compatible with skin secretions. Dusting powders must be able to protect the skin from irritation, caused by friction moisture and chemical irritates. In dusting powders along with medicament, other ingredients like adsorbents and lubricants are also incorporated, to adsorb the watery portion of the wounds and get stucks on the applied part of the body and enhance the spredability or flow property, so as to make powder to flow easily, on the affected part.[1]

The word 'antimicrobial' was derived from the Greek word anti (against), micros (little) and bio (life) and refers to all agents that act against microbial organisms. This is not synonymous with antibiotics a similar term derived from the Greek word anti (against) and biotikas (concerning life). An antimicrobial is an agent that kills microorganisms or inhibit their growth antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome.[2]

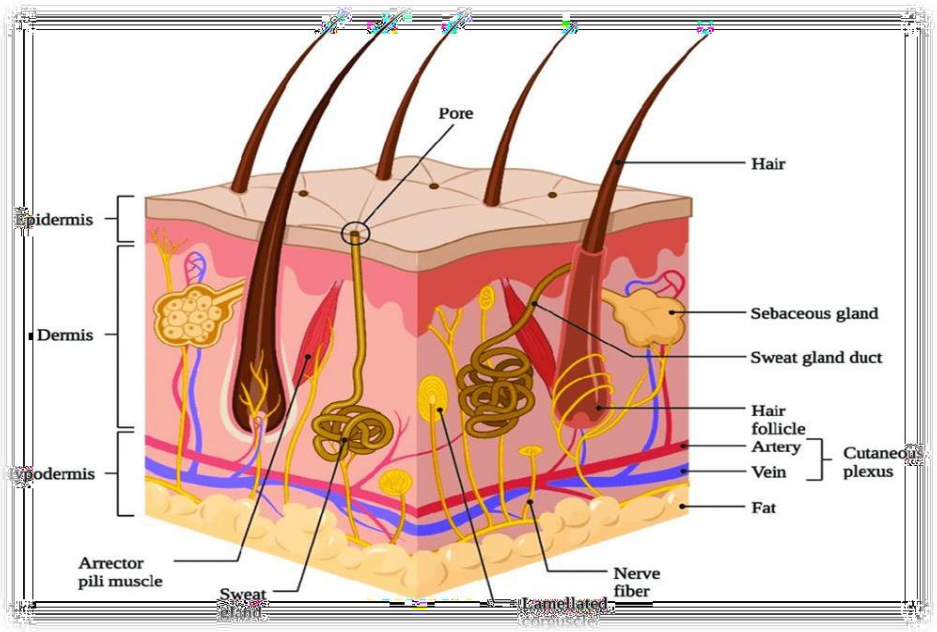
In this research, we focused on the use of antimicrobial testing method for the in vitro investigation of extracts as potential antimicrobial agent. In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. Therefore a greater attention has been paid to antimicrobial screening and evaluation methods, several bioassays such as disc diffusion, well diffusion or broth or agar dilution are well known and commonly used, but others such as flow cytoflurometric and bioluminescent methods are not widely used because they require specified equipment and further evaluation for reproducibility.[3]

SKIN :

1. The skin is a complex, dynamic organ.
2. The skin is the largest organ of the human body (1.75 m2), and the weight about 15% of the body
3. It consists of many cell types called Keratinocytes and Specialized structures like "the Basement Membrane"
4. Dermal- Epidermal junction is called basement membrane, the weakest part in the skin and the usual site of blisters.
5. It serves multiple functions that are crucial to health and survival.
6. It is divided into epidermis (ectoderm),basement membrane,dermis (mesoderm), subcutaneous fat and skin appendages (ectoderm and mesoderm).[4]

FUNCTIONS Of SKIN :

1. Prevents loss of water & proteins
2. Sensory organ protects against physical injury
3. Regulates body temperature
4. Important component of immune system
5. Vitamin D production by absorbing UVB
6. Has psychological and cosmetic importance such as hair, nails.**[5]**



TYPES OF SKIN :

The Dermis :

The dermis is an integrated system of fibrous, filamen-tous, and amorphous connective tissue that accommodates stimulus-induced entry by nerve and vascular networks, epidermally derived appendages, fibroblasts, macrophages, and mast cells. Other blood-borne cells, including lympho- cytes, plasma cells, and other leukocytes, enter the dermis in response to various stimuli as well. The dermis comprises the bulk of the skin and provides its pliability, elasticity, and tensile strength. It protects the body from mechanical injury, binds water, aids in thermal regulation, and includes receptors of sensory stimuli.

The dermis interacts with the epidermis in maintaining the properties of both tissues. The two regions collaborate during development in the morphogenesis of the dermal-epidermal junction[6]

LAYERS OF DERMIS:

The dermis is comprised of two layers:

1. *Papillary dermis:-*

The papillary dermis is the more superficial of the two, and lies just beneath the epidermal junction. It is relatively thin and is made up of loose connective tissue, which includes:

1. Capillaries

2. Elastic fibers
3. Reticular fibers
4. Collagen

2. *Reticular dermis:*

The reticular dermis is the deeper and thicker layer of the dermis, which lies above the subcutaneous layer of the skin. It contains dense connective tissue, which includes:

1. Blood vessels
2. Elastic fibers (interlaced)
3. Collagen fibers (in parallel layers)
4. Fibroblasts
5. Mast cells
6. Nerve endings
7. Lymphatics

Epidermis:

The epidermis is a stratified, squamous epithelium layer that is composed primarily of two types of cells: keratinocytes and dendritic cells. The keratinocytes differ from the "clear" dendritic cells by possessing intercellular bridges and ample amounts of stainable cytoplasm (Murphy, 1997). The epidermis harbors a number of other cell populations, such as melanocytes, Langerhans cells, and Merkel cells, but the keratinocyte cell type comprises the majority of the cells by far.

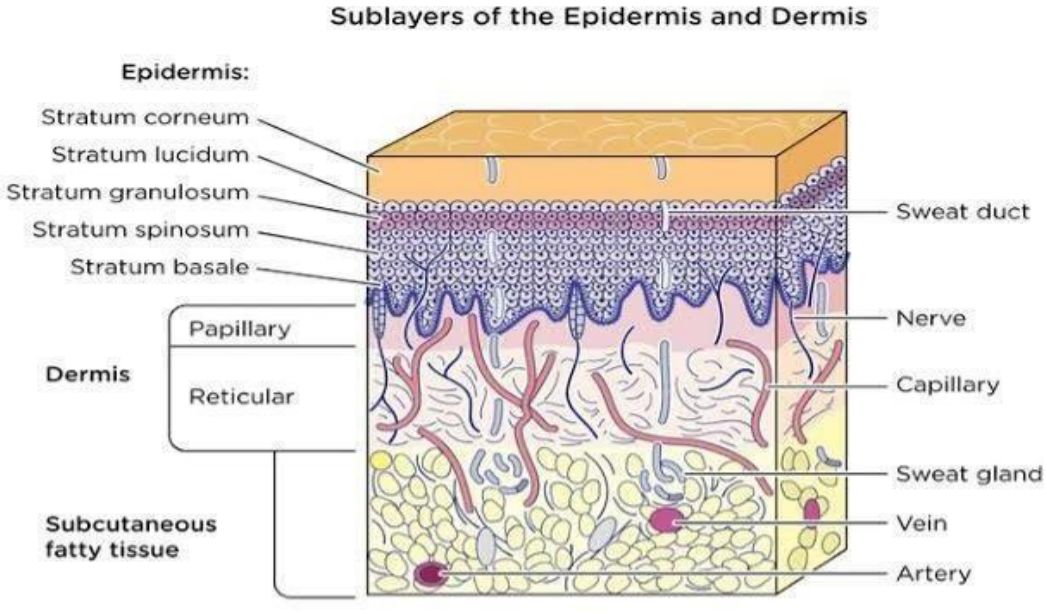
The epidermis commonly is divided into four layers according to keratino-cyte morphology and position as they differentiate into horny cells, including the basal cell layer (stratum germinativum), the squamous cell layer (stratum spinosum), the granular cell layer (stratum granulosum), and the cornified or horny cell layer (stratum corneum) The lower three layers that constitute the living, nucleated cells of the epidermis are sometimes referred to as the stratum malpighii and rete malpighii (Murphy).

The epidermis is a continually renewing layer and gives rise to derivative structures, such as pilosebaceous apparatuses, nails, and sweat glands. The basal cells of the epidermis un-dergo proliferation cycles that provide for the renewal of the outer epidermis. The epidermis is a dynamic tissue in which cells are constantly in unsynchronized motion, as differing individual cell populations pass not only one another but also melanocytes and Langerhans cells as they move toward the surface of the skin.[7]

LAYERS Of EPIDERMIS:-

The layers of the epidermis include the stratum basale (the deepest portion of the epidermis), stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum (the most superficial portion of the epidermis).

- **Stratum basale :-** Stratum basale, also known as stratum germinativum, is the deepest layer, separated from the dermis by the basement membrane (basal lamina) and attached to the basement membrane by hemidesmosomes. The cells found in this layer are cuboidal to columnar mitotically active stem cells that are constantly producing keratinocytes. This layer also contains melanocytes.
- **Stratum spinosum :-** Stratum spinosum, 8-10 cell layers, also known as the prickle cell layer contains irregular, polyhedral cells with cytoplasmic processes, sometimes called "spines", that extend outward and contact neighboring cells by desmosomes. Dendritic cells can be found in this layer.
- **Stratum granulosum :-** Stratum granulosum, 3-5 cell layers, contains diamond shaped cells with keratohyalin granules and lamellar granules. Keratohyalin granules contain keratin precursors that eventually aggregate, crosslink, and form bundles. The lamellar granules contain the glycolipids that get secreted to the surface of the cells and function as a glue, keeping the cells stuck together.
- **Stratum lucidum :-** Stratum lucidum, 2-3 cell layers, present in thicker skin found in the palms and soles, is a thin clear layer consisting of eleidin which is a transformation product of keratohyalin.[8]



Hypodermis:

The hypodermis (also called the subcutaneous layer or superficial fascia) is a layer directly below the dermis and serves to connect the skin to the underlying fascia (fibrous tissue) of the bones and muscles. It is not strictly a part of the skin, although the border between the hypodermis and dermis can be difficult to distinguish. The hypodermis consists of well-vascularized, loose, areolar connective tissue and adipose tissue, which functions as a mode of fat storage and provides insulation and cushioning for the integument.[9]

PHYSIOLOGY OF SKIN:

Number of topical or dermatological products are applied to the skin or mucous membrane, which enhance the fundamental function or pharmacologically alter the action in the underlined
tissues. Thus, to utilize the phenomenon of percutaneous absorption successfully, it is important to understand the anatomy, physiology, physicochemical properties of skin.The skin of an average adult covers a surface area of approximately 2 m2 and receives about one-third of the blood circulating through the body.

Microscopically skin constitutes three main histological layers: epidermis, dermis, and hypodermis (subcutaneous layer). The epidermis is 0.1-1.5 mm thick which is further divided into five parts: stratum germinativum, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum. It is responsible for the formation of the melanin pigment. The squamous cell layer is the thickest layer of the epidermis that helps to in movement of certain substances in and out of the body. The stratum corneum ("horny layers") is made up of 10 to 30 thin layers of the dead cells where the outermost layer is replaced by new layer.Just beneath the epidermis, lies the dermis, which is 1.5 to 4 mm thick. It contains collagen, elastin, sweat, oil glands, hair follicles, nerve endings, and blood and lymph vessels. Dermis acts as a water reservoir and contains scavenger cells from the immune system that engulf the foreign organisms.

The hypodermis (subcutaneous tissue) is the deepest layer of the skin. Subcutaneous tissue acts as an insulator and shock absorber protecting internal organs from injury. It stores fat and the blood vessels, nerves, lymph vessels, and hair follicles cross through these layers. The stratum corneum, which is the outermost layer of the skin, is an effective barrier for penetration of drugs into deeper layers.[10]

PENETRATION THROUGH SKIN:

The factors responsible for measuring the efficacy of TDDS include physicochemical characteristics of the drug and the type of the formulation. Whereas, the efficiency of treatment depends on the penetration of drug through the target layers of the skin at effective concentrations. Effective penetration of drug molecule plays an important role in TDDS. There are various routes of penetration of drug into skin.[11]

Sr no.	Drug molecule characteristics	Description
1	Drug molecule size and molecular weight	• Smaller molecules ensure good contact with stratum corneum and can pass easily through epithelial barrier leading easy penetration • Most commonly used drug molecules for transdermal route have molecular weight < 500 Dalton
3	Lipophilicity	• Important characteristic • It is closely related to partitioning free energy of drug molecule between two immiscible phases • lipid/water partition coefficient of a drug the basic determinant of drug permeability through the stratum corneum • low partition coefficient and low molecular weight drug molecules easy penetrated through skin • Example: liposomal amphotericin (Fungisome®, Ambisome®) has good penetrability and less toxicity in skin than conventional
Sr no.	**Drug molecule characteristics**	**Description**
4	Solubility	• Partially dependent on partition coefficient and molecular

		surface features • Molecule soluble in both lipid and water penetrates better than substances manifesting either high water or high lipid solubility
5	Ionization of drug molecule	• Depends on pH of solution as it alters the state of ionization of a drug and hence its hydrophilicity/lipophilicity as it crosses a membrane which ultimately affects into penetration into the skin
6	Stereochemistry and steric interaction	• It affects solubility of drug molecule and ultimately its permeation through skin • Steric hindrance of the hydroxyl groups determines aqueous solubility leading to its effect on penetration of drug molecule into skin

NEED AND OBJECTIVE:-

As we know very well that everything. In this world changes time by time, since thousands of year the era was of Ayurveda or herbal origin drug. But last few decades it was replaced by allopathic system of medicine, which was fastly accepted worldwide, but later due to its lots of adverse effect again men step down on Ayurveda because of its better therapeutic result and safety profile. Now the people are more believing in natural origin drug, looking to the scope of herbal drugs and increasing demand specially in disease of liver, hypertension, diabetes, cancer, arthritis and skin disease etc. It is decided that a scientific validation is to be carried out on Calotropis pricers, Aeglemarmelos, Annona squamosa in detail due to its effectiveness against microbial agent as claimed by tribal people.The plant selected for complete study was based on its easy availability, degree of research work which is not done and folkore claiming its therapeutic activity as antimicrobial. Till now only few studies has been done on this plant. Therefore, this plant is having wide scope for detailed pharmacognostical preliminary phytochemical and pharmacological investigation. All these three plant show antimicrobial property, so they can be used in microbial skin disease. We can make antimicrobial cream, lotion, oil, dusting powder.[12]

PLAN OF WORK :-

- Review of literature
- Selection of drugs
- Collection of excipients
- Selection of procedure
- Formulation of dusting powder
- Evaluation of dusting powder
    1. Angle of repose
    2. Bulk density
    3. Tapped density
    4. Carr's Index
    5. Hausner's Ratio
    6. PH
    7. Moisture content
    8. Ash value
    9. Extractive values
    10. Irritancy test .
- Result
- Conclusion
- Reference.

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- :- 23 oct 2020)

DEFINITION:-

Dusting powders are applied to various parts of the body as lubricants, protectives, absorbents, antiseptics, antipruritics, anti bromhidrosis agents, astringents and antiperspirants.[13]

CLASSIFICATION OF DUSTING POWDERS :-

1. Medical dusting powder:-

Medicated dusting powder are those powders in which one or more therapeutic agents are incorporated. They are mainly used for superficial skin conditions and sterility is rarely essential. However, they must be free from dangerous pathogens. Some natural ingredients such as talc, kaolin, etc. may be contaminated with spores of tetanus, gas gangrene, and anthrax and should be sterilized.
Medical dusting powders are not intended for application to open wounds or areas of broken skin. Since small particles are less likely to irritate sensitive tissue, all the ingredients are sieved through mesh 180 to get uniform size particles.

2. Surgical dusting powder:-
Surgical dusting powders are used mainly in body cavities and on burns and umbilical cords of infants as a result of major wounds, whereas medical dusting powders are used on superficial skin conditions. In contrast, medical dusting powders must be free of pathogenic microorganisms while surgical dusting powders must be sterilized before use. It is generally possible to prepare dusting powders by mixing two or more ingredients, of which starch, talc, or kaolin must be one element. The most common materials used are talc and kaolin because they are chemically inert. These ingredients are prone to contamination by pathogenic bacteria, however, so before using them they must be sterilized by dry heat method (160 degrees for 2 hours).[14]

PROPERTIES:

1. It should be homogenous.
2. It should not cause local irritation.
3. It should flow easily and spread uniformly.
4. It should cling to the skin on application.
5. It should have adsorptive and absorptive capacity.

ADVANTAGES:

- Good chemical stability compared with fluids.
- Easy to carry than the liquid dosage forms.
- Suitable for small children and elderly patients.
- Easy to apply over wounds
- Economical
- Rapid onset of action.

DISADVANTAGES:

- Difficult to protect powders containing hygroscopic or aromatic materials from decomposition.
- Not suitable for drugs which are unstable in normal atmospheric condition
- Susceptible to physical instability

PLANT PROFILE :

A. Calotropis procera:

Vernacular names: English – Calotropis, Hindi–Akada, Marathi – Arka,

Biological source:- Dried leaves and fibres are collected from the plant *Calotropis procera* belong to family Apocynaceae .

Taxonomy :

Kingdom – Plantae **Order** –

Gentianales **Family** –
Apocynaceae **Genus** –
Calotropis **Species** – C. procera

Calotropis procera are widely used traditional medicinal plant to treat various ailments. It is an erect, perennial shrub luxuriantly thriving in wastelands. Plants are the richest sources of secondary metabolites such as alkaloids, terpenoids, steroids and flavonoids etc.

B. Aegle marmelos:

Vernacular names: English–Aegle, Hindi–Bel, Marathi–Maredu,

Biological source:- Dried leaves and fibres are collected from the plant Aegle marmelos belong to family Rutaceae .

Taxonomy :

Kingdom – Plantae **Order** –
Sapincales **Family** – Rutaceae
Subfamily – Aurantioideae **Genus** –

Aegle

Aegle marmelos, commonly known as bael belonging to the family Rutaceae, is a moderate sized, slender and aromatic tree. It is indigenous india and is abundantly found in the Himalayan tract, Bengal, Central and south India. It is extensively planted near Hindu temples for its wood and leaves which are generally used for workship.It is deciduous shrub or small to medium sized tree.

C. Annona squamasa:

Vernacular names: English–Sugar apple, Hindi – Sharifa, Marathi – Sitaphal.

Biological source:- Dried leaves and fibres are collected from the plant Annona squamasa belong to family Annonaceae .

Taxonomy:

Kingdom – Plantae **Order** –
Magnoliales. **Family** –
Annonaceae **Genus** – Annona

Annona squomosa, belonging to the family Annonaceae is a small ever green tree commonly found in India and originates from West Indies and South America different parts of Annona squamosal are used in folkloric medicine for the treatment of various diseases. It is mainly grown in gardens for its fruits and ornamental value.
This plant is commonly called custard apple in English, sharifa in Hindi and sitaphalam in telungu in India. It is considered beneficial for cardiac disease, diabetes hyperthyroidism and cancer. It shows antibacterial, antifungal properties. Leaves are simple, alternative occur singly

MATERIAL AND METHOD :-

Collection of specimen:

The tree Calotropis procera, Annona squamosa and Aegle marmelos widely found throughout India.
The species for the proposed study that is Calotropis procera, Annona squamosa and Aegle marmelos were collected. Care was taken regarding the age and the health of the plant to obtain a best condition of leaves part.[15]

TREATMENT -

The leaves were washed with water, rinsed and dried in shade. The dried leaves were coarsely powdered by means of grinder and the powder was passed through the sieve no. 120#. Course powder was used for further studies.

COMPOUNDING METHODS:

1. Grinding
2. Weighing
3. Mixing
4. Wrapping

Sr no.	Ingredients	Quantity (gm)
1.	Calotropis procera	6

2.	Aegle marmelos	6
3.	Annona squamasa	6
4.	Starch	25
5.	Talc	17

PROCEDURE:-

1. First all the powders sieved through mesh 120# to ensure uniformity and fineness of particle size.
2. Weigh separately all the powder drug,purified talc and starch powder according to calculation
3. Perfume is absorbed onto a part of weighed quantity of talc.
4. All the powders are mixed in a geometric proportion and absorbed perfume is incorporated into powder mixture.
5. After uniform mixing, transfer this powder on a sheet of paper and spread as a thin layer with spatula , sterilize by keeping in hot air oven at 1600 for one hour.
6. After sterilization, cool the powder to room temperature and pass through once again mix the powder, lightly with spatula.
7. Dusting powder is then transferred to a labelled container.[16]

EVALUATION PARAMETERS :-

Angle of Repose:

Take a clean and dry funnel with a round stem of 20 – 30 mm diameter with flat tip and attach it to the burette stand. Place a graph paper sheet below the funnel on clean and dry platform. Adjust the distance between lower tip of the funnel and sheet to specified height 2 cm. Gently pour sample in funnel from top till a heap of powder forms and touches the lower tip of the funnel. Using a pencil draw a circle around the heap covering approximately 90% of total powder. Repeat the procedure four times to obtain average reading find out average diameter and radius of the each drawn circle.

θ= tan-1 (h/r)

1. Determination of Bulk Density and tap density

A. Weigh accurately 25 gm of powder (W1) place it in dried graduated measuring cylinder and note volume as V1 (ml).
B. Place the cylinder containing sample in bulk density apparatus. Adjust apparatus for 100 tapping and operate it. Record the volume occupied by the powder as V2 (ml).

Bulk Density = Mass/Bulk volume Tapped Density = Mass/Tapped volume

2. Hausner's Ratio:

Hausner's ratio is the ease of index of powder flow and calculated by using following formula,

Hausner's ratio = Tap Density / Bulk Density

Carr's Index:

percent compressibility of blend was determined by Carr's compressibility index, calculated by using following formula.:-

Carr's Index = $\frac{\text{Tap density – Bulk density}}{\text{Tap density}} \times 100$

3. Determination of pH:-

The pH of 1% solution of formulated powder and The pH of 1% solution of formulated powder and standard was determined using pH meter.

Determination of Moisture content :-

The moisture content of powder was determined by taking 3 gm of powder in hot air oven at 700C for 1 hr.

Determination of Ash values:-

Total ash value :-
2 gm of powder was weighed accurately in previously ignited and tarred silica crucible. The material was then ignited by gradually increasing the heat to 400 0C until it appeared white indicating absence of carbon. It is then cooled in a desiccator and total ash of air dried material is calculated.

Acid insoluble Ash value :-

Residue obtained after extracting the total ash treated with hydro alcoholic acid use to detect the contamination from sand or boil. Boil ash with 25 ml of 2 m HCl for 5 minutes, collect the residue on ashless filter paper, wash with hot water, ignite cool in desiccators and weigh.

Determination of Extractive values:-

Water soluble Extractive value :-
5 gm of powder was accurately weighed and placed inside a glass stoppered conical flask. It is then macerated with 100 ml of chloroform water for 18 hours. It was then filtered and about 25 ml of filtrate was transferred into a china dish and was evaporated to dryness on a water bath. It was then dried to 105 0C for 6 hours, cooled and finally weighed.

Alcohol soluble Extractive values:-
5 gm of powder was accurately weighed and placed inside a glass stoppered conical flask. It is then macerated with 100 ml of ethanol for 18 hours. It was then filtered and about 25 ml of filtrate was transferred into a china dish and was evaporated to dryness on a water bath. It was then dried to 105 0C for 6 hours, cooled and finally weighed.

Irritancy test:

Mark an area (1sq.cm) on the left hand dorsal surface. Definite quantities of dusting powder were applied to the specified area and time was noted. Irritancy, erythema, edema was checked if any for regular intervals upto 24 hrs and reported.[17]

Sr. No.	Physical parameters	Formulation (F1)	Standard
1.	Angle of repose		25.75
2.	Bulk density		0.53
3.	Tapped density		0.57
4.	Carr's Index		7.0%
5.	Hausner's Ratio		1.07
6.	PH		6.4
7.	Moisture content		15.0%
8.	Ash value Total ash value Acid insoluble Ash value		 12% 6%
9.	Extractive values Water soluble Extractive value Alcohol soluble Extractive values		 2% 3%
10.	Irritancy test:	Nil	Nil

RESULT AND DISCUSSION:-

The plant parts was identified and authenticated as Calotropis procera, Annona squamosa, and Aegele marmelos , And also done the evaluation parameters test.

CONCLUSION:

Antimicrobial activity of sample (F1) was less as compared to standard. Also the leaves of three plants contain the tannin, alkaloids and thus the formulation shows the antimicrobial activity.

F1< standard.

Thus, the formulation can be used as antimicrobial dusting powder.

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