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# Formulation and Evaluation of Anti microbial 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 $F_1$ and $F_2$ 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 both sample (i.e $F_1$ & $F_2$) was less as compared to standard. But the sample $F_2$ shows more antimicrobial activity than $F_1$. Also 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:** antimicrobial dusting powder, *Calotropis procera*, *Aegle marmelos*, *Annona squomosa,* hydro-alcoholic leaves extract

## 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. [17]

**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.

**Classification of dusting powders**

1) Medical dusting powder
2) Surgical dusting powder

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.

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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. In this research, we focused on the use of antimicrobial testing method for the in vitro investigation of extracts as potential antimicrobial agent. [1]

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.

Based on pathogenic infections, the patients are suggested to take antiviral agents, antibacterial agents, antifungal chemicals etc. Antimicrobial chemotherapy is the study to understand the mechanism using antimicrobial agents hence; antimicrobial agents that are used in treatment of infectious diseases are termed as antibiotics.

**Plant profile**

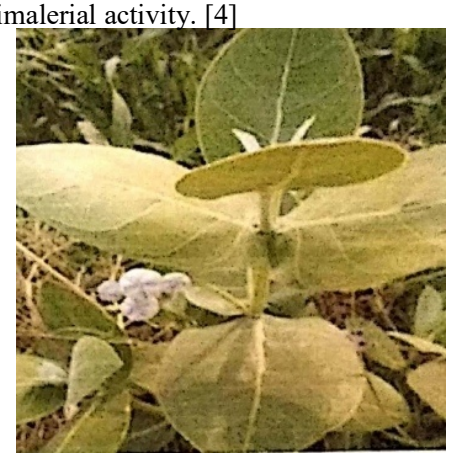
***Calotropis procera***

**Vernacular names:** English – Calotropis, Bengali – Akanda**,** Arabic – Usher**,** Hindi – Akada**,** Marathi – Arka**,** Sanskrit – Alarka

**Taxonomy [3]**

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. the traditional medicine involves the use of different plant extracts or bioactive chemicals. The result suggests that the phytochemical properties of the stem, leaves and flower for curing various ailments. The leaves are opposite – decussate, simple, subsessile and estipulate, the leaves are slightly to leathery and having a fine coat of soft hairs that sometimes sting too. The leaves were reported to use in sun worship from the vedic times.The leaves contain ascorbic acid , calactin , calotoxin , calatropagenin , calotropis, polysaccharide containing D-arabinose , D-glucose , D-glucosamine and L-rhamnose, calotropagenin , and 3-proteinase. The leaves shows antimicrobial, antidiarrheal, antifilarial, antihyperbilirubinemic, anti-implantation, antiplasmodial , antitumor, hypotensive , antimalerial activity. [4]



***Aegle marmelos***

**Vernacular names:** English – Aegle, Hindi – Bel, Marathi – Maredu, Urdu – Bel, Sanskrit – Adhararuha, Tripatra, Tamil – Vilvam

**Taxonomy**
Kingdom – Plantae
Order – Sapincales
Family – Rutaceae
Subfamily – Aurantioideae
Genus – Aegle
Species – *A. marmelos*

*Aegle marmelos*, commonly known as bael belonging to the family *Rutaceae*, is a moderate sized, slender and aromatic tree. It is indigenous to 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, up to 13 m tall with slender drooping branches and rather shabby crown. Leaf is trifoliate , alternate , each leaflet 5 – 14 × 2 – 6 cm , ovate with tapering or pointed tip and rounded bar , young leaves are pale green or pinkish , finely hairy while mature leaves are dark green and completely smooth. Each leaf has 4 – 12 pairs of side veins which are joined at margin. The end leaflet features a long stack , 0.5 – 3 cm white side stalks are typically shorter than 0.2 cm. Leaves contains flavonoids, alkaloids. The leaves show's hypoglycemic, antimicrobial, anti-inflammatory, radioprotective, anti-oxidative, anticancer, chemopreventive activity. [5]

***Annona squamasa***

**Vernacular names:** English – Custard apple, Sugar apple, Hindi – Sharifa, Marathi – Sitaphal**,** Tamil – Sitapalam

**Taxonomy**
Kingdom – Plantae
Order – Magnoliales
Family – Annonaceae
Genus – Annona
Species – *A. squamosa*

*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, 5 cm to 17 cm long and 2 – 6 cm wide rounded at the base and pointed at the tip, pale green on both surface . It contains alkaloids, glycoside. It is used in dysentery and urinary tract infection, applied to wounds. Leaves show antibacterial, antimicrobial, antioxidant, antitumor activity. [9]

**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 plants show antimicrobial property, so they can be used in

microbial skin diseases. We can make antimicrobial cream, lotion, oil, dusting powder.

## Material and Methods

### 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.

### Taxonomical Identification

The plant part was sent to authentication to botany department, Manoharbhai Patel College, Sakoli, dist- Bhandara.

### 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.

### Chemicals

Dragendroff's reagent(Loba), HCl(Loba), Mayer's reagent(Loba), Hager's reagent(Loba), Wagner's reagent(Loba), Distilled water , Lead acetate solution(Loba), Ferric chloride(Summer), Fehling solution A(K,R)& B(Apex), Benedict reagent(Loba), pyridine(Loba), sodium nitroprusside(Loba), Sulfuric acid(Summer), Millions reagent(Star), Chloroform(Loba), acetic anhydride, glacial acetic acid(Loba), Agar(Star), Peptone(Loba), Beef extract(Loba), sodium chloride(Loba), Talc(Loba) and starch(Loba).

### Apparatus

Beaker, Test Tube, Test Tube Holder, Conical Flask, Petri Plate, China Dish, Crucible, Funnel, wide mouth bottle.

### Instrument

Hot Air Oven, Hot Air Sterilization, Incubator, Autoclave, Laminar Air Flow, Moffle Furnace, Digital pH Meter(Modern scientific comp.), Bulk Density Apparatus(Delta electronic).

### Phytochemical screening (14)

Hydro-alcoholic extract of leaves were prepared by taking coarse powder of each leaves (20gm) separately in glass stopered wide mouth bottle and soaked it in menstrum that is water(60ml) and ethanol(100ml) for seven days with occassional shaking. Filter it, evaporate filterate upto 40ml. The extracts were subjected to preliminary phytochemical screening for possible presence of bioactive antimicrobial compounds.

**Test for Alkaloids:-**

**Dragendroff test-** Evaporate alcoholic extract, to residue add dilute HCL shake and filter. 2 – 3 ml of filtrate, add few drops Dragendroff's reagent. Orange brown precipitate indicates presence of alkaloid.

**Mayer's test-** 1 ml of extract, add 1 ml of Mayer's reagent (potassium mercuric iodide solution). Whitish yellow or cream colored precipitate indicates the presence of alkaloids.

**Test for saponins:-**Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1 cm layer of foam indicates the presence of saponins.

**Test for tannin and phenolic compound:-**

**Lead acetate solution test: -** Take little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

**$FeCl_3$:** To extract add neutral $FeCl_3$ – Intense coloration formed shows the presence of phenolic compound.

**Test for carbohydrate**

**Fehling's test –** To 1 ml of extract, add equal quantities of Fehling solution A and B, upon heating formation of brick red precipitate indicates the presence of sugars.

**Benedict's test –** To 5 ml of Benedict's reagent , add 1 ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

**Test for Glycoside**

**Le gal's test –**Dissolve extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink to red color shows the presence of glycoside.

**Test for Proteins and Amino Acids**

**Millon's test –** 1 ml of test solution is made acidify with sulphuric acid and add millon's reagent and boil this solution. A yellow precipitate is formed indicates the presence of protein.

**Ninhydrin test –** Add two drops of freshly prepared 0.2 % ninhydrin reagent (0.1% solution in n – butanol) to the small quantity of extract solution and heat. Development of blue color reveals the presence of proteins, peptides or amino acids.

**Test for Steroids**

**Lieberman – Burchard test –** 1 gm of test substance was dissolved in a few drops of chloroform, 3 ml of acetic anhydride, and 3 ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added, along the sides of test tube. Appearance of bluish – green color shows the presence of sterols.

**Preparation of dusting powder**

The dusting powder were formulated by using the formula as follows -

| Ingredient | Quantity (gm) | |
|---|---|---|
| | $F_1$ | $F_2$ |
| *Calotropis procera* | 10 | 20 |
| *Aegle marmelos* | 10 | 20 |
| *Annona squamosa* | 10 | 20 |
| Starch | 25 | 15 |
| Talc | 45 | 25 |

**Procedure [17]**

- First all the powders sieved through mesh 120# to ensure uniformity and fineness of particle size.
- Weigh separately all the powder drug, purified talc and starch powder according to calculation
- Perfume is absorbed onto a part of weighed quantity of talc.
- All the powders are mixed in a geometric proportion and absorbed perfume is incorporated into powder mixture.
- 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 $160^0$ for one hour.
- After sterilization, cool the powder to room temperature and pass through once again mix the powder, lightly with spatula.
- Dusting powder is then transferred to a labelled container.

**Evaluation of dusting powder**

**Flow Property [16]**

**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.

$$\theta = \tan^{-1} (h/r)$$

**Determination of Bulk Density and tap density –** Weigh accurately 25 gm of powder ($W_1$) place it in dried graduated measuring cylinder and note volume as $V_1$ (ml). 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 $V_2$ (ml)

$$\text{Bulk Density} = \text{Mass/Bulk volume} = W_1/V_1 \text{ g/ml}$$

$$\text{Tapped Density} = \text{Mass/Tapped volume} = W_1/V_2 \text{ g/ml}$$

**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-

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

**Determination of pH [11]**

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

**Determination of Moisture content [11]**

The moisture content of powder was determined by taking 3 gm of powder in hot air oven at $70^0$C for 1 hr.

**Determination of Ash values [13]**

**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 $^0$C 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 [13]**

**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 $^0$C 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 $^0$C 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.

**Antimicrobial Study**

Antimicrobial susceptibility testing was done using the well – diffusion method according to the standard of the National Committee for clinical laboratory standards Microorganism are broadly classified as bacteria, fungi, viruses, antinomytes etc. There are causative factors in the manifestation of various infectious diseases. In – vitro tests are used as screening procedure for new agents and for testing the susceptibility of individual isolates from infections to determine which of the available drug might useful therapeutically [12]

**Material**

- Dusting powder ($F_1$ and $F_2$)
- Marketed preparation(Neomycin)

**Study samples:** The antimicrobial study has been performed on three samples; one is powder ($F_1$), second is powder ($F_2$) and third is standard Neomycin powder.

**Microorganism:** In present study '*Staphylococcus aureus'* a gram positive bacteria, previously isolated, identified and stored in the laboratory of microbiology of College, was used.

**Preparation of sample:** For the sample preparation, 1 gm of each powder (including preparation & marketed preparation) is dissolved in 3 ml of distilled water and kept aside undisturbed to sediment. Then the suspension is taken as sample. [12]

**Agar diffusion method (Cup plate method)**

Agar medium is prepared by dissolving beef extract, peptone, sodium chloride in water and adjusting the pH to 7. Sterilize in autoclave at 121 $^0$C, 15 lbs pressure for 15 minutes. Now the medium to cool to 50 $^0$C then add culture and transfer the media in sterile petri plate under aseptic condition. Allow the medium to cool and to produce solid agar plates. [11]

Three wells of approximately 10 mm diameter was bored using well cutter and $F_1$, $F_2$ and standard sample added in the well. The plates where then incubated at 37 $^0$C for 24 hours. Antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. [12]

## Results and Discussion

The plant parts was identified and authenticated as *Calotropis procera, Annona squamosa, and Aegele marmelos* by Botany department, Manoharbhai Patel College, Sakoli Dist Bhandara.

**Table 1: Phytochemical screening**

| Plant constituents | *Calotropis procera* | *Annona squamosa* | *Aegle marmelos* |
|---|---|---|---|
| Alkaloids | + | + | + |
| Saponins | - | + | + |
| Tannin | + | + | + |
| Phenolic compound | + | + | + |
| Carbohydrate | + | + | - |
| Glycoside | + | + | + |
| Proteins & Amino acids | - | + | - |
| Steroids | - | + | + |

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**Table 2: Evaluation parameters**

| Sr. No | Physical parameters | Values | | |
|---|---|---|---|---|
| | | $F_1$ | $F_2$ | Standard |
| 1 | Angle of Repose | 29.57 | 27.45 | 25.75 |
| 2 | Bulk density | 0.51 | 0.50 | 0.53 |
| 3 | Tapped density | 0.53 | 0.53 | 0.57 |
| 4 | Carr's Index | 3.77% | 5.66% | 7.0% |
| 5 | Hausner's Ratio | 1.03 | 1.06 | 1.07 |
| 6 | pH | 5.2 | 5.7 | 6.4 |
| 7 | Moisture content | 18% | 17% | 15% |
| 8 | Ash values | | | |
| | i. i. Total ash | 16% | 19% | 12% |
| | i. ii. Acid insoluble ash | 7% | 8% | 6% |
| 9 | Extractive values | | | |
| | i. i. Water soluble extractive value | 4% | 2.5% | 2% |
| | i. ii. Alcohol soluble extractive value | 5% | 4.5% | 3% |
| 10 | Irritancy test | Nil | Nil | Nil |

**Anti-microbial activity**

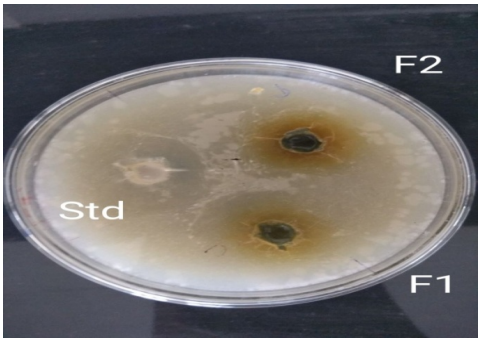


**Fig. 1: $F_1$ and $F_2$ Dusting powder compare with Neomycin**
**($F_1$ = formulation 1, $F_2$ = formulation 2, Std = Standard Neomycin powder)**

**Anti-microbial activity of $F_1$ and $F_2$ Dusting powder compare with Neomycin**

| Sample | Zone of Inhibition *S. aurues* |
|---|---|
| $F_1$ | 17 mm |
| $F_2$ | 21mm |
| Standard | 24 mm |

The various chemical constituent of leaves of *Calotropis procera, Aegle marmelos* and *Annona squamosa* have been reported. For the quality assured herbal product, the standardization is required. For standardization, the above mentioned parameters i.e, physical parameters were performed Zone of inhibition for $F_1$ - 17 mm, $F_2$ – 21mm for *S.aurues* was found while the standard dusting powder (Neomycin) shows the 24mm.

## Conclusion

Antimicrobial activity of both sample (i.e $F_1$ & $F_2$) was less as compared to standard. But the sample $F_2$ shows more antimicrobial activity than $F_1$. Also the leaves of three plants contain the tannin, alkaloids and thus the formulation shows the antimicrobial activity.

**$F_1 < F_2 <$ Standard.**

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

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