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# FORMULATION AND EVALUATION OF POLYHERBAL BASED ALCOHOL FREE HAND SANITIZER GEL

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**ABSTRACT** Clean hands are an essential practice for the control and prevention of infections. Antimicrobials of herbal origin represent an expanded source for medicines against all the known kinds of microbial infections. These are very potent to treat the infections besides limiting many of the side effects that are often associated with synthetic antimicrobials. Such active antimicrobial herbs can be safely incorporated in formulating polyherbal hand sanitizer. In the present research, agar well plate methods were employed. Extracts from hot aqueous, cold aqueous, ethyl acetate, and methanol were utilized to assess the activity of *Eucalyptus teriticornis, Coleus amboinicus, Mentha spicata, Azadirachta indica.* Hot aqueous and methanol extract of all plant is highly effective against C*itrobacter sp, Escherichia coli, Pseudomonas sp, Klebsiella sp,* and *Staphylococcus sp,* at different concentration. The phytochemical screening for all the plants was performed which confirmed the presence of alkaloid, flavonoid, tannin,and amino acid all in common. The present study concluded that hot aqueous and methanol extract of all plants can be used in formulating a herbal hand sanitizer gel.

**KEYWORDS :** Antimicrobial, phytochemical , Hand sanitizer gel.

## INTRODUCTION

Hygiene is defined as maintenance of cleanliness practices which carries the utmost importance in maintenance of health. These concepts highlight the need to maintain hygiene in the prevention of diseases [7].

The skin is the most exposed part of the body to the sunlight, environmental pollution and also to some protection against pathogens [3].

Hand sanitizers are available in the form of liquid, foam or easily flowing gel formulation. Both alcohol and alcohol-free hand sanitizers are available on the market. But theregular use of alcoholic sanitizer destroys our hand skin; therefore, there is a need to prepare an alcohol-free sanitizer and a herbal hand sanitizer which is mild to the skin and effective at killing germs [6].

## MATERIALS AND METHODS

### Collection Of Plant Sample

*Azadirachta indica, Eucalyptus teriticornis, Coleus amboinicus, Mentha spicata* were collected from in and around Tirupur district, Tamil Nadu, India. The freshly collected leaves were washed with water and dried under shade at room temperature. [1]

### Extraction Of Plant Material

### Extraction

2.5 grams of dried plant powder was suspended in 50ml (Hot distilled water, cold distilled water, Acetone, Methanol) and the mixture was soaked for 24 hours. The suspended solid was filtered through Whatman No.1 filter paper and kept in bath water at 80˚C for 2 hours. The dried crude extracts were stored at 4˚C for further use [4].

### Test Pathogens

The Strains of Clinical pathogens Gram negative (*E.coil, Pseudomonas sp., Klebsiella sp., Citrobacter sp.,)* Gram positive (*Staphylococcus sp*.,) collected from AWE CARE Analytical and Research Laboratory, Thindal, Erode.

### Confirmation Of Clinical Pathogen

### Selective Plate Method

The clinical pathogens were confirmed by selective plate method. *Escherichia coli* was streaked on EMB plate, *Staphylococcus* sp., on MSA plate, *Pseudomonas* sp., on Cetrimide agar plate & *Klebsiella* sp., and *Citrobacter* sp., on Macconkey agar plate.

### Biochemical Analysis

Clinical pathogens were characterized by using Bergey's manual of systematic bacteriology.

## ANTIBACTERIAL ACTIVITY OF PLANT EXTRACT

### Agar well diffusion technique [9]

The four plant extracts were tested for antibacterial activity by the standard agar well- diffusion method against clinical pathogen bacteria.

## COMBINATION OF ALL PLANTS

Based on the antibacterial activity, hot aqueous from all plants was selected for the combination. The plant Aqueous extracts was added at 4 different concentrations as given in the following table & named as A, B, C, D.

| Plant | A | B | C | D |
|---|---|---|---|---|
| *Azadirachta indica* | 125µl | 50µl | 150µl | 50µl |
| *Eucalyptus teriticornis* | 125µl | 200µl | 150µl | 150µl |
| *Coleus amboinicus* | 125µl | 50µl | 50µl | 150µl |
| *Mentha spicata* | 125µl | 200µl | 150µl | 150µl |

**Figure: 1**

### Antibacterial Activity For Combination [9]

Antimicrobial activity of 4 different concentrations were determined against pathogens *E.coli, Pseudomonas sp., Klebsiella sp., Citrobacter sp., Staphylococcus sp.,* by agar well diffusion Technique.

## MIC FOR COMBINATION AND PLANTS

Minimum inhibition concentration was done to determine the lowest concentration of extract, where it can show the bactericidal and bacteriostatic effect.

## FORMULATION OF HAND SANITIZER [10]

The formulation of hand sanitizer is prepared by adding all ingredients with sterile tools. The carbopol was added in 0.5 gram hot aqueous (70°C) until completely dissolved. Then the best combination of extract was added with 7.5 ml of Glycerin into the carbopol solution, stirred until homogeneous. To the homogenated solution, the remaining water was added.

## PHYSICAL EVALUATION OF HERBAL SANITIZER GEL

The nature, color, pH, odour, texture of the dried powder in the combined form was checked manually.

## SPREADABILITY TEST [2]

The spreadability test of the gel preparations was carried out by weighing 0.5 grams of the gel, which was placed in the middle of a petridish spiked with millimeter block of paper, then put in another petridish for 1 minute. Then the average diameter was measured. Then add 50– 200 grams of weight, each left for 1 minute, and calculate the average diameter. Replication was carried out three times.

## COMPARATIVE STUDY (Agar well diffusion technique) [9]

The herbal sanitizer and commercial sanitizer were tested for antibacterial activity by bacteria well-diffusion method against clinical pathogen bacteria. The pure cultures of bacterial pathogens

were subcultured in nutrient broth; 20 ml of nutrient agar were poured into petriplates. The cultures were swabbed uniformly using sterile cotton swab. Using gel puncture, 6 mm diameter wells were created on nutrient agar, and 100µl of plant extract solution was subsequently added to each well. After incubation at 37ºC for 24 hours, the inhibition was measured.

### Stability Test [8]

The Prepared herbal sanitizer was stored at room temperature (37ºC) and prepared (4°C) for 10 days. pH, Texture, Odour, Color Parameters were checked on 1st, 3rd, 5th, 7th &10th day.

## RESULT

### Confirmation Of The Microorganism

The 5 strains of clinical pathogens were collected and confirmed by a selective plate method that showed results of metallic green colonies seen on the EMB plate indicate *E. coli* colored green colored colonies on the MSA plate indicate *Staphylococcus* sp, yellow colored colonies on cetrimide agar plate indicate *Pseudomonas* sp, pink mucoid colonies on MacConkey agar plate indicates *Klebsiella* sp and *Citrobacter* sp pink Colored Colonies. According to Bergey's manual of system, all the clinical strains were analyzed by biochemical characterization for further confirmation.

### Isolation And Identification Of Pathogens

The 5 different strains of clinical pathogens were isolated from clinical samples. All the strains were identified as *Klebsiella* sp, *Staphylococcus* sp, *Pseudomonas* sp, *E.coli*, and *Citrobacte*r sp, based on morphological and biochemical characters.

### Antibacterial Activity

The hot aqueous extract of *Azadirachta indica* showed the maximum zone of inhibition against pathogens ranging from *E.coli* (17mm), *Pseudomonas* sp., (10mm), *Citrobacter* sp., (22mm), *Klebsiella* sp., (15mm), *Staphylococcus* sp.,(10mm). The hot aqueous extract of *Eucalyptus teriticornis* showed maximum zone of inhibition against pathogens ranging as *E.coli* (15mm), *Pseudomonas* sp., (20mm), *Citrobacter* sp., (25mm), *Klebsiella* sp.., (20mm), *Staphlococcus* sp., (26mm). The hot aqueous extract of *Coleus amboinicus* showed a maximum zone of inhibition against pathogens ranging from *E.coli* (16mm), *Pseudomonas* sp., (16mm), *Klebsiella* sp.,(17mm), *Staphylococcus* sp., (12mm). The hot aqueous extract of *Mentha spicata* showed a maximum zone of inhibition against pathogens ranging from *E.coli* (20mm), *Pseudomonas* sp., (11mm), *Citrobacter* sp., (25mm), *Klebsilla* sp., (25mm), *Staphylococcus* sp.,(30mm).

### Antibacterial Activity Of Plant Extracts Combination

The Hot Aqueous extract of A showed the maximum zone of inhibition against pathogens ranged as *E.coli* (11mm), *Pseudomonas* sp., (15mm), *Klebsilla* sp., (15mm), *Citrobacter* sp.,(14mm), *Staphylococcu*s sp., (16mm) and Sp.,. The hot aqueous extract of B showed the maximum zone of inhibition against pathogens ranged as *E.coli* (15mm), *Pseudomanas* sp., (17mm), *Klebsilla s*p., (10mm), *Citrobacter* sp., (15mm), *Staphylococcus* sp., (15mm). The hot aqueous extract of C showed a maximum zone of inhibition against pathogens ranging as *E.coli* (15mm), *Pseudomonas* sp.,(11mm), *Klebsiella* sp., (15mm), *Citrobacter* sp.,(12mm), *Staphylococcus* sp., (15mm) . The hot aqueous extract of D showed a maximum zone of inhibition against pathogens ranging from *E.coli* (13mm), *Pseudomonas* sp., (10mm), *Klebsiella* sp., (13mm), *Citrobacter* sp., (15mm), *Staphylococcus* sp., (16mm).

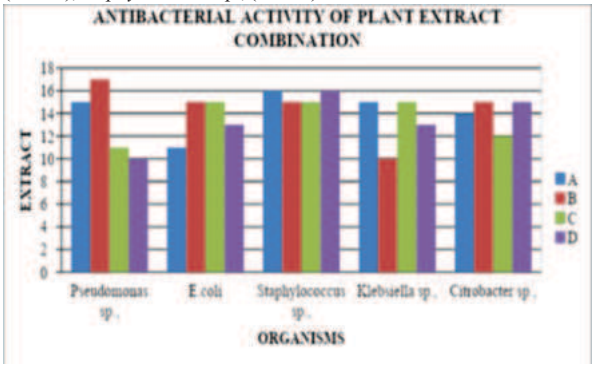


**Figure: 2**

### MIC Of Plant Extract Combination (A, B, C, D)

The Hot Aqueous extract of A showed MIC value of strains like E.coli (125mg/ml), Pseudomonas sp.,(125mg/ml), Citrobacter sp.,(62.5mg/ml), Klebsiella sp.,(125mg/ml), Staphylococcus sp.,(125mg/ml). The hot aqueous extract of B showed MIC value of strains like E.coli (125mg/ml), Pseudomonas sp.,(31.25mg/ml), Citrobacter sp.,(125mg/ml), Klebsilla sp.,(125mg/ml), Staphylococcus sp.,(125mg/ml). The Hot Aqueous extract of C showed MIC value of strains like E.coli (500mg/ml), Pseudomonas sp., (125mg/ml), Citrobacter sp., (250mg/ml), Klebsiella sp.,(250mg/ml), Staphylococcus sp.,(250mg/ml). The Hot Aqueous extract of D showed MIC value of strains like E.coli (62.5mg/ml), Pseudomonas sp.,,Klebsiella sp., and Staphylococcus sp.,(125mg/ml), Citrobacter sp.,(250mg/ml),

### Formulation And Characterization Of Poly Herbal Hand Sanitizer

The herbal sanitizer was yellowish brown, gave it a smooth feel and transparent. PH also maintained throughout the study, was found to be 6 and 6.2.

## COMPARATIVE STUDY

The commercial sanitizer showed a maximum zone of inhibition against pathogens ranging from *E.coli* (10mm), *Pseudomonas* sp., (10mm), *Citrobacter* sp., (9mm), *Klebsilla* sp., (9mm), *Staphylococcus* sp., (10mm). The hand sanitizer showed the maximum zone of inhibition against pathogens ranged as *E.coli* (13mm), *Pseudomonas* sp., (11mm), *Citrobacter* sp., (10mm), *Klebsilla* sp., (10mm), *Staphylococcus* sp., (11mm).

| S. No | ISOLATES | Zone of inhibition mm | |
|---|---|---|---|
| | | Herbal sanitizer | Commercial sanitizer |
| 1. | *E.coli* | 13 | 10 |
| 2. | *Pseudomonas sp.,* | 11 | 10 |
| 3. | *Klebsiella sp.,* | 10 | 9 |
| 4. | *Citrobacter sp.,* | 10 | 9 |
| 5. | *Staphylococcus sp.,* | 11 | 10 |

**Figure: 3** Antibacterial Activity For Sanitizer Compared With Commercial Sanitizer

### Stability Test

The stability of hand sanitizer gel on 1st, 3rd, 5th, 7th and 10th days was observed.

## CONCLUSION

The microorganisms commonly present in hands were isolated and identified as *Escherichia coli*, *Staphylococcus* sp., *Klebsiella* sp., *Citrobacter* sp., and *Pseudomonas* sp. They were used to determine the phenotypic characterization. The *Azadirachta indica, Mentha spicata, Coleus amboinicus* and *Eucalyptus teriticornis* were used for the preparation of hot aqueous, cold aqueous, methanol, and acetone extraction for further study. Antibacterial activity of hot aqueous, cold aqueous, methanol and acetone were studied. Then the plant extracts were made into combination and checked for its antimicrobial activity and MIC. From that, the better combination was made to formulate a hand sanitizer. The antibacterial activity of the Formulated Hand sanitizer was checked, which shows equal activity with handicapping.

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