Phytochemical and Antibacterial Evaluation of Formulated Hand Sanitizer from different Natural Sources and Waste materials.

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AIM OF THE PROJECT: Formulation of A Hand Sanitizer From Different Natural Sources And Waste Materials along with their Phytochemical and antibacterial Analysis.

PROCEDURAL DETAILS:
Material and Method
Sample Collection: Lemon grass (Cymbopogen citratus) was collected from the local market, Kalyan. Lemon peels (Citrus limon) and Custard Apple seeds (Annona squamosa) were collected from local juice shops, Kalyan. All the materials were washed thoroughly with tap water and then were dried for several hours to remove the moisture completely. All the materials were powdered separately in a blender. Aloe Vera was obtained from household plant.

Extract preparation:
The extract was prepared by Hot water Extraction method. 20 gm of all the powdered materials were soaked in Acetone for 24 hours. It was then filtered out using Whatmann’s Filter paper no.1 into the pre-weighed beaker. The filtered extract/Supernatant was then evaporated using boiling water bath technique and the remaining pellet obtained was dried and weighed. The dried pellet was regenerated using acetone and the volume was made upto 20 ml. This extracts were used for phytochemical analysis and antimicrobial tests. It was stored in refrigerator was further use (Kalaivani et al., 2018).

Phytochemical analysis:
Phytochemical screening refers to the extraction, screening and identification of the medically active substances found in plants. Phytochemical are naturally present in plants and shows biological significance by playing an essential role in the plants to defend themselves against various pathogenic microbes by showing antimicrobial activity or killing mechanism. The following test were carried out to check the phytochemical constituents present in the plant extracts (Bansode and Salalkar, 2015).

1. Test for Flavonoids:
   1. Few drops of 10% Lead Acetate was added to 1ml of extract
   2. Formation of yellow colored precipitate confirms the presence of Flavonoids.

2. Test for Reducing Sugar
   1. Molisch Reagent was added to 1 ml of extract and kept in BWB for 5 minutes. Concentrated H₂SO₄ was added from the corner of tube.
   2. Formation of ring indicates the presence of Reducing Sugar

3. Test for Saponins :
   1. 20 ml of Distilled Water was added to 1ml of extract .Shake for 15 minutes in Gradient cylinder
   2. Formation of a layer of foam of 1 cm indicates presence of Saponins.

4. Test for Tannins :
   1. 1ml of 5% FeCl₃ was added to the extract
   2. Formation of greenish black precipitate indicates the presence of Tannins.

5. Test for Potassium Dichromate :
   1. 0.5ml of 10% K₂Cr₂O₇ was added to the 2ml of extract
   2. Formation of yellow precipitate indicates the presence of Potassium Dichromate.

6. Test for Protein :
   1. 4-5 drops of concentrated Nitric acid was added to the extract.
   2. Yellow Coloration indicates the presence of Proteins.

7. Test for Glycosides:
   1. 3 ml chloroform was added to 1ml extract and 10% Ammonium solution was added.
   2. Pink Coloration indicates the presence of Glycosides.

8. Test for Terpenoids :
   1. 2 ml chloroform was added to 1ml extract and Concentrated H₂SO₄ was added.
   2. Red Brown coloration indicates the presence of Terpenoids .

9. Test for Quinones:
   1. 1 ml concentrated H₂SO₄ was added to the extract.
   2. Red coloration indicates the presence of Quiniones

10. Test for Coumarins :
    1. 1ml of 10% NaOH was added to 1 ml of extract.
    2. Yellow coloration indicates the presence of Coumarins.

11. Test for Phenols :
    1. 2 ml distilled water was added to 1 ml of extract. Few drops of 10% Ferric chloride was added.
    2. Blue/Green coloration indicates the presence of Phenols.

12. Test for Steroids :
    1. 2 ml chloroform was added to 1 ml of extract. 1 ml of concentrated H₂SO₄ was added from the corner of tube.
    2. Reddish Brown ring at interface indicates the presence of steroids.

13. Test for Anthocyanin and Betacyanin :
    1. 1ml of 2 N NaOH was added to 1 ml of extract. Heat it for 5 minutes at 100°C (Boiling water bath)
    2. Bluish Green coloration indicates the presence of Anthocyanin. Yellow coloration indicates the presence of Betacyanin.

Quantitative test for Tannins, reducing sugars and Phenols:
The quantitative analysis showed the presence of Tannins (Folins-Ciocalteau Method), Reducing Sugars (DNSA Method) and Phenols (Folins-Ciocalteau Method). Quantitative analysis for these phytoconstituents was estimated and the concentration was estimated (Bansode and Salalkar, 2015).
The concentrations for standards for Tannins (Tannic Acid), Reducing Sugars (Dextrose) and Phenols (Gallic acid) was 0.01 mg/ml, 1.0 mg/ml and 0.1 mg/ml respectively

**Antimicrobial evaluation of individual plant extract:**

The plant extracts were tested for their antimicrobial activity against organisms like (Escherichia coli (Ec), Klebsiella pneumoniae (Kp), Staphylococcus aureus (Sa), Corynebacterium diptheriae (Cd)). The cultures were collected from the Department of Biotechnology, B.K. Birla College, Kalyan. Sterile Nutrient agar plates were prepared by adding 1 ml of each plant extract into it. The plates were kept for 24 hours for sterility checking. 24 hr old culture suspensions of the bacterial cultures were spot inoculated on the Nutrient agar plates containing the plant extracts in a grid manner (Figure 1). The plates were incubated for 24 hours at 37°C in the incubator. Further zone of inhibition was checked for separate plant extracts w.r.t. different organisms (Stanley et al., 2017).

![Figure 1: Grid pattern for inoculation](Image)

**Preparation of Hand-Sanitizer**

All the plant extracts were mixed together in a ration of 1:1:1:2 (Lemon peels: Lemon grass: Custard Apple seeds: Aloe Vera) into the beaker under sterile conditions. Acetone was used as the base. The sanitizer was stored at room temperature in a bottle (Figure 2). Antimicrobial analysis of the hand sanitizer was tested against laboratory culture and hand flora (bacteria) from hand.

![Figure 2: Sanitizer](Image)

**Antimicrobial evaluation of Hand Sanitizer on laboratory culture and hand flora:**

1. **Laboratory Cultures**

The sanitizer was tested against the hand flora as well as the bacterial cultures (Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Corynebacterium diptheriae). Sterile Nutrient agar plates were prepared by adding 1 ml of sanitizer into it. 24 hr old culture suspensions of the bacterial cultures were spot inoculated on the Nutrient agar plates containing the sanitizer in a grid manner. The plates were incubated for 24 hours at 37°C in the incubator. Further zone of inhibition was checked for sanitizer w.r.t. different organisms (Stanley et al., 2017).

2. **Hand Flora**

Sterile Nutrient agar plates were prepared by adding 1 ml of sanitizer into it. The plates were kept for 24 hours for sterility checking. Sterile Cotton swabs were used to streak the hand flora onto the sterile Nutrient Agar plates. The hand was kept steady for 10 minutes after that cotton swab was rubbed on the hand and streaked onto the Nutrient Agar plate. Sanitizer was applied on the same hand and was kept steady for 10 minutes after which St. Cotton swab was rubbed on the hand and streaked onto the different St. Nutrient Agar plate. These plates were kept for 24 hours incubation at 37°C and were checked for the reduction in number of colonies before and after applying the hand sanitizer.

**INFEERENCE:**

The plant extracts were prepared in acetone and tested for the phytochemical constituents. The qualitative analysis showed the presence of Reducing sugars, Phenols, Steroids, Tannins, Betacyanins, Quinones, Terpenoids and Coumarins (Table 1). The Quantitative analysis of Tannins, Reducing sugars and Phenols was carried out. High amount of tannins were found in Lemon peels extract (2.75 mg/ml). High amount of Reducing sugars were found in Lemon grass extract (0.72 mg/ml). High amount of Phenols were found in Custard apple seeds extract (0.65 mg/ml) (Figure 3).

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<thead>
<tr>
<th></th>
<th>Lemon Peels</th>
<th>Lemon Grass</th>
<th>Custard Apple Seeds</th>
<th>Aloe Vera</th>
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<tr>
<td>Flavonoids</td>
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<td>Reducing Sugars</td>
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<td>K2Cr2O7</td>
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<td>Coumarins</td>
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![Figure 3: Concentrations of Phytoconstituents in Plant extracts](Image)

**Concentrations of Phytoconstituents in Plant extracts**

The plant extracts were tested for their antimicrobial activity against organisms like (Escherichia coli (Ec), Klebsiella pneumoniae (Kp), Staphylococcus aureus (Sa), Corynebacterium diptheriae (Cd)). Lemon peel extract showed antibacterial activity against the four bacterial cultures tested. Custard apple seeds extract showed antibacterial activity...
against *Escherichia coli*. Lemon grass extract showed antibacterial activity against *Klebsiella pneumonia* (Figure 4).

**Figure 4: Antimicrobial evaluation of plant extracts of (A) Lemon Peels, (B) Custard apple Seeds and (C) Lemon Grass**

**Antimicrobial evaluation of Hand Sanitizer on laboratory culture and hand flora**

1. **Laboratory culture**

   The sanitizer was tested against the hand flora as well as the bacterial cultures (*Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Corynebacterium diptheriae*). The hand sanitizer showed antibacterial activity against the four bacterial cultures (Figure 5).

**Figure 5: Antimicrobial evaluation of Hand Sanitizer on laboratory culture**

2. **Hand Flora**

   The effect of the hand sanitizer was tested on the hand flora. There was a visible decrease in the number of bacteria after the use of the hand sanitizer (Figure 6).

**Figure 6: Sanitizer against hand flora**

**CONCLUSION**:

Sanitizer was prepared using plant extracts of Lemon peels, lemon grass and custard apple seeds. The extracts were prepared by Hot water extraction method and checked for their phytochemical constituents. The qualitative analysis showed the presence of Reducing sugars, Phenols, Steroids, Tannins, Betacyanins, Quinones, Terpenoids and Coumarins. The quantitative analysis showed the high amount of Tannins in lemon peels, high amount of Reducing sugars in Lemon Grass and high amount of phenols in Custard Apple seeds. In antimicrobial evaluation Lemon peels and the Hand sanitizer showed antibacterial activity against all the four cultures (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Corynebacterium diptheriae*). Lemon grass extract showed antibacterial activity against *Klebsiella pneumonia*. Custard apple seeds extract showed antibacterial activity against *Escherichia coli*. The sanitizer was tested against the hand flora and there was a visible decrease in the number of colonies after the use of sanitizer.

**REFERENCES**: