EFFICACY OF PONGAMIA OIL AND HERBAL BASED FORMULATION SOAP AGAINST CONVENTIONAL - TO ENCOURAGE HYGIENE PRACTICE AMONG RURAL FOLK FOR ENTEROPATHOGENS.

Project Reference No.: 42S_B_MSC_020

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INTRODUCTION: Pongamia pinnata is a fast growing leguminous tree with potential for high oil seed production and the added benefit of the ability to grow on marginal lands. More recently, the effectiveness of P. pinnata as a source of biomedicines has been reported, specifically as both an antimicrobial agent and as a therapeutic agent targeting host pathways and processes. Occurrence of most of the infections is by hand to mouth inoculation of microorganisms leading to dysentery, diarrhoea, typhoid, cholera and others. In order to overcome this problem (especially among rural persons) posed by these microorganisms and reduce the intensity of infections, a herbal based formulation of soap made from pongamia oil and neem extract, was prepared. This would encourage rural folk to maintain hygienic practices thereby reduce the disease burden and through soap preparation enter into small scale entrepreneurship. The usage of this formulated soap would reduce microbial load transferred from intestine to mouth and thus health issues would be minimized. In this context, pongamia and neem based herbal products have wide range of applications industrially and possess several biological and pharmacological properties which are beneficial for hygienic practices among rural folk.

OBJECTIVES

1. Development of herbal (Neem extract) based soap with pongamia oil using formulation technique.
2. Isolation and characterization of (Biochemical) enteropathogens using hand scrub method from rural folk.
3. Optimization of herbal (Neem extract) based soap with pongamia oil based soap.

METHODOLOGY

Formulation of herbal based soap.
The basic saponification reaction between neutral fatty acid and alkali used to form soap. Hence, Pongamia oil as a neutral fatty acid and lye as an alkali (NaOH) has been used for the soap preparation. Eighteen grams of alkali (Sodium hydroxide) pellets were dissolved in a beaker containing 35 ml of ethanol and 35 ml of deionized water. About 140 ml of Pongamia oil was added to the mixture slowly with stirring under 60° C until it forms homogenous solution. Then the Neem extract was added (2g of extract/100g soap mixture) and mixed thoroughly. The formulated mixture was allowed to cool and filtered using Whatmann No.1 filter paper. Then the residue was washed with saturated NaCl solution to remove impurities and followed by acid wash using 10 ml of 0.1N HCl. Then formulated mixture was transferred into mould and allowed to solidify for 4 days (Afsar et al., 2016; Ruckmani et al., 2014).

Physicochemical parameters of the formulated soap.
Various physicochemical parameters which are mentioned below were performed to establish quality of the prepared formulations (Afsar et al., 2016). Determination of clarity, color and odour, pH, Determination of percentage free alkali, Moisture Content, Foam Height, Foam Retention, Alcohol Insoluble Matter, High Temperature Stability.

Antimicrobial efficacy of herbal formulated soap

- Total Microbial count
Sterile cotton swab was soaked in physiological saline (0.85%) which was used for collecting the sample among human subjects before and after hand wash (From Tholahunse & Kurki village, near Davangere University) and determined the total microbial plate count using pour plate method. For which one mL of the sample was transferred aseptically to sterile petri plate and later 15 ml molten liquefied soybean casein digest agar was poured (45°C) and later gently mixed on a flat surface clockwise and anticlockwise. Allowed the agar to set at room temperature and incubated at 37°C for 24 to 48 hours.

- Isolation of pathogenic bacteria.
The hand wash sample was inoculated on different selective media such as Mannitol salt agar (Staphylococcus aureus), Bismuth sulfate agar (Salmonella sp.), Eosin Methylene blue Agar (Escherichia. coli) and Cetrimide agar (Pseudomonas sp.). Later the
The sample was inoculated by spread plate method, incubated at 37°C for 24 hours and the colonies were chosen based on their characteristic properties. The isolated colonies were sub-cultured onto nutrient agar slants and pure cultures were maintained.

**Identification of pathogenic bacteria.**
The isolated colonies were identified by morphological studies using gram staining, motility and various biochemical tests such as catalase test, carbohydrates fermentation test (glucose, sucrose, and lactose), Indole test, Methyl red test, Voges-Proskauer test, Citrate utilization test, gelatin hydrolysis test and nitrate reduction test.

**Antibacterial activity of soap against isolated pathogens**

**Agar Well diffusion assay**
To determine the antimicrobial activity of neem extract and pongamia oil based herbal soap against the pathogenic bacteria isolated from hand wash sample, agar well diffusion assay method was used. The pathogenic isolates were inoculated into the nutrient broth and incubated at 37°C for 24 hours. Mueller Hinton Agar medium was autoclaved (121°C/15min/15 lbs/sq in), then poured into sterilized petri plates, and allowed to solidify. The agar plates were inoculated by spread plate method with 0.1ml of 24 hour old broth culture of pathogenic bacterial isolates. Then 10 mm diameter wells were made using sterile cork borer. Different concentrations like 50 mg/ml, 100 mg/ml and 150mg/ml of soap solutions were added into the well. After overnight incubation at 37°C, the diameter of zone inhibition (including the diameter of the well) formed were measured and recorded.

**Turbidometric measurement.**
This method was based on comparison of intensity of light scattered by the sample under defined conditions with the intensity of light scattered by the solution. The higher intensity of scattered light, higher is the turbidity. Spectrophotometer was used to find the optical density. To 9 ml of autoclaved, cooled Nutrient broth, 100µL of 1X10^5 bacterial cells/ml inoculated according to Mc Farland standard. Later, 1ml of different concentrations of formulated soaps samples (50 mg, 100 mg, 150 mg) was added and incubated at 37°C for a time interval of 5 min, 10 min, 15 min and optical density (OD) was measured (590nm) (Bhat et al., 2011).

**RESULTS AND DISCUSSION**
The herbal soap preparation by general formulation technique was done and successfully completed.

**Physicochemical parameters of the formulated soap.**
Various physicochemical parameters of herbal soap were determined and the Physicochemical characters were recorded.

**Antimicrobial efficacy of Formulated herbal Soap.** Antimicrobial efficacy of formulated herbal soap was determined by total bacterial plate count by collecting various hand scrub samples from human subjects before and after hand wash. The Formulated herbal soap exhibited antimicrobial activity with lowering the colony forming units (CFU) in samples after hand wash.

**Isolation and identification of pathogenic bacteria.** A total of four human pathogenic bacteria were isolated from different human subjects by hand scrub method, using selective media. The isolated bacteria were identified using morphological and biochemical test.

**Antibacterial activity of soap against the isolated pathogens.** The effect of soap on growth of different species of bacteria was measured by presence/ absence of clear inhibition zones by well diffusion method. All the bacterial isolates exhibited sensitivity to the test soap, the zone of inhibition was greater at maximum concentration and vice versa. Conventional soaps showed minimum inhibition zone formation compared to pongamia oil based soaps. Formulated herbal soap containing neem extract and pongamia oil showed greater inhibition zone against tested pathogenic organisms compared to conventional as well as Pongamia oil based soap.

**Turbidity analysis method:** The growth rate of pathogenic organisms in the broth medium varied with different concentrations of soaps at different time intervals were determined as turbidometric measurements. The results were in concordant with well diffusion method.

**OUTCOME OF THE PROJECT**
- Spread of awareness to control possible disease causing bacteria, particularly of their own intestinal origin.
- Encouragement to rural folk to grow more pongamia trees for biofuel production.
- Pongamia oil production and preparation of soaps and effectively used to maintain hygiene.

**APPLICATIONS OF THE PROJECT**
- **Health:** Maintenance of personal hygiene avoid number of infectious disease
- **Agriculture and small scale industry:** Encouragement to grow pongamia trees for water conservation and biofuel / oil / soap production. Particularly to encourage the small scale industry or home makers to develop the herbal soap.
- **Societal:** Particularly to women folk, encouragement to become self-employed with the development of soap using available herbal products.