



## Research Article

### FORMULATION AND EVALUATION OF POLY HERBAL SOAP AND HAND SANITIZER

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#### ABSTRACT

A poly herbal soap and hand sanitizer was formulated using the leaf and bark extracts of *Cassia fistula*, *Ficus religiosa* and *Milletia pinnata*. The antimicrobial efficacy of the prepared formulations was tested using agar well diffusion method against the organisms *E coli* (MTCC-1698), *S aureus* (MTCC-1143) and *P aeruginosa* (MTCC-2453). The prepared poly herbal formulations exhibited good antimicrobial effect. The prepared formulations were evaluated for various physicochemical parameters for which good characteristics were observed. Hence owing to good antimicrobial effect and acceptable parameters, the prepared formulations can be further standardized and used as effective antiseptics and disinfectants.

**Keywords:** *Cassia fistula*, *Ficus religiosa*, *Milletia pinnata*, Hand sanitizer, Zone of inhibition.

#### INTRODUCTION

Opportunistic microorganisms primarily cause nosocomial infections; and multidrug-resistant pathogens are commonly involved in nosocomial infections. Multidrug resistant infectious diseases of bacterial and fungal origin are leading killers and account for approximately 25% of global deaths and are difficult to treat.<sup>1</sup> The alarming rate at which the human pathogens like *Staphylococcus aureus* (Methicillin & Multiantibiotic resistant), *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Cryptococcus neoformans* are evolving themselves as multidrug resistant “Superbugs” towards the newly generated classes of antibiotics, demands for exploration of new chemical sources from biodiversity and develop therapeutic regimens to combat infectious superbugs.<sup>2</sup> Hand hygiene is a vital principle and exercise in the prevention, control, and reduction of healthcare acquired infections. Right hand washing and drying methods stop the chain of transmission of deadly pathogens (from the contaminated surface/site) from hands to other parts of the body. Hand sanitization is the preeminent aid in preventing nosocomial infections caused by different opportunistic microorganisms.<sup>3</sup> The hands of health care workers are the primary mode of transmission of these multidrug-resistant pathogens and infections to patients. Skin being the most exposed part of our body requires protection from skin pathogens. Hence it brings up the use of hand sanitizers and antiseptic soaps for hand wash purposes.<sup>3</sup> Many of chemical antiseptics available in the market are alcohol based sanitizers. These formulations including soaps and solutions reduce health care associated transmission of contagious diseases but they have some short comings or adverse effects, their frequent use can lead to skin irritation and also resistance among pathogens.<sup>3</sup> Plants are rich in a wide variety of secondary metabolites, such as phenolic compounds, tannins, terpenoids, alkaloids, and flavonoids, which have been found to have antimicrobial properties. Hence there is an upsurge of developing herbal disinfectants and evaluate its efficacy.<sup>4</sup> The chemical analysis of

different parts of *Cassia fistula* has been reported. It was found to contain Flavonoids, phenolic compounds, and proanthocyanidins.<sup>5</sup> Literature survey of this plant shows that it is reported to possess good antimicrobial properties.<sup>6, 7</sup> The chemical composition of *Milletia pinnata* was studied. It was reported to contain saponins, tannins, carbohydrates, alkaloids, sterols and flavonoidal glycosides.<sup>8</sup> The pharmacological properties of *Milletia pinnata* reported in literature also suggest it has good antimicrobial properties.<sup>9</sup> The phytochemical screening of the bark extracts of *Ficus religiosa* showed the presence tannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides.<sup>10</sup> The pharmacological properties of *Ficus religiosa* showed that the methanolic extracts showed to possess good antimicrobial properties.<sup>11</sup>

The aim of the present study was to prepare hand sanitizer and soap formulations using the extracts of *Cassia fistula*, *Milletia pinnata* and *Ficus religiosa* and to investigate the antimicrobial activity of the extracts against the common organisms which cause nosocomial infections. Furthermore to evaluate the stability and phytochemical parameters of the prepared formulations so that they can be further standardized and used commercially.

#### MATERIALS AND METHODS

##### Collection of samples

The plants *Cassia fistula*, *Milletia pinnata* and *Ficus religiosa* were collected from Mysore district, the specimen were authenticated at RRL, Bangalore.

##### Preparation of extracts

The leaves and bark of *Cassia fistula*, *Milletia pinnata* and *Ficus religiosa* were dried in hot air oven at 35°C for three days, powdered to a mesh size of # 40 and stored in air tight

containers. The powder was then extracted successively by refluxation for eight hours using five different solvents with increasing polarity. Viz: - Petroleum ether, Chloroform, Ethyl acetate, Methanol, and 40% methanol.

#### Preliminary antimicrobial screening of the extracts

All the extracts of leaf and bark were subjected to preliminary antimicrobial screening by agar well diffusion method against the organisms *E coli* (MTCC-1698), *S aureus* (MTCC-1143) and *P aeruginosa* (MTCC-2453). The extracts which exhibited maximum activity were selected for the formulation.

#### Preparation of formulations

Three extracts that exhibited maximum antimicrobial activity were prepared in combinations in two different concentrations i.e. 250 mg each (750 mg) and 500 mg each (1500 mg) and these combinations of extracts were incorporated in the prepared formulations.

#### Herbal soap

Solidified basic glycerine soap was broken down to smaller pieces and melted on water bath. 1.5grams of the extract combinations were added to the melted soap along with 5ml of ethanol. 0.033 g of stearic acid, 1ml each of cinnamon oil and citronella oil was added to the melted soap. The melted soap was gently mixed for about 30 minutes and moulded in circular moulds. The soap was allowed to solidify at room temperature until set and kept under physical observation for any characteristic changes.

#### Preparation of Hand sanitizer

The hand sanitizer was prepared using the following formula.

Ingredients	Quantity taken (10ml)
Extract combination	0.75 g
Citronella oil	1.0 ml
Cinnamon oil	1.0 ml
Carbopol	0.1 g
Triethanolamine	0.1 g
Glycerine	0.5 ml
Polysorbate-20	0.1 ml
Perfume	Qs
Methyl paraben	0.1 mg
Alcohol	4.0 ml
Water	2.0 ml

#### Procedure

The extract combinations were added in water and all the ingredients were added and stirred well except triethanolamine, alcohol and perfume. Citronella oil and cinnamon oil were added to triethanolamine along with perfume stirred well and both the contents were mixed together thoroughly and the volume was made up using alcohol.

#### Evaluation of physicochemical parameters of the prepared formulations

Various physicochemical parameters which are mentioned below were performed to establish quality of the prepared formulations.

**Determination of clarity, color and odor:** Clarity and color was checked by naked eyes against white background, the odor was smelled.

**pH:** The pH of all the prepared formulations was determined by using Digital pH Meter. The formulations were dissolved in 100 ml of distilled water and stored for two hours. The measurement of pH of formulation was done in previously calibrated ph meter.

**Determination of percentage free alkali:** About 5 gm of sample was taken in a conical flask and added to it into 50 ml of neutralized alcohol. It was boiled under reflux on a water bath for 30 minutes, cooled and 1 ml of phenolphthalein solution was added. It was then titrated immediately with 0.1N HCL.

**Foam Height:** 0.5gm of sample of soap was taken, dispersed in 25 ml distilled water. Then, transferred it into 100 ml measuring cylinder; volume was make up to 50 ml with water. 25 strokes were given and stand till aqueous volume measured upto 50 ml and measured the foam height, above the aqueous volume.

**Foam Retention:** 25 ml of the 1% soap solution was taken into a 100ml graduated measuring cylinder. The cylinder was covered with hand and shaken 10 times. The volume of foam at 1- minute intervals for 4 minutes was recorded.

**Alcohol Insoluble Matter:** 5 gm of sample was taken in a conical flask. Added it to 50 ml of warm ethanol and shaken vigorously to dissolved The solution was filtered through a tarred filter paper with 20 ml warm ethanol and dried it at 105°C for 1 hour. The weight of dried paper was taken.

#### Formula

$$\% \text{ alcohol insoluble matter} = \frac{\text{Wt. of residue} \times 100}{\text{Wt. of sample}}$$

**High Temperature Stability:** Liquid soap was allowed to stand at 50°C for one week. The stability of liquid soap was observed during this period. The sample which was homogeneous and stable liquid after standing was indicated as stable and the sample in which the crystals were roughened and the sample in which precipitation was caused; then liquid was said to be as unstable.

#### Antimicrobial testing of the prepared formulations

The prepared soap and hand sanitizer were subjected to antimicrobial screening by agar well diffusion method. Organisms used were *E coli* (MTCC-1698), *S aureus* (MTCC-1143) and *P aeruginosa* (MTCC-2453). One gram of soap was mixed with 5 ml of sterile water; 1ml of sanitizer was mixed with 5 ml DMSO and used for evaluating the antimicrobial activities. The plates were incubated at 37°C for 24 hours and the zones of inhibition were recorded.

## RESULTS

#### Preliminary antimicrobial screening of the extracts

The initial susceptibility testing of the different extracts of leaves and stem bark of *Cassia fistula*, *Milletia pinnata* and *Ficus religiosa* was done by using agar diffusion method. The stem bark extracts of *Cassia fistula* showed considerable antimicrobial activity in terms of zones of inhibition. The extracts of leaves showed no inhibition zones. The leaves and bark of *Milletia pinnata* and *Ficus religiosa* also showed significant zones of inhibition. The observations are recorded in **Table 1**.

**Table 1: Zones of inhibition (mm) of leaf and bark extracts of *Cassia fistula*, *Milletia pinnata* and *Ficus religiosa***

Extracts	<i>E coli</i>	<i>S aureus</i>	<i>P aeruginosa</i>
CB-PET	8.0	6.0	16.0
CB-CHL	14.0		16.0
CB-ETH	18.0	18.0	16.0
CB-MOH	20.0	18.0	20.0
CB-40MOH	12.0	8.0	6.0
CL-PET	---	---	---
CL-CHL	---	---	---
CL-ETH	---	---	---
CL-MOH	---	---	---
CL-40MOH	---	---	---
MB-PET	6.0	10.0	12.0
MB-CHL	12.0	8.0	16.0
MB-ETH	16.0	12.0	16.0
MB-MOH	18.0	14.0	18.0
MB-40MOH	12.0	10.0	16.0
ML-PET	12.0		8.0
ML-CHL	4.0	---	6.0
ML-ETH	8.0	---	8.0
ML-MOH	6.0	---	10.0
ML-40MOH	6.0	---	12.0
FL-PET	---	---	---
FL-CHL	---	---	---
FL-ETH	---	---	---
FL-MEOH	8.0	14.0	10.0
FL-40MOH	15.0	---	17.0
FB-PET	---	---	---
FB-CHL	---	---	---
FB-ETH	18.0	16.0	16.0
FB-MEOH	10.0	---	12.0
FB-40MOH	---	8.0	---

Note: CB= *Cassia fistula* bark, CL= *Cassia fistula* leaf, MB= *Milletia pinnata* bark, ML= *Milletia pinnata* leaf, FL= *Ficus religiosa* leaf, FB= *Ficus religiosa* bark, PET= Petroleum ether, CHL= Chloroform, ETH= Ethyl acetate, MOH= Methanol, 40MOH= 40% Methanol. --- indicates no activity.

**Preparation and evaluation of physicochemical parameters of formulations**

The physicochemical parameters of the prepared soap and hand sanitizer were determined. Parameters such as color, odour, appearance, pH were tested. The formulations exhibited good

appearance characteristics as well as the pH was found in the range of 6.5 to 7.5 which is the desired pH. Other parameters such as percentage free alkalie, foam height, foam retention, alcohol insoluble matter, test for chlorides and high temperature stability were determined; the results are tabulated in **Table 2**.

**Table 2: Physicochemical parameters of hand sanitizer and soap formulations**

Formulation	Color	Odour	Appearance	pH	% free alkalie	Foam height (cm)	Foam retention (min)	Alcohol insoluble matter	High temperature stability
Hand sanitizer	Cream-brown	Aromatic	Good	7.5	0.15	10	2.5	4.5	Good
Soap	Light brown	Fragrant	Good	7.0	0.27	27	6.0	18.0	Soap melts above 60°C

**Antimicrobial screening of the prepared formulations**

The extracts that exhibited maximum antimicrobial activity were ethyl acetate bark extracts of *Cassia fistula*, *Ficus religiosa* and methanolic bark extracts of *Cassia fistula* and *Milletia pinnata* with zones of inhibition ranging from 14 to 20 mm. Hence these

three extracts were prepared in combinations and incorporated in formulations in two different concentrations i.e. 250 mg each (750 mg) and 500 mg each (1500 mg). Both the formulations exhibited good zones of inhibition ranging from 18 to 26 mm. The results are tabulated in **Table 3**.

**Table 3: Antimicrobial screening of the prepared formulations**

Sl no	Formulation	Zones of inhibition in mm		
		<i>E coli</i>	<i>S aureus</i>	<i>P aeruginosa</i>
01	HS-1	18.0	20.0	22.0
02	HS-2	26.0	22.0	24.0
03	SP-1	20.0	18.0	20.0
04	SP-2	22.0	24.0	22.0

Note: HS-1 = Hand sanitizer with 750 mg concentration of extracts, HS-2 = Hand sanitizer with 1500 mg concentration of extracts, SP-1 = Soap with 750 mg concentration, SP- = Soap with 1500 mg concentration.

## DISCUSSION AND CONCLUSION

The plants *Cassia fistula*, *Milletia pinnata* and *Ficus religiosa* were extracted using four different solvents of increasing polarity and the extracts were subjected to antimicrobial screening. Results revealed that most of the extracts exhibited good antimicrobial effect among which the ethyl acetate bark extracts of *Cassia fistula* and *Ficus religiosa* and methanolic bark extracts of and *Milletia pinnata* and *Cassia fistula* exhibited maximum activity with zones of inhibition ranging from 14 to 18 mm. This is in accordance with the antimicrobial activities of these plants listed in the literature<sup>6,7,9,11</sup>. Furthermore those extracts exhibiting maximum activity were selected and their combinations were included in our prepared soap and hand sanitizer formulations. The prepared formulations when tested for antimicrobial activity exhibited zones of inhibition ranging from 18 to 26 mm which was far better than the zones of inhibition of individual extracts. This enhancement of antimicrobial properties may be attributed to the synergistic effect or total sum of effects produced by the combinations of extracts. Furthermore the prepared soap and hand sanitizer formulations were standardized by evaluating various physico chemical properties such as pH, spreadability, appearance, extrudability, high temperature stability, in which they exhibited satisfactory characters. However these formulations need to be further standardized as good antiseptics and disinfectants.

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