



# Formulation of Soaps by mixing varying proportions of Non-Edible Oils and investigation of their Antimicrobial activity on different microbes

Juzer Ali Rangwala\* and Geetha Sarasan

Department of Chemistry, Govt. Holkar Science College, Indore, M.P., India  
jzr.rgw@gmail.com

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## Abstract

*In this investigation, different samples of soap were prepared by mixing non-edible Jatropha Oil with non-refined Cottonseed oil in varying proportions. Spectroscopic Technique like Infrared spectroscopy method was used to investigate the molecular structure of the synthesized product. Different parameters related to the quality evaluation of soap, such as soap yield, moisture content (%), pH, foam ability, etc., were determined using standard protocols. The alkalinity of Soap samples was expressed in terms of Phenolphthalein alkalinity, and Total Alkalinity (in terms of % of carbonate) was determined using the pH meter titration method. To study the medicinal efficacy of the derived product, three different microbes, Staphylococcus aureus, Salmonella enterica and Candida albicans were chosen as test organisms, and the antimicrobial activity of soap solutions at different dilutions was evaluated by the Muller-Hinton agar Plate method. Soaps made from non-edible oils have good efficacy, and these soaps can be used to produce cosmetic formulations, household cleansing agents and antibacterial/antifungal solutions valuable in the pharmaceutical sector.*

**Keywords:** Jatropha Oil, Cottonseed Oil Salmonella, Candida albicans, Liquid Cleansers.

## Introduction

Soaps are anionic surfactants used as cleansing agents in daily life and are usually derived from animal fat such as beef tallow, lard or edible oils of plant seeds such as coconut (palm), olive oil, Soybean Oil etc. However, with the growing population, there is a huge gap in demand and supply of food items in several developing or developed countries, due to which prices of food commodities are continuously increasing; hence, sustainable alternatives such as non-edible oils seem a viable option for making soaps and surfactants. With the increasing awareness about animal cruelty, several people demand skin care products of plant/herbal origin that are free from animal fat or other kinds of derivatives obtained from animal body parts. Especially in a country like India, where a large number of people observe vegetarianism and advocate a non-violent mode of lifestyle, the production of soaps from plant-based material is a quintessential demand<sup>1-6</sup>.

The Indian economy is agriculture-based, and the majority of the population in India depends on agricultural production to earn their livelihood. Jatropha plant is hardy in nature, and it can be cultivated in semi-arid regions, easily producing a large number of oleaginous seeds. Oils obtained from Plants such as Jatropha are non-edible in nature due to the presence of certain poisonous esters, which harm organ systems. Jatropha Oil is primarily used for the production of biodiesel, but besides fuel production, crude Jatropha oil is suitable for making soaps of medical efficacy because phytochemical investigation on Jatropha oil has revealed broad-spectrum antimicrobial activity<sup>7</sup>.

Besides, Jatropha crude cottonseed oil obtained after separating lint from cotton seeds is a suitable raw material for making soaps. Crude oil is not suitable for human consumption because it contains Gossypol, a toxic compound that can cause multiple organ failure if consumed in large excess. Both Jatropha and Cottonseed Oil have saponification value in the range of 180-200 mg KOH/g, which makes them a suitable raw material for saponification reaction<sup>8,9</sup>.

## Materials and Methods

**Saponification of Jatropha and Cottonseed oil<sup>10</sup>:** Jatropha Oil was purchased from a dealer in Madhya Pradesh and non-refined Cottonseed oil obtained after crushing cottonseed kernels were collected from a local village of Dewas District in Madhya Pradesh. Sodium Hydroxide pellets (AR) were dissolved in distilled water to prepare concentrated lye solution equivalent to 40% w/v NaOH solution. Jatropha Oil and Cotton seed oil were filled in two separate clean beakers. Three separate oil mixtures were prepared by mixing Jatropha Oil and Cottonseed Oil in the ratio of 7:3, 5:5, 3:7 combining in total 50 g of oil mixture in each case. Before adding lye, each oil mixture was heated gently over a hot plate and hot lye solution was added with gradual stirring over the period of 25 to 30 minutes. Formation of thick paste indicates trace point. Beaker was removed from hot plate and left for cooling overnight. Next day crude soap was washed with small quantity of distilled water and soaps were transferred in petri dish and dried in hot air oven first at 70°C followed by 105°C for 1 hour so as to remove maximum quantity of water. Soft soap was left for

hardening for 7-10 days. Each soap was removed from Petri dish and weighed to record the yield.

**Estimation of Moisture Content in soap samples:** During the hardening period dry soap tends to absorb water vapours from air. In order to calculate the amount of humidity retained, percentage of moisture was determined in each soap sample by literature reported method. Each sample was dried in hot air oven at 105°C for 1-2 hours and hot silica crucible containing soap sample was immediately transferred in an air tight dessicator for cooling. Weight in loss on drying was recorded and moisture content was calculated in terms of % w/w.

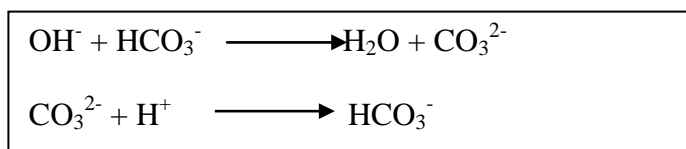
**Determination of melting point:** Melting Point of each soap sample (dried in hot air oven) was determined by using digital melting point apparatus.

**Determination of pH:** Dry soap samples were dissolved in boiling deionized water and diluted to the strength of 5% w/v. After cooling at 25°C, pH was checked with Universal indicator paper and well calibrated pH meter.

**Determination of foam height/foam ability:** 5 grams dry soap sample was added in 50 ml boiling distill water and stirred with glass rod till a clear solution was formed. This solution was cooled at room temperature and transferred in 100 ml graduated measuring cylinder slowly so as to avoid frothing. After plugging the stopper, each cylinder was shaken vigorously for 30 seconds and foam height was measured (in centimeters) with the help of marked ruler. After 10 minutes of frothing, foam height was measured again so as to check the persistence of foam height.

**Determination of Phenolphthalein Alkalinity in Aqueous Solution:** During Saponification lye was added in slight excess and reaction was carried out in the pH range of 12-12.5 so as to ensure complete conversion of fatty acids into sodium salt. Thus, each soap sample has some amount of unreacted alkali responsible for pH values above 9 in aqueous solution.

When soaps are dried in open air for hardening and curing purpose, unreacted sodium hydroxide tend to absorb CO<sub>2</sub> from air forming bicarbonate and carbonate. As we have not added any kind of fillers for the solidification of soap, in the present investigation a modified method was used to calculate the phenolphthalein alkalinity of soap. 50 ml deionized water is boiled in a borosil beaker so as to expel any Carbon-di-oxide gas dissolved in solvent. 1 gram dry sample was added and mixture was agitated till a clear solution is formed. This solution was cooled and transferred in a 100 ml volumetric flask, after dilution up to standard mark, entire solution was titrated against standard 0.5 N H<sub>2</sub>SO<sub>4</sub> solution using Phenolphthalein indicator. (pH of solution at end point is around 8.3, this alkalinity represents partial neutralization of carbonate ions in aqueous phase).



**Determination of Total alkalinity of Soap sample by pH meter/potentiometric method:** Total alkalinity of Soap is defined as the total amount of alkaline elements present in soap samples. The value of Total Alkalinity is higher than that of Phenolphthalein alkalinity, as it includes alkalinity combined as a result of hydroxides, bicarbonates and carbonates. 1% w/v soap solution was titrated against 0.5 N H<sub>2</sub>SO<sub>4</sub> solution using a well-calibrated pH meter. Change in value of pH and EMF was noted after each addition of 0.5 ml H<sub>2</sub>SO<sub>4</sub>, and the equivalence point of titration was obtained after plotting a graph between pH v/s Volume of Titrant i.e. 0.5 N H<sub>2</sub>SO<sub>4</sub> and ratio of change in EMF and change in volume of titrant (ΔE/ΔV) v/s Volume of Titrant.

**Fourier Transform Infra Red (FTIR) Analysis<sup>11</sup>:** FTIR spectrum of dry soap sample (obtained by mixing Jatropha and Cottonseed oil in the ratio of 5:5) was recorded with the help of Bruker Spectrophotometer.

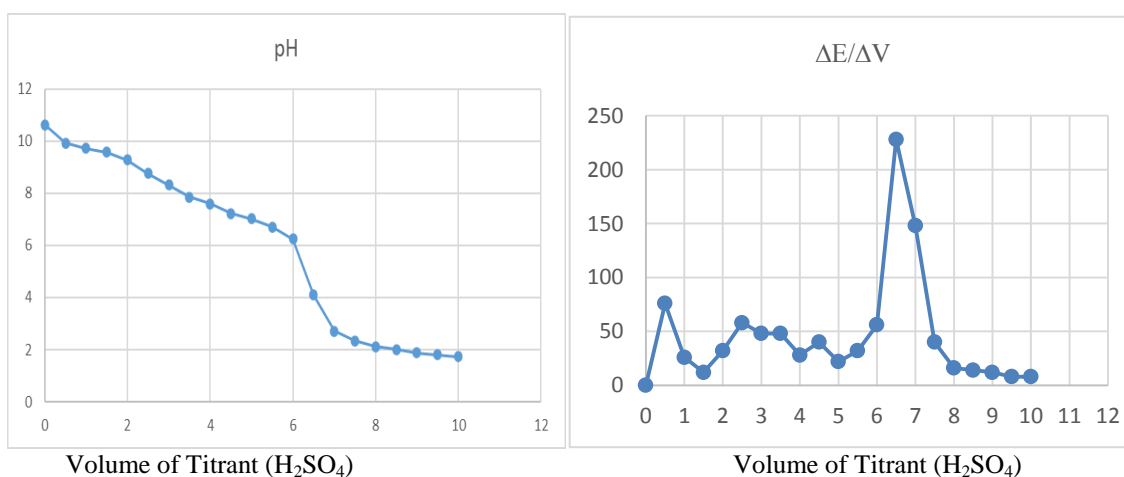


Figure-1: pH meter/potentiometric titration of total alkalinity of soap 5:5 J:CSO.

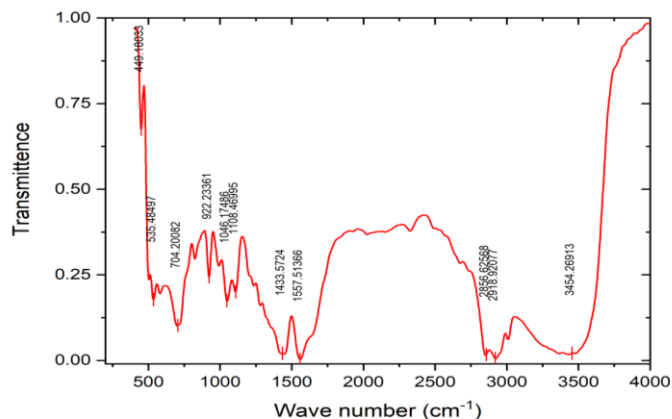


Figure-2: FTIR Spectrum of Soap sample.

**Determination of Antimicrobial activity of soap samples<sup>12-14</sup>:** Antimicrobial activity of prepared soap samples was studied on test organisms *Staphylococcus aureus*, *Salmonella enterica* and *Candida albicans*. Sample solutions were prepared by dissolving soap samples in sterile distilled water at a concentration of 100 mg/ml (10% w/v). Serial dilutions were prepared from the stock solution @ 5%, 2%, 1% w/v concentrations. *Staphylococcus aureus* & *Salmonella enterica* were cultured on Muller-Hinton Agar Plate whereas *Candida albicans* was cultured on Potato Dextrose Agar Plate. 20 µL of each sample was poured into well of 4mm diameters and bacterial plates were incubated at 37°C for 24 hours where as fungus for 5 days. Value of Zone of Inhibition was noted in mm for each sample.



Figure-3: Determination of Antimicrobial activity of Soap samples.

## Results and Discussion

In every case, % yield is calculated on the basis of only quantity of oil used during saponification. It excludes amount of lye consumed and moisture retained during soap solidification and hence in each case % yield is significantly higher than the theoretical value.

Table-1: % Yield of Soaps.

Type of soap (Jatropha: cotton seed) oil ratio	Input weight of oil (in g)	Output weight of soap obtained (in g)	% Yield [weight of soap/ weight of oil consumed] x 100
7:3	50.03	66.50	133 %
5:5	50.02	71.50	143 %
3:7	50.05	62.30	124.60 %

**% Moisture Content in Soap:** % Moisture =  $C_w - C_s / W_s \times 100$   
Where,  $C_w$  = Weight of Sample + Crucible (before drying),  $C_s$  = Weight of Sample + Crucible (after drying),  $W_s$  = Weight of soap sample before drying (in grams).

Table-2: % Moisture Content in Soap.

Type of soap (Jatropha: cotton seed) oil ratio	$C_w$ (in g)	$C_s$ (in g)	$W_s$ (in g)	% Moisture $([C_w - C_s] / W_s) \times 100$
7:3	70.0015	68.9622	5.1081	20.346 %
5:5	62.9461	61.8980	5.0875	20.601 %
3:7	80.0354	79.2730	5.2123	14.626 %

Table-3: Melting Point/pH/Foam Height of Soaps.

Type of soap (Jatropha: cotton seed) oil ratio	Melting point (in °C)	pH		Foam height (in cm)	
		Universal indicator	pH meter	Initial foam height	After 10 min.
7:3	90-95	9-10	10.05	13	10
5:5	90-95	9-10	10.32	15	12
3:7	85-90	9-10	9.53	14	12

**Calculation of Phenolphthalein Alkalinity in Soap samples:**

Volume of Titre (soap solution) x N<sub>p</sub> = Volume of Acid consumed x Normality of Acid

100 ml x N<sub>p</sub> = V<sub>H<sub>2</sub>SO<sub>4</sub></sub> x 0.493 (where, N<sub>p</sub> = Normality of Phenolphthalein Alkalinity).

In terms of CO<sub>3</sub><sup>2-</sup> equivalence

= N<sub>p</sub>x30 = X g CO<sub>3</sub><sup>2-</sup>/L= V<sub>H<sub>2</sub>SO<sub>4</sub></sub> x 0.493x 30/100

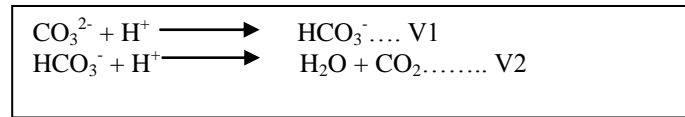
For 100 ml Soap solution containing W grams of soap

% of CO<sub>3</sub><sup>2-</sup> = X\*100 \*100/1000 \*W

% of CO<sub>3</sub><sup>2-</sup> = X\*10/W

**Calculation of Total Alkalinity in Soap samples by pH**

**meter/Potentiometric Titration:** From the analysis of pH v/s Volume of Titrant two inflexion points were observed, first V1 at pH around 8.3 which represents partial neutralization of Carbonate ions into bicarbonate ions and V2 at pH around 4 that represents complete neutralization of bicarbonate into H<sub>2</sub>O and CO<sub>2</sub>.



**Table-4:** Phenolphthalein Alkalinity in Soap samples (%).

Type of soap (Jatropha: cotton seed) oil ratio	Weight of soap sample (W) in g	Volume of acid consumed (in ml)	Xg CO <sub>3</sub> <sup>2-</sup> /L	% of CO <sub>3</sub> <sup>2-</sup>
7:3	1.0045	2.4	0.3549	3.53%
5:5	1.0070	2.8	0.4141	4.11 %
3:7	1.010	1.8	0.2662	2.64 %

Thus, total volume of acid consumed. i.e. V1 +V2 gives the total amount of alkaline substance in soap solution.

Volume of Titre (Soap Solution) x N<sub>B</sub> = Total Volume of Acid consumed x Normality of Acid

100 ml x N<sub>B</sub>=(V1+V2) x 0.493

Where: N<sub>B</sub> is total Normality of Base (in terms of carbonate).

**Table-5:** Total Alkalinity in Soap samples (in % carbonate).

Type of soap (Jatropha: cotton seed) oil ratio	Weight of soap sample (W) in g	First equivalence point (V1) in ml at pH 8.3	Second equivalence point (V2) in ml at pH 4.0	Total volume of acid consumed (V1+V2)	X g CO <sub>3</sub> <sup>2-</sup> /L [N <sub>B</sub> x 30]	% of CO <sub>3</sub> <sup>2-</sup> [X*10/W]
7:3	1.008	2.5	3.0	5.5	0.8134	8.07 %
5:5	1.004	3.0	3.5	6.5	0.9613	9.57 %
3:7	1.009	2	3	5	0.7395	7.33 %

**Table-6:** FTIR interpretation of Soap prepared by mixing Jatropha and Cottonseed oil.

Wave number (cm <sup>-1</sup> )	Functional Group	Compound	Remark
3454.27	O-H stretching	Water /Glycerol	Broad band due to intermolecular H-bonding in water and glycerol
2918.92	C-H stretching	Hydrocarbon chain	Anti-symmetric Stretching Vibration
2856.62	C-H stretching	Hydrocarbon chain	Symmetric Stretching Vibration
1557.51	C-O stretching	Carboxylate ion	Bond between carboxylate ion and sodium metal
1433.57	C-H bending	Alkane	Methylene group
1108.46	C-O Stretching	Ester	Triglyceride ester
704.20	--C-H- out of the plane Bending	Alkene	Disubstituted (cis)

**Table-7:** Results of *in vitro* antimicrobial activity (sensitivity or MIC) of the test sample 7:3 J: CSO at 4 different concentrations against test ATCC microbial cultures.

Test Organisms	Antimicrobial Efficacy as Zone of Inhibition (in mm) at different sample concentrations against			
	10 %	5%	2%	1%
<i>Staphylococcus aureus</i>	12	10	nil	nil
<i>Candida albicans</i>	12	nil	nil	nil
<i>Salmonella enterica</i>	nil	nil	nil	nil

**Table-8:** Results of *in vitro* antimicrobial activity (sensitivity or MIC) of the test sample 5:5 J:CSO at 4 different concentrations against test ATCC microbial cultures.

Test Organisms	Antimicrobial Efficacy as Zone of Inhibition (in mm) at different sample concentrations against			
	10 %	5%	2%	1%
<i>Staphylococcus aureus</i>	11	10	nil	nil
<i>Candida albicans</i>	14	11	nil	nil
<i>Salmonella enterica</i>	10	nil	nil	nil

**Table-9:** Results of *in vitro* antimicrobial activity (sensitivity or MIC) of the test sample 3:7 J:CSO at 4 different concentrations against test ATCC microbial cultures.

Test Organisms	Antimicrobial Efficacy as Zone of Inhibition (in mm) at different sample concentrations against			
	10 %	5%	2%	1%
<i>Staphylococcus aureus</i>	10	10	nil	nil
<i>Candida albicans</i>	10	nil	nil	nil
<i>Salmonella enterica</i>	10	10	nil	nil

**Discussion:** In the present investigation, the FTIR spectrum of soap (Figure-2) displays a sharp peak at  $1557\text{ cm}^{-1}$  because of the metal carboxylate bond. Saponification involves the hydrolysis of triglyceride esters in an alkaline medium, due to which the peak of C-O stretching in the ester bond shifts from its base value (around  $1750\text{ cm}^{-1}$ )<sup>2,15</sup>.

pH and Total Alkalinity of the prepared soap sample are slightly above the prescribed limit of BIS norms for toiletry soaps because, in the present investigation, alkalinity of soap has been determined in aqueous medium in terms of % of carbonate instead of hydroxide without involving the use of alcoholic solvents like ethanol. The modified titrimetric method is relatively easy to perform and doesn't require tedious steps like preparation of neutral alcohol and separation of fatty acids, as prepared soap samples don't contain any synthetic fillers like silicates, rosin, or borates. The titrimetric method, performed in deionized water with the aid of a pH meter, yields satisfactory results (Figure-1).

The foam height remains persistent even after 10 minutes of agitation, indicating the effective lathering property and cleansing power of the prepared surfactant materials. % of

Moisture content, % yield, and Antimicrobial activity are very satisfactory @ 10% w/v, and the prepared soap formulation in terms of pH is suitable for the development of laundry-based cleansing agents such as detergents (ideal range 6-11) or surface cleaners useful for cleaning surgical items, clothing, and cooking utensils. Soap having composition of Jatropha and Cottonseed Oil in the ratio of 50 percent each displays antimicrobial activity on *Candida albicans* a pathogenic yeast responsible for skin infection at the dilution of 5% as well as 10% w/v<sup>16</sup>.

Promising results on *Salmonella enterica* (Figure-3), a pathogenic bacterium responsible for transmission of Zoonotic Salmonellosis in humans from animals like fish, reptiles, birds, etc., show that surface cleansing agents from Jatropha and Cottonseed Oil that are water-soluble in nature can prove to be a good alternative and have the potential to substitute disinfectant compounds prepared from aromatic hydrocarbons and less eco-friendly in nature. These cleansing agents can be used for washing aquariums, reptile terrariums, bird enclosures, and more, without posing a risk of causing harmful side effects to animals or humans, thereby preventing the high risk of *Salmonella* transmission<sup>17-21</sup>.

Anion surfactants having long-chain fatty acids are biodegradable in nature and tend to be depleted by the process of aerobic respiration through bacteria or fungi living on organic substrates.

## Conclusion

Non-edible oil-based soaps are effective and can be used to make household cleaning products, cosmetic formulations, and antibacterial/antifungal treatments that are useful in the pharmaceutical industry. Further work on the improvisation of soap quality to make a skin-friendly product by adding fillers to reduce pH without altering the foaming capacity or antimicrobial activity, varying the combinations of different kinds of non-edible oils and studying biodegradability to assess suitability as an eco-friendly alternative to synthetic detergents<sup>22,23</sup>.

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