ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

SYNTHESIS AND ANTIMICROBIAL EVALUATION OF DIFFERENT TYPES OF SOAP SAMPLES FROM JATROPHA OIL

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ABSTRACT

Study on soaps made from Non Edible Oil such as Jatropha Oil revealed that these surfactants have bactericidal effects. In present research different soap samples (solid as well as liquid) were prepared by mixing lye solution with blend of Non Edible Jatropha oil with Castor oil and Coconut Oil. Essential oils like Tea tree Oil, Thyme oil, Peppermint and D-limonene were included in these soaps to improve their quality and impart pleasant fragrance. pH of final product was tested with the help of Universal indicator and pH meter and it was in the range of 7-9 which is under prescribed limit of BIS. Antimicrobial activities of all the soaps were determined quantitatively at concentration range of 50 ppm to 200 ppm by using Cup Borer method in nutrient agar medium. Test organisms selected were *Bacillius cereus, B. megaterium, Staphylococcus aureus*, *Eschericia coli, Pseudomonas aeruginosa*, *Aspergillus niger and Candida albicans*. Liquid Surfactants made by combining Jatropha oil and Essential oils have remarkable antibacterial activity on *Pseudomonas* and it could be developed as disinfectant to sterilize catheters and surgical equipments to control nosocomial infections in Hospitals. Study on *Aspergillus* confirms fungicidal activity of these surfactants. As these surfactants are biodegradable in nature they could be considered as a better eco friendly (green) alternative to harmful chemical fungicides which are used in agricultural fields and have several adverse effects on ecosystem. Solid as well as liquid surfactants from Jatropha oil have a potential to become significant product in Cosmetic, Pharmaceutical and Agrochemical sector.

Keywords: Jatropha Oil, Antimicrobial activity, Liquid soaps, Medicinal Soaps, Eco friendly Fungicide.

1. INTRODUCTION

Soaps, chemically defined as Sodium or Potassium salts of fatty acids have been a household material used for cleansing purposes. Ever since its invention, method of making soap has been modified by Chemists and still a huge amount of research is being conducted by Research and Development wing of Cosmetic industries to produce new variety of soaps not only with better efficacy but also from resources which are more economical and provide desirable properties. However, it has been observed that most of the production of soap relies on utilization of animal fats such as beef tallow or lard or edible oils such as palm oil, olive oil etc. but research on making of soap from non edible oils such as Jatropha Oil revealed that these soaps have antibacterial properties better than commercial soaps which are sold in market under various brand names [1]. So far very little effort has been done to explore pharmaceutical derivatives from Jatropha oil based surfactants [2-4]. In this research an attempt has been made to improve quality of Jatropha oil soap by mixing it with Castor oil [5, 6], Coconut oil and various essential oils of medicinal importance. Three different types of soap samples (including one transparent soap) were prepared by blending different ingredients with Jatropha oil. A sample of liquid soap was prepared by mixing Jatropha Oil with hot KOH solution. These soap samples were screened for antimicrobial activity against *Bacillius cereus, B.megaterium, E.coli, S.aureus, Candida albicans, Aspergillus niger, Pseudomonas aeruginosa.* Foremost aim of this study was to ascertain utility of Jatropha oil based surfactants in the production of medicinal soaps, disinfectants, hand sanitizers and agro chemical eco friendly products as an alternative to harmful chemical based fungicides.

2. MATERIAL AND METHODS

Jatropha Oil, Castor Oil and Coconut Oil was purchased from authentic dealers through e-portals. Essential Oils of high purity (as certified by quality analysis report provided by dealers) were obtained from

VDH organics. Chemical Reagents like NaOH, KOH, Ethanol, Glycerin etc were of Analytical Grade. Reagents required for preparation of nutrient media (Peptone, Sodium Chloride, Beef extract, Yeast Extract, Agar etc,) were of high quality as per protocol.

2.1. Preparation of Soap

2.1.1. Soap Type I

Jatropha oil, Castor oil and Coconut oil were mixed in the ratio of 4:1:1. This mixture was heated gently over hot plate (without boiling) and warm lye (40% NaOH) solution was added with continuous stirring till it reached trace point. Whole mixture was slightly heated to ensure complete Saponification and transferred in Silicone moulds. It was left undisturbed for next 7 days for hardening and curing purpose. After one week, soap was unmolded and spray washed 3-4 times with cold water so as to remove un-reacted lye and dried in open air. After drying, resultant soap bar was weighed. Around 25.37 gm of soap was obtained from 20 gm of Jatropha oil.

2.1.2. Soap Type II

Jatropha oil, Castor oil and Coconut oil were mixed in the ratio of 4:1:1. This type soap was prepared by "superfatting method" *i.e.* amount of oil was kept in slight excess as compared to amount of lye. This helps in reducing Alkalinity of Soap and minimizing its harsh effect on skin. After adding lye, reaction mass was cooled up to room temperature and 1-1.5 ml of Tea tree oil was added to mixture and whole mixture was stirred with glass rod to ensure homogenous mixing. Soap mixture was molded, hardened, washed and dried. Around 27.55 gm of soap was obtained from 20 gm of Jatropha oil.

2.1.3. Soap Type III

A transparent soap was made from Jatropha Oil. Again 20 gm of Jatropha oil was blended with Castor and Coconut oil in ratio of 4:1:1. After adding warm lye reaction mass was thoroughly mixed with the help of glass rod and boiled for 30 min. over hot water bath. During the period initial viscous mass gradually changed into a thick translucent paste. Saturated solution of glycerin dissolved in absolute Ethanol was added; on stirring a clear solution was formed. Beaker was covered with a plastic wrap and a watch glass was placed to avoid loss of ethanol during heating over a water bath, 50% Sugar solution was added and again mixture was boiled. Now a mixture of essential oils of medicinal importance *i.e.* Tea Tree Oil with D-Limonene, Thyme and Peppermint oil [7-10] was added to soap solution. Immediately a thick transparent gel like paste was formed which was transferred in silicone mould and allowed to dry gradually at room temperature. After hardening and curing for 10 days a semi transparent soap was obtained. After unmolding it was washed, dried and weighed. Around 34 gm of soap was obtained.

2.1.4. Soap Type IV

Sample of liquid soap was made by mixing 50 gm of Jatropha oil with Castor and Coconut oil in the ratio of 5:1:1. Lye Solution was made by dissolving KOH in Distilled water (~50%). After adding lye (in slight excess) to the mixture of oils it was stirred till it reached trace point. Then soap mixture was boiled for approximately 3 hours to ensure complete Saponification. After 3 hrs, a small quantity of test sample was withdrawn and it was added to boiling distilled water. A murky or translucent solution ensured complete Saponification. A viscous soap paste was formed with few solid chunks, this paste was diluted by adding hot boiling distilled water and large pieces of chunks were dissolved by breaking with glass rod and boiling the solution over water bath. After cooling upto room temperature a blend of essential oils (as in Soap Type III) was added and after mixing with glass rod liquid soap was left undisturbed overnight. pH of this soap was checked by using Universal indicator. Since highly alkaline Soap is harmful for skin a conc. Citric Acid Solution was added gradually and carefully with continuous stirring till soap attained pH~7 as indicated by Universal indicator paper. Excess of citric acid was avoided as it may lead to precipitation of fatty acids.

2.2. Determination of pH

Five (5) gm of dry soap was dissolved in hot boiling 100 ml distilled water and after cooling pH was tested by using Universal Indicator Paper and pH meter.

2.3. Determination of Antimicrobial activity

In total seven different test organisms were selected *i.e. Bacillius cereus, B. megaterium and Staphylococcus aureus* as gram +ve bacteria. *Eschericia coli, Pseudomonas aeruginosa* as gram-ve bacteria. Two fungi *Aspergillus niger and Candida albicans* were included to study antifungal activity of all the soap samples.

Antimicrobial activity of all the samples were determined by calculating "Zone of Inhibition" (it is

defined as a diameter or circular area around a spot or well in nutrient agar plate in which microbial colonies do not grow due to the presence of antimicrobial agents). "Zone of Inhibition" is a qualitative as well as quantitative indicator of effectiveness of antimicrobial agent. Larger size of zone of inhibition indicates greater efficiency of a compound or substance under consideration. In this research cup plate method or cup borer method [11] was adopted. First of all nutrient agar medium (suitable for colonial growth of test organism) was prepared by dissolving all the necessary ingredients in distilled water. pH of solution was adjusted around 7.4 Organisms to be tested were sub cultured in nutrient agar medium. Solution of different concentrations of each soap sample was prepared by dissolving in hot distilled water. The nutrient agar medium was sterilized above 120°C in autoclave. All necessary equipments such as Petri plates, Inoculation Tubes and flasks plugged with cotton were sterilized in hot air oven above 150°C. In each sterilized Petri plate molten nutrient agar medium along with inoculated microbial organisms was transferred aseptically. After solidification in each plate 4 wells or cups of diameter 6mm was punched with the help of sterile borer. The test solution (either I, II, III or IV) of conc. 50 ppm, 100ppm, 150 ppm, 200 ppm were added aseptically in each plate. All the four samples were tested against all the seven organisms. After 2 hours all the plates were transferred in incubator and maintained at 37°C (±1°C) for 24 hours. Value of Zone of Inhibition was measured in mm with the help of calibers.

3. RESULTS AND DISCUSSION

3.1. pH

In terms of suitability for application on human skin pH is an important quality parameter. Highly alkaline soaps prove harmful towards skin and causes rashes or itching in case of people having skin. Ideally pH of a normal soap should be below 9. As indicated from observation (table 1) Soap Type I has highest pH (8.64) while Soap Type II has least (~7). Desirable pH could be attained by maintaining proper Oil: Lye ratio, keeping oil in slight excess as compared to lye helps in reducing amount of un reacted lye in final product. Also addition of slightly acidic fillers like citric acid, boric acid etc. helps in reducing alkalinity of soap.

3.2. Antimicrobial activity

As evident from observation tables 2-5, soaps prepared by different recipes have significant difference in Bactericidal or Fungicidal action. Inclusion or Exclusion of one or another sort of ingredient leads to some sort of change in biological activity of soap sample. Except for fungus *Candida albicans* (an opportunistic pathogenic yeast which causes disease called Candidiasis in immune compromised patients) all the four soap samples exhibited some sort of antimicrobial activity against 2-6 different test organisms. This negative result although seems ambiguous but it is consistent with the fact that Jatropha oil is reported to have Fungicidal activity against *Candida* sp. at Minimum inhibitory concentration (MIC) values above 200 ppm [12]. So in case of soap solution having concentration of Jatropha oil below 200 ppm it is admissible that Soaps doesn't have any remarkable antifungal activity. Further studies at higher concentration levels are recommended to determine exact concentration of these surfactants required to generate fungicidal action.

Table 1: pH of soap samples

Sample	pH (Universal Indicator)	pH (pH meter)
I	9	8.64
II	8	7.70
III (Transparent)	8	8.21
IV (Liquid)	7	7.08

Bacillius cereus is a Gram +ve rod shaped spore forming toxin producing pathogen mainly responsible for Food poisoning or Diarrhea. Except for Soap Type I all the other samples exhibited bactericidal property against B. cereus. This indicates that inclusion of essential oil like Tea tree oil [13, 14] lead to improvement in biological activity of soap from Jatropha oil. There is remarkable increase in value of zone of inhibition of *B. cereus* when more essential oils like Thyme oil, Peppermint Oil, D-Limonene were included which enhanced effectiveness of soap against *B. cereus*. Highest reported value was in Soap Type IV (around 19mm @ conc. of 200 ppm).

In case of *B. megatarium* also a similar trend was observed but value of zone of inhibition was less in case of liquid soap IV as compared to solid Soap Type II and III. This could be explained by the fact that in case of Liquid soap active phytochemical contents responsible for antimicrobial action are in much more diluted state, also prolonged heating required for preparation of liquid soap affects structure of certain phytochemical ingredients which lead to decline in biocide activity. Hence temperature during production of soap could be

regarded as one of the crucial limiting factor which determines extent of antimicrobial activity.

Staphylococcus aureus is a Gram +ve rod shaped bacteria which causes skin infections like abscesses, boils, impetigo etc. and is reported as a causal agent of wound infections after surgery in hospital. All the four samples exhibited antimicrobial activity against *S. aureus*. Thus soap made from Jatropha Oil, Castor oil and essential oils could be used in making antiseptic soaps for treating surgical wounds. Liquid soaps could be used to make hand wash and hand sanitizers for cosmetic as well as clinical purpose.

Table 2: Antimicrobial Activity at Conc. 50 ppm (Zone of inhibition in mm)

S.No.	Micro organism	Soap Type I	Soap Type II	Soap Type III	Soap Type IV
1)	*Bacillus cereus*	-ve	6	6	10
2)	*Bacillus megaterium*	-ve	10	12	7
3)	*Eschericia coli*	-ve	14	17	4
4)	*Staphylococcus aureus*	3	5	5	6
5)	*Pseudomonas aeruginosa*	-ve	-ve	-ve	6
6)	*Aspergillus niger*	14	15	7	2
7)	*Candida albicans*	-ve	-ve	-ve	-ve

Note: In each table-ve means value of zone of inhibition is either zero or negligible i.e. test samples are giving negative result in terms of bactericidal or fungicidal activity.

Table 3: Antimicrobial Activity at Conc. 100 ppm (Zone of inhibition in mm)

S.No.	Micro organism	Soap Type I	Soap Type II	Soap Type III	Soap Type IV
1)	*Bacillus cereus*	-ve	6	8	12
2)	*Bacillus megaterium*	-ve	12	15	9
3)	*Eschericia coli*	-ve	17	17	4
4)	*Staphylococcus aureus*	7	6	6	7
5)	*Pseudomonas aeruginosa*	-ve	-ve	-ve	7
6)	*Aspergillus niger*	16	17	9	4
7)	*Candida albicans*	-ve	-ve	-ve	-ve

Table 4: Antimicrobial Activity at Conc. 150 ppm (Zone of inhibition in mm)

S.No.	Micro organism	Soap Type I	Soap Type II	Soap Type III	Soap Type IV
1)	*Bacillus cereus*	-ve	9	14	18
2)	*Bacillus megaterium*	-ve	18	18	12
3)	*Eschericia coli*	-ve	22	24	10
4)	*Staphylococcus aureus*	12	8	6	9
5)	*Pseudomonas aeruginosa*	-ve	-ve	-ve	12
6)	*Aspergillus niger*	19	21	11	7
7)	*Candida albicans*	-ve	-ve	-ve	-ve

Table 5: Antimicrobial Activity at Conc. 200 ppm (Zone of inhibition in mm)

S.No.	Micro organism	Soap Type I	Soap Type II	Soap Type III	Soap Type IV
1)	*Bacillus cereus*	-ve	15	16	19
2)	*Bacillus megaterium*	-ve	23	25	18
3)	*Eschericia coli*	-ve	24	26	15
4)	*Staphylococcus aureus*	27	10	9	13
5)	*Pseudomonas aeruginosa*	-ve	-ve	-ve	15
6)	*Aspergillus niger*	20	24	14	9
7)	*Candida albicans*	-ve	-ve	-ve	-ve

Eschericia coli (commonly known as *E.coli*) is a Gram-ve rod shaped coliform bacterium which is normally present in gut and keeps digestive tract healthy. Although it is considered harmless few pathogenic strains are reported to cause food poisoning, diarrhea and Urinary Tract Infection (UTI). Except for Soap Type I all the four soap samples displayed bactericidal property, especially solid soaps were more active as compared to liquid soap.Highest value of zone of inhibition was in Soap Type III (26mm @ conc. 200ppm) and least was in Soap Type IV (15mm @ conc. 200 ppm). Prolonged boiling or heating required for production of liquid soaps and excessive dilution affects structure and activity of phyto active ingredients. *Pseudomonas aeruginosa* is a pathogenic Gram-ve rod shaped bacteria known for nosocomial (hospital acquired) infections. It gets easily transmitted in immune compromised patients through surgical items and leads to severe clinical problems such as septic in burning wounds, respiratory tract infections and UTI. It develops drug resistance against various antibiotics. Only liquid soap Type IV exhibited bactericidal activity against *Pseudomonas aeruginosa* at all the concentrations (Minimum 6 mm @ conc. 50 ppm and Maximum 15 mm @ 200 ppm). This result could be utilized for the development of high quality antiseptics as well as disinfectants from liquid Jatropha Soaps, which could be used for the sanitization of hospitals and Surgical Instruments. Further laundry based products could be developed from these liquid surfactants which could be used to clean and sterilize hospital clothings and bandages.

Aspergillus niger is a fungi which is less virulent in Humans but a potent Plant pathogen and causes diseases like 'Black rot' in fresh harvested fruits like Apricots, Mangoes, Strawberries, Grapes etc. and vegetables like onion. All the four soap samples showed Fungicidal activity against *Aspergillus* (Max. in Soap Type II 24mm @ conc. 200 ppm and Minimum in Soap Type IV 9 mm @ conc. 200 ppm). Thus Jatropha oil based surfactants could be used as fungicide at higher concentrations to prevent black rot disease in various fruits and vegetables.

In all the cases it was observed that there is an increase in antimicrobial activity with an increase in concentration of soap from 50 ppm to 200 ppm.

Liquid soaps although being more soluble suffers from limitation of dilution which is indicated by low bactericidal activity in case of *E.coli, S.aureus and Aspergillus*. However these soaps at higher concentration may show phenomenal antimicrobial activity. Thus as an extension of present work it is suggested that Minimum Inhibitory Concentration (MIC) values of these surfactants (solid as well as liquid) should be determined at different dilution level so as to figure out the perfect ratio of different ingredients.

4. CONCLUSION

From this study we can conclude that soaps prepared from non edible Jatropha oil has various medicinal properties. Soap Type I had best effect on *S.aureus* @ conc. of 200 ppm. Soap type II had remarkable effect on *B. megaterium, E.coli* and highest effect in *Aspergillus niger*. Soap Type III was most effective against *E.coli* and least against *S.aureus*. Soap Type IV was effective against all six organisms. It was most effective in case of *B. cereus* and least effective in case of *Aspergillus*. It was only liquid soap (Type IV) which was effective against *Pseudomonas*. From reported value of Zone of Inhibition in different microbes it can be concluded that physical state of surfactant, dilution and temperature of preparation of various surfactants has a remarkable effect on its Antimicrobial activity. As an extension of work we propose that further study on surfactants made from Jatropha oil after detoxification could lead to development of effective and safe medicinal soaps, liquid disinfectants and Eco friendly Fungicides as well.

Declaration of conflicting interests

The authors declare that there is no conflict of interest in preparing this Article.

5. ACKNOWLEDGEMENT

We would like to thank Mr. Vikram Chaturbhai Solanki for providing Microbial strains and his valuable assistance in Microbiological Experiments.

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