ABSTRACT

Objective: To prepare soap under laboratory condition using fruit peels of Citrullus lanatus, Citrus lemon, Citrus maxima, Carica papaya, Ananas comosus and Punica granatum, to view the antibacterial property and pH of the soap prepared and to study the phytochemical content of the fruit peels.

Methods: The homemade soap is prepared in the laboratory using fruit peelings. Antibacterial properties of the prepared soap were then tested using disc paper inhibition method against Escherichia coli, Staphylococcus aureus, and Staphylococcus epidermidis. The Extract was prepared using distilled water and ethanol. The pH of the fruit peels was tested and screened for phytochemical properties.

Results: Overall best results for the antibacterial property was shown by Citrullus lanatus, Carica papaya followed by Citrus aurantifolia and Ananas comosus for water extract. Citrus maxima followed by and Ananas comosus and Citrus aurantifolia for ethanol extract. All six fruit peels showed the presence of Alkaloids, Terpenes, Saponins, Glycosides, Quinones, and Tannins. pH of the soap ranges from 7-10.

Conclusion: The peels of the fruit shows good results against the anti-bacterial activity for skin bacteria studied. Also, the soaps are less prone to the addition of the harmful chemicals and their derivatives.

Keywords: Fruit peels, Phytochemicals, Antibacterial, Zone of inhibition, Ethanol extract, Water extract

INTRODUCTION

Fruits are nature’s wonderful gift to mankind. Since time immemorial fruits have been the source of food for human beings as they used to reap the harvest of fruits and enjoy their flavour and taste. Fruits are also the chief source of some of the essential vitamins and minerals. Fruits are one of the largest groups of food that contain antioxidants and serve as a natural remedy for a multitude of ailments such as soothing itchy skin, healing wounds, and protecting our skin from sun damage.

Fruits and vegetable wastes and their by-products are formed in great amounts during industrial processing and hence represent a serious problem, as they exert a harmful effect on the environment. So they need to be managed or they can be utilized [1]. Apart from consumption, fruits are used in cosmetics, soaps, shampoos, toothpaste, etc.

Soap: The first concrete evidence we have of soap-like substance is dated around 2800 BC., the first soap makers were Babylonians, Mesopotamians, Egyptians, as well as the ancient Greeks and Romans. All of them made soap by mixing fat, oils and salts. Soap wasn’t made for bathing and personal hygiene but was rather produced for cleaning cooking utensils or goods or was used for medical purposes.

Today, there are different soaps made for a vast array of purposes and is available for personal, commercial, and industrial use. There are handmade and commercially produced soap, which are used to wash clothes, dishes, cars etc. Overall, soap is a remarkably useful substance, just as it has been for thousands of years [2].

Phytochemicals are plant chemicals that have protective or disease preventive properties. Plants produce these chemicals to protect themselves but recent research demonstrates that they can also protect humans against diseases. There are many phytochemicals and each works differently. These are some possible actions: Antioxidant, Hormonal action, Stimulation of enzymes, Interference with DNA replication, Anti-bacterial effect and Physical action.

MATERIALS AND METHODS

Six different locally available fruits (Punica granatum, Carica papaya, Ananas comosus, Citrus aurantifolia, Citrus maxima, and Citrullus lanatus) from various families were selected for preparation of soaps, and screening the fruit soap for antibacterial activity, phytochemical aspects and pH.

Preparation of soap

• Fruits were collected from the Mapusa market, Bardez, Goa.
• Fruit peels were separated to prepare three different types of soaps of each fruit.
• The peels were then cut into smaller pieces (i.e. chopping, blending, etc.)
• Making the soap
• 15 ml of each: virgin coconut oil and Castor oil were put in a container.
• 5g of lye (NaOH pellets) was measured and dissolved in 30 ml of water.
• The oil mixtures and the lye solution were separately heated between 95-105 °C (this temperature is critical for soap making).
• When both the oils and lye mixture are at the right temperature, the oil and the lye were poured into the blender, till it lightens and becomes into a thick paste
• Immediately 60-150 ml of the blended fruit parts were added to the mixture and blended until it turns into a batter (the weight of the cut fruit parts is proportional to the amount of coconut oil and lye solution).
• The soap batter was then poured into the moulds and allowed the soap to cure for 2-6 w till it hardens.
• After it hardens it was wrapped and kept aside.

Preparation of soap extract for antibacterial property

Two solvents were used for the preparation of the extract. 0.2g of six fruit soaps each (i.e. soap from fruit peel) was dissolved in 10 ml of 90% ethanol and distilled water. The content was well mixed and used for screening antibacterial properties.
Preparation of media
To prepare one liter of nutrient agar medium: Peptone 5g, Sodium Chloride 5g, Beef extract 3 gm was weighed and dissolved in 1000 ml of distilled water. The pH was adjusted to 7.4 and 15g agar was added and sterilised in an autoclave at 15lbs for 20 min at 121 °C [3] and used for preparing media plates.

Test organisms used
Pure cultures of Escherichia coli, Staphylococcus aureus and Staphylococcus epidermidis were procured from Department of Microbiology, St. Xavier’s College, Mapusa-Bardez, Goa. They were maintained by inoculating a loop full of the culture of the respective organism on a nutrient agar medium using Streaking Culturing technique and incubated for 24 h at 27 °C. Loop full of test organism grown on media were suspended in sterile saline and used to determine antibacterial activity.

Determination of antibacterial activity by paper disc method
Paper disc approximately 6 mm in diameter was cut from Whatman Filter Paper No.1. The disc was put in a petri dish and autoclaved. The sterilised discs were placed in the oven at 120 °C for 1 hr before use. Discs saturated with Penicillin were dried and then used as the positive control. Other sterilised discs saturated with soap extract were placed aseptically using sterile forceps in the center of each media plate inoculated with 0.1 ml of the test organism. Ethanol and distilled water were taken as normal control. These plates were incubated for 24 h at room temperature and diameter of the inhibition zone was measured. The results were documented by taking photographs of plates. Analysis of antibacterial activity was carried out using Microsoft Excel 2010.

Phytochemical tests
Fruit peels were air dried for several weeks and powdered using grinder. The powder was used to carry out the different phytochemical tests [4].

Test for alkaloids
2 ml 2N HCl+2 ml of plant extract shaken vigorously, kept aside for 5 min after which a few drops of Mayer’s reagent (Hg Cl₂) was added. The mixture was kept at room temperature and the color of the mixture was observed. Next, 2 ml distilled water was added, shaken, and the mixture was kept for comparison with standard solution of ammonium hydroxide.

Test for saponin
1 ml solvent extract+1 ml conc. H₂SO₄ was shaken vigorously, kept aside for 5 min after which a few drops of Mayer’s reagent (Hg Cl₂) was added. The mixture was kept at room temperature and the color of the mixture was observed. Next, 2 ml distilled water was added, shaken, and the mixture was kept for comparison with standard solution of ammonium hydroxide.

Test for quinone
2 ml 2N+KI in 2 ml H₂SO₄ solution was shaken vigorously, kept aside for 5 min after which a few drops of Mayer’s reagent (Hg Cl₂) was added. The mixture was kept at room temperature and the color of the mixture was observed. Next, 2 ml distilled water was added, shaken, and the mixture was kept for comparison with standard solution of ammonium hydroxide.

Test for terpenes
Ethanol extract+plant extract+conc. H₂SO₄ was added, shaken so that creamy precipitation

Test for glycosides
The powder was used to carry out the different phytochemical tests [4]. Fruit peels were air dried for several weeks and powdered using grinder. The powder was used to carry out the different phytochemical tests [4].

Test for tannins
Ethanol extract was stirred separately with a few drops of dilute HCl and filtered. Filtrate was used to carry out the iodine test. 1 ml filtrate+few drops of iodine solution, red colour developed.

Test for saponin
Methanol extract (0.5 ml)+5 ml distilled water will form persistence frothing and foaming.

Test for glycosides
Methanolic extract was treated with conc. H₂SO₄+benzene to check the presence of glycosides in the sample.

Test for quinone
1 ml solvent extract+1 ml conc. H₂SO₄ to give red or deep green colouration.

pH of fruit soap
0.2 gms of fruit soap was weighed and dissolved in 10 ml of distilled water was tested using Universal pH Indicator to check the pH.

RESULTS AND DISCUSSION
The fruit peel soaps prepared from different fruits were analysed for the antibacterial properties, using paper disc method against three bacteria Escherichia coli, Staphylococcus aureus and Staphylococcus epidermidis in two extract water and ethanol and pH studied.

Peel fruit soap in water extract against
i) S. aureus (Sa): The clearing zone against S. aureus was maximum in Citrus maxima (2.3 cm), followed by Citrus aurantifolia (2.0 cm), Ananas comosus (1.5 cm), Carica papaya (1.3 cm) and Punica granatum (1.1 cm). Least result was shown by Citrus maxima (1.0 cm).

ii) S. epidermidis: The zone of inhibition against S. epidermidis was maximum in Carica papaya (2.0 cm) and Citrullus lanatus (2.0 cm) followed by Ananas comosus (1.0 cm). No result was shown by Citrus aurantifolia (0 cm), Citrus maxima (0 cm) and Punica granatum (0 cm).

iii) E. coli: Only Citrus aurantifolia showed zone of clearing of about 1.1 cm against E. coli. (table 1 fig. 1).

Peel fruit soap in ethanol extract against
i) S. aureus: The zone of inhibition against S. aureus was maximum in Ananas comosus (2.1 cm) followed by Citrus aurantifolia (2.0 cm), Punica granatum (1.2 cm) and Citrus maxima (1.1 cm).

ii) S. epidermidis: The zone of clearing against S. epidermidis was maximum in Citrus maxima (2.4 cm) followed by Carica papaya (2.1 cm) and Ananas comosus (2.1 cm), Punica granatum (1.4 cm). Least result was shown by Citrullus lanatus (1.2 cm).

No result was shown by Citrus aurantifolia.

iii) E. coli: The zone of clearing against E. coli was maximum in Citrullus lanatus (1.7 cm), followed by Ananas comosus (1.6 cm), Citrus aurantifolia (1.5 cm) and Citrus maxima (1.5 cm) which
showed similar results. *Punica granatum* (1.0 cm) showed least result. No result was shown by *Carica papaya* (table 2, fig. 2).

**Phytochemical test**

All six fruit peels showed the presence of Alkaloids, Terpenes, Saponins, Glycosides, Quinones, and Tannins (table 3).

**pH of fruit soaps**

The pH of the fruit soap prepared ranged from pH 7 in *Punica granatum* to pH 9.5 in *Citrullus lanatus*. Majority showing pH 7.5 in the fruit soap of *Citrus aurantifolia*, *Citrus maxima* and *Carica papaya* (table 4).

| Table 1: The fruit peel soaps from different fruits and the inhibition zone against three bacteria in water extract (WE) |
| --- |
| **Fruit peel soap** | **S. aureus** | **E. coli** | **S. epidermidis** |
|  | **W. E. (cm)** | **W. E. (cm)** | **W. E. (cm)** |
| Ananas comosus | 1.5 | 0 | 1.0 |
| Carica papaya | 1.3 | 0 | 2.0 |
| Citrus aurantifolia | 2.0 | 1.1 | 0 |
| Citrullus lanatus | 2.3 | 0 | 2.0 |
| Citrus maxima | 1.0 | 0 | 0 |
| Punica granatum | 1.1 | 0 | 0 |
| Control | 1.2 | 1.0 | 0.6 |

| Table 2: The fruit peel soaps from different fruits and the inhibition zone against three bacteria in Ethanol Extract (EE) |
| --- |
| **Fruit peel soap** | **S. aureus** | **E. coli** | **S. epidermidis** |
|  | **E. E. (cm)** | **E. E. (cm)** | **E. E. (cm)** |
| Ananas comosus | 2.1 | 1.6 | 2.1 |
| Carica papaya | 0 | 0 | 2.1 |
| Citrus aurantifolia | 2.0 | 1.5 | 0 |
| Citrullus lanatus | 0 | 1.7 | 1.2 |
| Citrus maxima | 1.1 | 1.5 | 2.4 |
| Punica granatum | 1.2 | 1.0 | 1.4 |
| Control | 1.3 | 1.1 | 0.7 |

| Table 3: Phytochemical test for fruit peels |
| --- |
| **Fruits peel** | **Alkaloids** | **Terpenes** | **Saponins** | **Glycosides** | **Quinones** | **Tannins** |
|  | + | + | + | + | + | + |
| Citrus aurantifolia | + | + | + | + | + | + |
| Citrus maxima | + | + | + | + | + | + |
| Carica papaya | + | + | + | + | + | + |
| Ananas comosus | + | + | + | + | + | + |
| Punica granatum | + | + | + | + | + | + |
| Citrullus lanatus | + | + | + | + | + | + |

All six fruit peels showed the presence of Alkaloids, Terpenes, Saponins, Glycosides, Quinones, and Tannins (table 3).

| Table 4: pH of different fruit soaps |
| --- |
| **Fruits peels** | **pH** |
| Citrus aurantifolia | 7.5 |
| Citrus maxima | 7.5 |
| Carica papaya | 7.5 |
| Ananas comosus | 8.5 |
| Punica granatum | 7 |
| Citrullus lanatus | 9.5 |
| Control | 10 |
Peel fruit soap in water extract
Overall best results for antimicrobial activity was shown by peel soap of Citrullus lanatus, Carica papaya, followed by Citrus aurantifolia and Ananas comosus.

Peel fruit soap in ethanol extract
Overall best result for antimicrobial activity was shown by peel soap of Citrus maxima, followed by Ananas comosus and Citrus aurantifolia. All fruit peels showed alkaline pH ranging from 7-9.5, which will not have much harm on the skin. While, all fruit peels also showed positive results for the phytochemicals such as Alkaloids, Terpenes, Saponins, Glycosides, Quinones, and Tannins. Alkaloids have analgesic, antispasmodic and antibacterial properties [5]. The properties of tannins include anti-inflammatory, regeneration, antiacarthal, antimicrobial and soothing effect [6]. Elsewhere, tannins have been reported to contain antiviral, antitumor, anti-inflammatory and wound healing properties among other organs [7],[8]. The action of saponins includes expectorant, antiacarthal, antimicrobial and coughs suppressant properties. The glycosides have laxative and carminative effects [9]. The terpenoids possess soothing relief, antimicrobial, carminative effect and antiseptic properties [10]. It has been found that peels of fruits and vegetables hold tremendous potential to serve as a source of newer, effective, safer and better antioxidant and antimicrobial agents [11]. The soaps so prepared from the different peels of fruits will serve to fight against some of the skin diseases.

CONCLUSION
In the present study, we have shown that soaps prepared from the peels of the fruit which are rejects, shows good results against the anti-bacterial activity for skin bacteria studied. Also the soaps are less prone to the addition of the harmful chemicals and their derivatives, which are normally done for the industrially prepared soaps. This soap includes the addition of the natural products of the fruits and thus less harmful to the human skin. The pH of all the tested soaps is basic, making the soap mild for the skin. The presence of phytochemicals such as terpenes, alkaloids and saponins contents are seen in the fruit, which may be the reason the soaps showing good anti-bacterial activity with peel soap which otherwise is a waste product.

AUTHORS CONTRIBUTIONS
All the author have contributed equally

CONFLICT OF INTERESTS
Declare none

REFERENCES
1. Duda Chodak A, Tarko T. Antioxidant properties of different fruits seeds and peels. Acta Sci Pol Technol Aliment 2007;6:29-36.
2. Brady James E, Russell, Joel W, Holam John R. Chemistry: matter and its changes. 3rd edition. New York: Wiley; 2000.
3. Aneja KR. Experiments in microbiology, plant pathology and biotechnology, New Age International Publishers; 2003.
4. Trease GE, Evans WC. Pharmacology. 11th Ed. Bailliere Tindall Ltd. London; 1978. p. 60-75.
5. Stray F. The national guide to medicinal herbs and plants. Tiger Books International London; 1998. p. 12-6.
6. Manikandan L, Senithilkuman GP, Rajesh LT, Shuresh RR. Cancer Chemopreventive agents from medicinal plants. In: Trivedi PC. (Ed). Medicinal plants: Ethnobotanical approach. Agrobios, India; 2006. p. 410.
7. Amakaha RA, Uba ST, Otoplea M, Shenja G. Phytochemical screening of danta sramo seeds. J Chem Soc Niger 2002;27:105-7.
8. Zhejlakov VD, Amber C, Charles LC. Yield and oil composition of 38 Basil (Ocimum basilicum L.) accessions grown in mississippi. J Agric Food Chem 2008;56:241-5.
9. Scalbert A, Rajiedran K, Dineh KC. Dietary polyphenol and prevention of diseases. critical review. Food Sci Nutr 2005;45:287-306.
10. Sofawara A. Medicinal and traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria; 1993. p. 289.
11. Sonia Parashar, Hitender Sharma, Munish Garg. Antimicrobial and antioxidant activities of fruits and vegetable peels: a review. J Pharmacogn Phytochem 2014;3:160-4.
12. https://www.diynatural.com/how-to-make-soap-2/[Last accessed on 10 Jan 2019]