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Physicochemical and Microbiological Characteristics of Natural Antiseptic Solid Soaps Formulated from Coconut Oil, Palm Oil, and Olive Oil incorporating Tea Tree Essential Oil as Antibacterial Agent

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ABSTRACT: The objective of this paper is to evaluate the physicochemical and microbiological characteristics of natural antiseptic solid soaps produced from coconut oil, palm oil, and oliveoil incorporating tea tree essential oil as antibacterial agent using appropriate standard methods. The water content analysis revealed that all samples were within the recommended range for water content. The pH analysis results indicated that all samples exhibited an alkaline nature. The results of the hardness test revealed that sample D exhibited the highest value of 86674.5gram-force. The infrared spectra analysis of the solid soap formulations revealed consistent results, displaying prominent bands representing the υ (C=O) frequency of the keto group at 1558 cm^{-1} and a strong band at 1450 cm^{-1} corresponding to the υ (C-O) frequency of the ester oxygen. The various samples underwent in-vitro antimicrobial screening against multiple bacterial strains. The in-vitro antimicrobial screening of the different samples showed that sample D displayed significantly greater activity against the tested microorganisms compared to samples A, B, and C. Furthermore, the antiseptic efficacy test demonstrated that sample D achieved an outstanding bacterial reduction rate of approximately 98%.

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Hand hygiene practices in the patient care setting have a long history, originating in the early 19th century(Hassan *et al*., 2019, Gammon and Hunt, 2019). In 1847, Dr. Semmelweis, made a groundbreaking observation that contaminated hands were the primary means of transmitting contagious diseases. In response, he implemented a mandatory hand washing practice using chlorinated lime water for all hospital staff, including medical students, irrespective of their roles. This simple yet effective intervention led to a significant reduction in death rates(Haque, 2020). Since then, hand hygiene practices have evolved and gained recognition for their vital importance in infection control. Extensive evidence supports their effectiveness in reducing the presence of pathogens associated with nosocomial or hospital-acquired infections when combined with other hand-hygiene measures(Gammon and Hunt,

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2019, McMichael, 2019).Hand washing is an effective measure to prevent infectious diseases, including hepatitis, malaria, tuberculosis, and coronavirus(Olajuyigbe*et al*.,2017;Kim and Rhee, 2016, Gardner and Luciw, 2008, Glaser *et al*, 2006). Tuberculosis and coronavirus 2019 remain highly dangerous, with approximately 10.0 million new cases of tuberculosis infections and around 1.5 million deaths reported worldwide(Haw and Uy, 2020;Battin 2020;Team, 2013).Today, several soaps labeled as 'antibacterial' or 'antimicrobial' are available on the market(Olajuyigbe *et al*., 2017, Boyce and Pittet, 2002). The term "antibacterial soap" refers to soap that contains antiseptic active ingredients. Many studies have reported the inhibitory potential of antimicrobial and non-antimicrobial soaps in clinical cases, indicating that antimicrobial soap containing active ingredients can remove more bacteria compared to plain soap. This type of soap has been shown to remove up to 65% to 85% of bacteria present on human skin (Boyce and Pittet, 2002;Toshima *et al.,* 2001,Osborne and Grube, 1982).Commercial soaps commonly include antiseptic active ingredients obtained through chemical synthesis, such as Triclocarban (TCC), triclosan, and chloroxylenol, which are frequently found in antimicrobial soaps(Kim and Rhee, 2016, Handayani *et al.,* 2018). However, the excessive use of these antimicrobial soaps can pose significant health risks. These risks involve the potential development of bacterial resistance, allergenic properties leading to skin problems like irritation, redness, sensitivity, dehydration, and eczema, as well as the persistent presence of these compounds as environmental pollutants(Kim and Rhee, 2016).Natural antibacterial alternatives are needed in soap production(Handayani *et al.,* 2018).Essential oils are highly concentrated oils that possess potent aromas. They are extracted from various plants and have been used for centuries in aromatherapy, traditional medicine, and as soap-making ingredients(Naeem *et al*., 2018, Tongnuanchan and Benjakul, 2014).Tea tree (*Melaleuca alternifolia*) is an essential oil derived primarily from the native Australian plant *Melaleuca alternifolia* used largely for its antiseptic and anti-inflammatory actions(Carson *et al*., 2006, Yasin *et al.*, 2021).Many topical formulations incorporated Tea tree essential oil which is powerfully anti-infective, anti-parasitic and antifungal as the natural active ingredient for cutaneous infections treatment(Carson *et al*., 2006).The antimicrobial activity of tea tree oil against bacteria is attributed to multiple mechanisms. Firstly, tea tree oil, which contains compounds like terpenes (example: terpinen-4-ol), impacts the permeability of bacterial cell membranes by interacting with their lipid components, resulting in structural and functional disruptions, increased permeability, and the release of cellular content (Carson *et al*., 2006).Additionally, tea tree oil interferes with essential enzymes and proteins within bacterial cells, inhibiting their activity and impairing normal functions necessary for bacterial growth and metabolism(Hammer*et al.,* 1999).Furthermore, there is evidence suggesting that tea tree oil can disrupt the cell walls of bacteria, causing cell leakage and eventual cell death. These combined mechanisms contribute to the antibacterial activity of tea tree oil(Hammer *et al*., 1999, Cox *et al.,* 2000).In the literature, several studies have been conducted to characterize and evaluate the effectiveness of different natural active compounds in solid soap formulations.Ikotun *et al*.,(2017) conducted research on the phytochemistry and antimicrobial properties of African black soap and its modified samples.Olajuyigbe *et al*.,(2017) on the other hand, compared the antibacterial activity of African black soap with medicated soaps commonly used for treating bacteria-infected wounds.Pratama and co-workers (2021) focused on examining the characteristics and microbiological quality of solid soap formulated with citronella oil and the addition of Lactobacillus brevis. Additionally, Handayani *et al*.,(2018) conducted a study specifically investigating the standard quality and antibacterial activity of clove oil in the production of solid soap, targeting *Staphylococcus aureus* and *Escherichia coli*.

However, there is currently no report on the investigation of the inhibitory effects of tea tree essential oil in solid soap formulations. Consequently, the objective of this paper is to evaluate the physicochemical and microbiological characteristics of natural antiseptic solid soaps produced from coconut oil, palm oil and olive oil incorporating tea tree essential oil as antibacterial agent.

MATERIALS AND METHODS

Materials: Coconut oil, palm oil,olive oil, tea tree essential oil and geranium hydrosol were certified and purchased from a local market. The remaining chemicals and solvents were obtained in analytical gradefrom Sigma-Aldrich and VWR chemicals.

Test microorganisms: Nine strains including *Staphilococus épidermique ATCC 12228;Micrococusflavus ATCC 10240 ; Staphylococcus aureus ATCC 6538p; Bacillus subtilis ATCC 6633; Sacharomyces cerevisiae ATCC 9763; Escherichia coli ATCC 8739; Pseudomonas aeruginosa ATCC 9027; Aspergillus brasiliensis ATCC 16404; Condida albicans ATCC 10231* were

obtained from theantibiotic complex SAIDAL of Medea, Algeria.

Preparation of solid soap: The procedure of Pratama *et al.,* (2021) was employed for the preparation of solid soap with some modifications. Vegetable oils (80g olive oil, 20g coconut oil, 10g palmist oil) were combined in a glass beaker and heated in a water bath at 40 °C. NaOH was accurately weighed and dissolved either in distilled water or geranium hydrosol. The resulting NaOH solution was then mixed with the vegetable oils and stirred until the mixture was homogenized. Before incorporating 1% tea tree essential oil or geranium hydrosol into the prepared soap mixture, it is essential to maintain a temperature of approximately 40°C. To investigate the impact of adding Tea tree essential oil and geranium hydrosol on the properties of the solid soap, four samples were prepared: Sample A is control soap without any additions, Sample B has Tea tree essential oil added, Sample C has geranium hydrosol added, and Sample D has both Tea tree essential oil and geranium hydrosol added. These samples were poured into molds and left to harden for 30 days at room temperature. The presence and absence of chemical compounds in the different solid soap samples were documented and presented in Table 1.

Table 1: Chemical compound in the different solid soap samples.

Material	A	B	C	D
Geranium hydrosol	-	-	+	+
Tea tree essential oil	-	+	-	+

(-) indicates absence, (+) indicates presence

Solid Soap analysis

*Infrared Spectra Analysis:*The surface functional groups of the solid soap were analyzed using Fourier Transform Infrared (FTIR) spectroscopy with an IR spectrophotometer Fourier Transform, Perkin Elmer Precisely, Model Lambda 25. Spectra were recorded in the wavelength region between 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1} with 10 scans.

The soap antiseptic power evaluation: An experimental protocol was designed to evaluate the efficacy of the different soap samples. Participants for this test were selected from students and voluntary employees at the University of Medea, Algeria. The experimental protocol consisted of several stages. Bacteriological samples were collected from the hands of the participants before and after washing with the prepared soap. The distal ends of the phalanges of fingers 2, 3, and 4 (index, middle, and ring fingers) of each hand were gently pressed onto the agar of three sample boxes for a duration of 5 seconds. These procedures were conducted over the course of a single day.

The collection boxes were labelled as "R" for the right hand and "L" for the left hand. Afterward, the agar boxes containing the samples were incubated at a temperature of 37°C for duration of 24 hours. Subsequently, the bacterial colonies present in each box were counted. The bacterial reduction rate (TR) was calculated as following:

$$\mathrm{TR}\ (\%) = \left[1 - \frac{\text{Number of colonies after washing (CFU)}}{\text{Number of colonies before washing(CFU)}}\right] \times 100 (1)$$

*Antimicrobial activity:*The antimicrobial activity of the samples was evaluated using the following strains: *Staphilococusépidermique ATCC 12228;Micrococus flavus ATCC 10240 ; Staphylococcus aureus ATCC 6538p ; Bacillus subtilis ATCC 6633 ; Sacharomyces cerevisiae ATCC 9763 ; Escherichia coli ATCC 8739 ; Pseudomonas aeruginosa ATCC 9027 ; Aspergillus brasiliensis ATCC 16404 ; Condida albicans ATCC 10231.*

The antimicrobial activity was determined using Ikotun *et al*., (2017) with little modifications.A 1g portion of solid soap was combined with 5 ml of sterile water and centrifuged at 10,000 rpm for 5 minutes at 4°C. The resulting supernatant was filtered through a Millipore disk with a 0.22µm porosity. The antimicrobial activity of the prepared soap solutions was evaluated using the disk diffusion method. This involved applying 100 µL of a suspension containing the tested microorganisms, each at a concentration of 10^6 cells/mL. Sterile Hinton Agar (MHA) and Sabouraud media were prepared and cooled to a temperature between 45-50°C. The media were then poured into sterile Petri dishes with a 9 cm diameter. Filter paper discs measuring 9 mm in diameter were individually impregnated with 5 µL of the soap solution and placed on the surface of the agar media already inoculated with the tested microorganisms. The Petri dishes containing the impregnated discs were incubated at 37°C for 24 hours for bacterial strains and at 28°C for 48 hours for fungal strains. Following the incubation period, the diameters of the inhibition zones (in mm), including the diameter of the discs, were measured.

Statistical analysis: The data analysis was carried out using Minitab software (version 22.1), employing a one-way ANOVA test along with Dunnett's comparison. Differences in water content, pH, hardness, and reduction rate were considered significant when the p-value was less than 0.05.

RESULTS AND DISCUSSION

Water Content determination: To prevent product contamination, it is recommended to maintain a water

content range of 10-20% in the prepared soap(Idoko *et al.*, 2018) Excessive water content in the soap can lead to hydrolysis during storage(Widyasanti and Hasna, 2016), resulting in the reaction of free fatty acids and glycerol in the saponification process, known as hydrolysis process, occurring in the soap during storage(Pratama *et al*., 2021, Widyasanti and Hasna, 2016). The findings presented in Table 2 demonstrate that the water content of the prepared soap falls within the recommended range of 10 to 20%.(Idoko *et al*., 2018) This indicates that the soap is not prone to excessive growth of harmful microorganisms. Similar results have been reported in other studies, such as those conducted by Sany and Fahmi,(2019) and Pratama *et al*.,(2021) The water content results of the solid soap are summarized and presented in Table 2.

Table 2: Chemical characterization results of the prepared solid soap.

Samples	Water content (%)	pH	Hardness (gf)	Reduction rates (%)
A	12.15127 ± 0.152977	9.23 ± 0.71	71379.0	39 ± 0.04
B	12.04336 ± 0.239377	9.23 ± 0.167	79536.6	80 ± 0.59
C	12.06211 ± 0.289213	9.18 ± 0.112	66028.5	59 ± 0.53
D	12.63515 ± 0.572181	9.16 ± 0.179	86674.5	98 ± 0.42

pH determination: Table 2 shows the pH values of the various prepared solid soaps. All the prepared soaps have an alkaline pH which varies between 9.18 to 9.23. The prepared soap with 5% surgras allow the recovery of the protective film of the skin and it will help to avoid irritation. The pH of the prepared soap should be basic to help the skin open the pores during the cleansing process so that the dirt on the surface is bound to the soap foam(Praptiwi *et al*., 2014).The pH value should not be too alkaline and not be too acidic. The forte alkaline soap can cause the skin peeling and the forte acid soap cause skin irritation(Agustini and Winarni, 2017). According toPratama *et al*.,(2021)the pH would be relatively safe in a range of 9 to 11.

Hardness test: The hardness results of the solid soap are presented in Table 2. It can be observed that sample D exhibits the highest hardness value of 86674.5 gram-force, whereas sample A has the lowest value of 66028.5 gram-force. Additionally, the table indicates that the hardness values for all four samples are relatively close. It is noteworthy that the obtained results surpass those reported by Sany and Fahmi, (2019)for *Eucheuma cottoni* extracted soap, which had a hardness of only 1446.29 gram-force. Soap hardness is a crucial factor that significantly impacts its resistance and durability. Typically, the inclusion of saturated fatty acids containing double bonds results in heightened hardness at room temperature. Conversely, saturated fatty acids lacking double bonds tend to solidify, thereby augmenting the soap's hardness even further (Sany and Fahmi, 2019).

Antiseptic power soaps: The purpose of this test was to assess the antibacterial activity of the soaps, which is attributed to the oils incorporated in their formulation. The bacterial reduction rates (TR) were determined using Equation 1. The results obtained from the analysis are presented in Table 2 and Figure 1. According to Table 2, samples B, C, and D exhibited the highest capacities for bacterial reduction, with reduction rates exceeding 50%. Notably, sample D demonstrated the highest efficacy with a remarkable reduction rate of 98%.

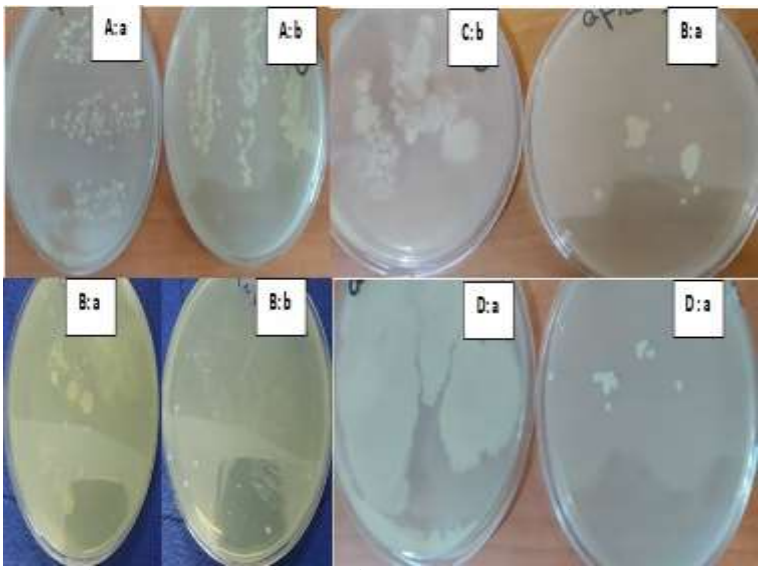

Fig.1: Comparison of bacterial samples before and after hand washing (a: Before Washing, b: After Washing).

Infrared Spectra Analyses: The FTIR spectra of the samples were collected and are presented in Table 3 and Figures 2-5. The characteristic vibrational frequencies were identified by comparing the spectra of the modified samples with the unmodified solid soap (sample A).

Table 3: FTIR spectra analyses of solid soap.

Compounds	υ(OH) (cm^{-1})	υ(C-H) (cm^{-1})	υ (C=O) (cm^{-1})	υ(C-O) (cm^{-1})
Sample A: (Solid soap without modification)	3379.40	2924.18	1558.54	1442.80
Sample B: Solid soap modified with addition of Tea tree essential oil	3402.54	2924.18	1558.54	1450.52
*Sample C: (*solid soap modified with geranium hydrosol)	3425.69	2924.18	1558.54	1450.52
*Sample D: (*solid soap modified with addition of Tea tree essential oil and geranium hydrosol)	3410.26	2924.18	1558.54	1450.52

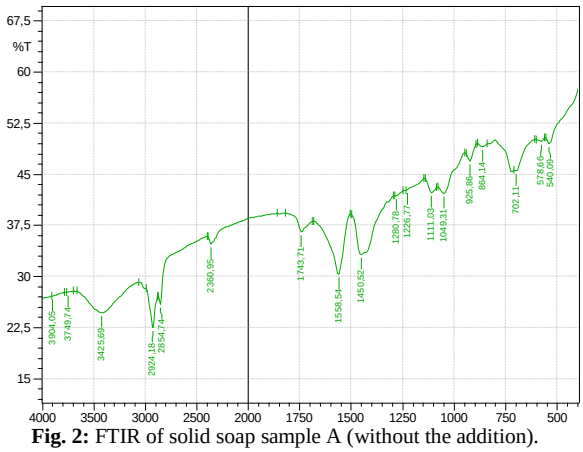

Fig. 2: FTIR of solid soap sample A (without the addition).

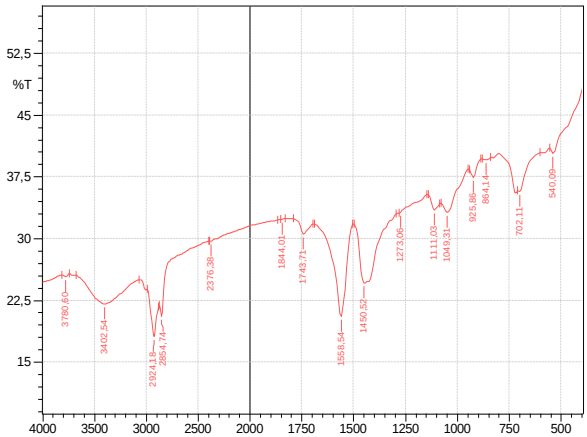

Fig.3: FTIR of solid soap sample B (with Tea tree essential oil).

In the infrared spectrum of sample A, a broad and strong band at 3379.40 cm^{-1} was observed, which corresponds to the stretching vibration of υ (OH) resulting from hydrogen bonding. This band appeared as a broad band, either medium or strong, with a shift to higher frequencies of 3402 cm^{-1}, 3425 cm^{-1}, and 3410 cm^{-1} in Samples B, C, and D, respectively. The peaks around 2924 cm^{-1} in all the different samples are attributed to the C-H vibrations of methyl groups. The strong bands observed at 1558 cm^{-1} in the spectra of the different solid soap samples indicate the presence of the υ (C=O) frequency of the keto group. Additionally, the strong band around 1450 cm^{-1}corresponds to the υ (C-O) frequency of the ester oxygen. This band remains unchanged in all the samples.

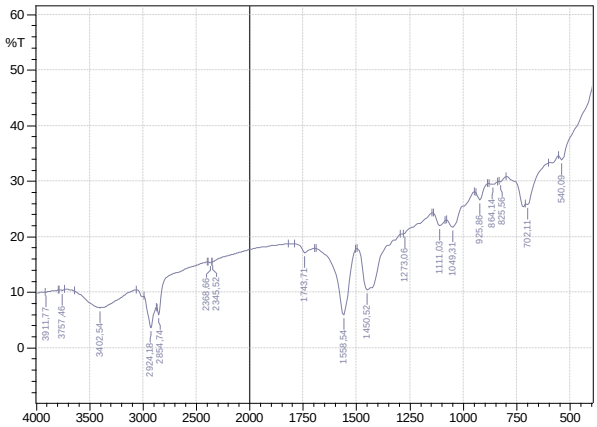

Fig. 4: FTIR of solid soap sample C (with geranium hydrosol).

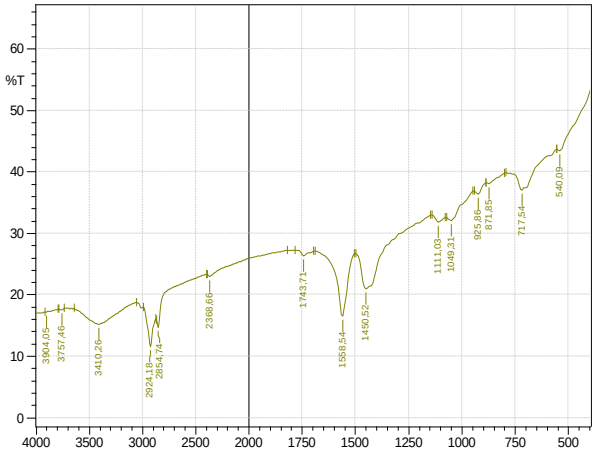

Fig. 5: FTIR of solid soap sample D (with Tea tree essential oil and geranium hydrosol)

Solid soap antimicrobial activity: The results of the antimicrobial activity of the solid soap samples are displayed in Table 4. It is evident that samples B and D exhibit the highest antimicrobial activities, with inhibition of 7 (77.7%) and 8 (88.8%) tested

microorganisms, respectively. Additionally, these samples display the largest antimicrobial zones. The *Candida albicans ATCC 10231* and *Escherichia coli ATCC 8739* strains exhibited resistance to sample B, while sample D displayed the largest antimicrobial zone among the solid soap samples (Table. 4). This can be attributed to the presence of 1% Tea tree essential oil (*Melaleuca Alternifolia*) and geranium hydrosol, which possess a broad-spectrum antimicrobial effect.(Cox *et al.*, 2000) The antimicrobial activity in sample C is primarily attributed to the essential oil component present in the geranium rose hydrosol, which becomes dissolved in the distillation water during the distillation process(Rao, 2012). Conversely, sample A, which serves as the control soap without any additional ingredients, demonstrated the weakest antimicrobial activity compared to the other samples.

Table 4: Antimicrobial Activity of Solid Soap Samples

Strain	Zone (mm)			
	A	B	C	D
Staphilococus épidermique ATCC 12228	12	16	12	15
Micrococus flavus ATCC 10240	11	16	17	22
Staphylococcus aureus ATCC 6538p	10.6	16	14	15
Bacillus subtilis ATCC 6633	11	14	13	14
Sacharomyces cerevisiae ATCC 9763	10	13	12	12
Escherichia coli ATCC 8739	/	/	/	12
Pseudomonas aeruginosa ATCC 9027	/	13	/	15
Aspergillus brasiliensis ATCC 16404	11.8	12	10.8	12.3
Condida albicans ATCC 10231	/	/	/	/

Zone of inhibition includes diameter of disc (9 mm).

Conclusion: In conclusion, the natural antiseptic solid soap made from a combination of coconut oil, palm oil, and olive oil with incorporated Tea tree (*Melaleuca alternifolia*) has shown to be a powerful antibacterial soup with the benefits eradicating harmful bacteria and viruses on the skin. This can be particularly advantageous for maintaining proper hand hygiene and reducing the risk of infections. The antimicrobial activity was assessed against specific microorganisms, and future research could focus on improving the soap's formulation by incorporating additional naturally derived active ingredients to enhance its antibacterial effects and broaden its spectrum of activity.

Declaration of Conflict of Interest: The authors declare that there is no conflict of interest.

Data Availability Statement: The authors declare that data for this research are available upon request from the corresponding author

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