**Antiseptic properties of Cocoa Pod Husk Herbal soap**

**Abstract**

The antiseptic properties of two soaps prepared with the leaves of *Azadirachta indica* (NEM) and *Moringa oleifera* (MRG) were evaluated. The study employed Inhibitory activity sensitivity test using Agar-well Diffusion Method to test the antimicrobial activities of the soap samples on four bacteria (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeuroginosa*) and two fungi (*Trichophyton spp. and Candida albicans*) and minimum Inhibitory concentration (MIC) obtained using regression analysis. The physical properties of the prepared soap including hardness, foamability and pH were evaluated and results were in consonant with the standard. The soap samples, MRG and NEM exhibited higher antiseptic properties against the bacteria and fungi tested. Regression analysis showed that Moringa soap exhibited lower MIC against *E. coli* (0.00077 mg/mL) and C. *albicans* (0.0851 mg/mL), Neem soap showed higher antifungal activity against *T. rubrum* (0.00000099 mg/mL) and *B. subtilis* while both soaps exhibited poor efficacy against *P. aeruginosa*. The contact time of the soap samples on the test organisms was not a determining factor in the inhibitory behavior of the samples. The ability of these soaps to inhibit pathogens at low concentrations highlights their potential as natural alternatives to synthetic antiseptics which will warrant the assessment of their synergistic effect, long-term stability and efficacy.

**Keywords**: Antisepsis, MIC, Herbal soap, Antimicrobial, Regression

**Introduction**

Soap constitutes the largest group of the detergents that are in common use today as they constitute about 95% of all the detergents(Wilkinson,1974). It is a molecule of long hydrocarbon chain with a carboxylic acid group on one end which is ionic bonded to a metal ion, usually a sodium or potassium. Soap play an important role in human hygiene and health. There are arrays of cleaning agents available in the market, which are presented in various forms with distinct formulations. Among these formulations are Triclosan, trichlorocarbamide and p-chloro-m-xylenol (PCMX/ chloroxylenol) which are the commonly used antibacterial in antiseptic soaps, most of which are known to be carcinogenic, mutagenic and able to generate allergic reactions (Ameh *et al.,* 2013). These compounds have low toxicity, their 2-hydroxy isomers have been shown to undergo thermal and photochemical ring closure to form polychlorinated dibenzo-p-dioxins which are toxic in nature, thus making it unsafe for human skin (Okumura and Nishikawa, 1996). Furthermore, it is believed that some bacteria could develop resistance to triclosan and this could lead to development of resistance and change in microbial community structure (Aiello *et al*., 2007).

The term “antiseptics” is used to describe products with the property of preventing and/or inhibiting the growth of microorganisms on the skin and mucosa up to levels considered safe and within an adequate period. This class includes degerming soaps, antiseptics, products that contain alcohol in concentrations used for antisepsis, sanitizing products for domestic and hospital use, personal hygiene products, and mouthwash, among others (ABDI, 2015). The choice of the ideal product has been of great interest to people, considering the diversity of products, the large market offers, and the variations in guidelines regarding indications and use (Toigo *et al.,* 2020).

While many of the synthetic antiseptic soaps seems exorbitant and not easily affordable especially in developing countries, herbal soaps provide an affordable and sustainable cheap means with comparative health and safety benefits (Joshi and Pawal, 2015; Sharma *et al.,* 2008). These shortcomings of antiseptic soap - treated with synthetic antiseptics has led to a search for a more health and environment - friendly herbal soaps with antiseptic properties which can be sourced from medicinal plants of natural origin.

Most medicinal plants in Nigeria are embedded with fatty acids, amines, proteins and esters which are essential for maintaining body skin health. Among these medicinal plants are *Moringa oleifera* and *Azadirachta indica* which are readily availble. *Moringa oleifera* leaves, flower, fruit, seed contains range of antioxidants (phenolic acids and ascorbic acids), isothiocyanates and saponin which have anti-inflammatory and antimicrobial properties. *Azadirachta indica* on the other hand contains azadirachtin, a triterpenoid with antifungal and antibacterial properties. It also contains quercetin and some glucosides with anti-inflammatory properties. (Sutar *et al.*, 2008)

Herbal soaps contain antioxidant, anticancer and antimicrobial agents that could help in the management of various skin and hair conditions. The presence of phytochemicals such as vitamins, proteins, tannins, terpenoids and other bioactive ingredients rejuvenate, freshen and protect the hair and skin from various skin and hair conditions such as psoriasis, eczema, skin dryness, skin cancers, sun burn, skin dryness. More awareness has been given to the use of natural antioxidants in the prevention of certain disease caused by free radicals. The eco-friendliness, health benefits, efficacy and cheap cost of these herbal soaps also explain its preference over the orthodox medicated soaps despite their readily availability and popularity (Lifongo *et al*, 2014., Nasir *et al* 2015., Adebayo and Krettli, 2011).

In adherence to the principle of green chemistry, herbal soaps prepared from plant-based renewable sources must align with the United Nation 17 sustainable development goals in ensuring protection of the planet (SDG 13), having good health (SDG 3), sustainable community (SDG 7), clean energy and making life on land (SDG 7&15) and in water safer (SDG 14) (UNDP-SDG, 2000). This paper therefore evaluates the antiseptic properties of cocoa pod husk soaps blended with *Moringa oleifera* and *Azadirachta indica*.

**2. Materials and Methods**

*2.1 Preparation of Antiseptic herbs*

The herbs (*Moringa oleifera* and *Azadirachta indica*) used for the study were obtained from the fruiting tree within the Ibadan metropolis of Nigeria, Ibadan. The leaves of these herbs were dried at room temperature, pulverized and retained until ready for use.

*2.2 FTIR Analyses of Moringa oleifera and Azadirachta indica*

The Fourier Transform Infrared Spectroscopy (FTIR) analyses of *Moringa oleifera* and *Azadirachta indica* were carried out using FTIR (8400S Shimadzu, Japan). The range was 4000 to 400 cm-1 wavelength and the matrix used was KBr pellet.

*2.3 Preparation of Cocoa Pod Husk Antiseptic soaps*

Cocoa Pod Husk Soaps were prepared according to the methods of Yahaya et al., 2002, 2012. In a typical experiment, one-part weight of Palm kernel oil was reacted with two-part weight of lye extracted from the pod husk of cocoa. The mixture was boiled through saponification process. Boiling continued until a semi-solid mass was obtained. The antiseptic soaps were prepared by incorporating varied amount (% w/w) of *Moringa oleifera* and *Azadirachta indica* powder. Variation of blends was made at 0, 2, 6, 12 % (w/w) which are coded MRG-0, MRG-2, MRG-6, MRG-12 and NEM-0, NEM-2, NEM-6, NEM-12 for *Moringa oleifera* and *Azadirachta indica* soaps respectively.

*2.4 Soap characterization*

*2.4.1 pH analysis* The pH values of the produced soaps were analyzed using a pH meter (Kent EIL 7055). 2.0 g of the produced soaps were dissolved in 50 ml of deionised water and the pH determined using the meter. This was done twice for each soap sample and the mean value computed.

*2.4.2 Hardness test*. To determine the hardness of the soap, a needle (6.4 cm in length; 1 mm in diameter) to which a lead fishing weight (130 g) was attached was lowered unto the soap, the distance into which the needle penetrates the soap, after 30 s, was recorded as a measure of its hardness. This was replicated to obtain mean value.

*2.4.3 Foamablity test*. This involved dissolving 2.00 g of the soap in 50 ml of distilled water in a 100 ml measuring cylinder and shaken vigorously for 2 min. It was allowed to stand for 10 min after which the height of the foam was determined. This was repeated thrice for each soap sample and the mean computed.

*2.4.4 Solubility tests*: Into 100 mL measuring cylinder containing 10 mL of distilled water was added 0.2 g of each soap. The duration of the dissolution of the soap after continuous shaking was recorded.

2.5 Determination of minimum inhibitory concentration (MIC)

Pure cultures of test organisms were obtained from the Department of Pharmaceutical microbiology, faculty of Pharmacy, University of Ibadan. Bacteria: *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeuroginosa and* Fungi: *Trichophyton spp. Candida albicans*. The organisms were purified using nutrient agar (bacteria) and sabourad dextrose agar. Broth suspensions were prepared using McFarland standard (1 × 10⁶ CFU/µl) (Schön, 2020).

Using Agar Diffusion Technique, nutrient agar (for bacteria) and Sabouraud dextrose agar (for fungi) were sterilized and poured into 85 mm sterile petri dishes and allowed to set (Balouiri, 2016). 50 µl of the prepared pathogen suspension was spread evenly on the surface of the agar. Five millimtere diameter wells were bored into the agar using a sterile cork borer. Fifty microlitire of each soap solution (MRG and NEM) was pipetted into the wells in duplicate. Distilled water used as negative control and Lactic acid (antimicrobial agent) as positive control. The plates were incubated at room temperature for 18–48 hours for bacteria and 48 hours for Fungal plates. The diameter of the zones of inhibition (millimeter) around the wells was measured using a meter rule. Mean zones of inhibition obtained by dividing the sum of the replicates by two. While minimum inhibitory concentration (MIC) of the soap on test pathogens was analyzed using linear regression plotting the logarithm of the soap concertation against the mean zone of inhibition at 5mm threshold (Bora, 2016).

**3. Results and Discussion**

 *3.1 FTIR Analyses of Moringa oleifera and Azadirachta indica*

Figures 1 and 2 shows the FTIR spectrum of *Moringa oleifera* and *Azadirachta indica* respectively while table 1 and 2 shows the peaks and their assignments. The functional group (C=O, C=H and CH stretch) identified in Tables 1 and 2 are typical of most organic compounds. For example, absorption at 3282.9 cm-1, 3292 cm-1 are characteristics of hydroxyl groups which are possibly from phenolic compounds or the glycosides. Absorption at 2918.5, 2851, 2918.5, 2851cm-1 indicates the presence of methyl (-CH3) and methylene (-CH2-) groups possibly from the fatty acids or terpenes. The absorption at 1598.6 cm-1 may likely indicate the presence of C=C, from unsaturated fatty acids. Peaks at 1607.9, 1367.8, 1318 cm-1 for *Azadirachta indica* may suggests the presence of alkaloids such as nimbin and nimbidin.



 Fig. 1 FTIR Spectrum of *M. oleifera*



Fig. 2. FTIR Spectrum of *Azadirachta indica*

Table 1: FTIR Spectrum Peaks and assignment of *Moringa oleifera*

|  |  |  |
| --- | --- | --- |
| **Peaks (cm-1)** | **Assignment** | **Remark** |
| 3282.9 | O-H Stretching | Vibration (aliphatic) |
| 2917.1, 2849.1 | C-H Stretching | vibration (aliphatic) |
| 1733 | C=O Stretching | vibration (ester |
| 1598.6 | C=C Bending | vibration (aliphatic) |
| 1239.7 | C-O-C Stretching | vibration (ester |
| 720 | C-H group  | vibration (aliphatic) |

Table 2*:* FTIR Spectrum peaks and assignment of *Azadirachta indica*

|  |  |  |
| --- | --- | --- |
| **Peaks (cm-1)** | **Assignment** | **Remark** |
| 3292 | O-H Stretching | vibration (aliphatic) |
| 2918.5, 2851  | C-H Stretching | vibration (aliphatic) |
| 1725 | C=O Stretching | vibration (ester) |
| 1607.9, 1367.8, 1318 | C= C bending | vibration (aliphatic) |

**3.2 Characteristics of soap samples**

The physical characteristics of soap samples (Table 3) revealed that the pH value of all the soaps fell between 9.50 -9.81 and are within acceptable limit for household bathing soap of 9- 11 (Mak-Mensah and Firempong, 2011). These pH Values are also in consonant with those obtained by (Ogunsuyi and Akinnawo, 2012; Vivian *et al*., 2014). The implication of this is that the corrosive effect of the soaps would be minimal and is expected to produce mild effect on the skin. Soaps with high pH are known to be corrosive to the skin as they can increase dehydration, irritation and destruction of the bacterial flora in the body (Tarun *et al.*, 2014). Interestingly, the use of lye from cocoa pod husk, a source of alkali in the production of these soaps is noteworthy. The hardness of the soaps which is the extent of penetration of the loaded needle on the soap samples revealed that the soaps are generally soft. This is so because potash from cocoa pod husk produces soft soap. However, results indicated that level of hardness is a function of the amount of herb introduced into the sample. It is observed that RMG-12 and NEM-12 were relatively harder compared to other soaps evaluated with degree of hardness of 3.91 and 5.51 cm respectively, while NEM-0 and RMG-0 were softer with 4.63 and 6.19 cm degree of hardness. The implication of this is that the herb must have imparted certain level of hardness on the soap samples. Table 3 also showed the foamability of the various soap samples evaluated. As indicated, soaps with zero concentrations of herb were more foaming than those with herbs. Soaps RMG-0 and NEM-0 exhibited high foamability with foam heights of 18.31 and 18.22 cm respectively. It is obvious that inclusion of herbs in the soap formulation decreased the foamability. The solubility and hardness of the soaps is an indication of the ability of the soaps to last longer when used due to its ability to slowly dissolve in water.

Table. 3 Physical characteristics of the Soap samples.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Soap Sample** | **pH** | **Hardness (cm)** | **Foam height (cm3)** | **Solubility (sec)** |
| RMG-0 | 9.79 | 4.63 | 18.31 | 620 |
| RMG-2 | 9.72 | 4.51 | 17.22 | 622 |
| RMG-6 | 9.69 | 4.12 | 16.91 | 504 |
| RMG-12 | 9.60 | 3.91 | 16.80 | 615 |
| NEM-0 | 9.81 | 6.19 | 18.22 | 605 |
| NEM-2 | 9.77 | 6.11 | 18.01 | 609 |
| NEM-6 | 9.50 | 5.63 | 17.11 | 544 |
| NEM-12 | 9.50 | 5.50 | 16.96 | 551 |

**3.3 Minimum inhibitory concentration (MIC) of soap**

Antiseptics are active against a large number of microorganisms, while others may only be active for one species. However, there is no ideal antiseptic soap for all purposes (Costa *et al.,* 2018). Table 4 presents the results of the sensitivity of the various soap concentrations on the pathogens *S. aureus*, *B*. *subtilis,* *E. coli*, *T. rubrum*, *P. aeruginosa*, and *C. albican* after 18 hours of contact. All the prepared soaps (MRG and NEM) showed appreciable level of antiseptic property against some tested pathogens. The tests showed effectiveness of antiseptic action of the soap samples, which is an indicated by the clear zone around the well. Wider zone diameter is an indication of higher activity of the soap sample to inhibit the growth of the pathogen. One of the pathogens used for this test for example is *S. aureus* which is commonly found in public toilets and human genitals. According to the result, NEM soap exhibited more inhibitory action against *S. aureus* than MRG as evident with a zone of inhibition of 30.25 mm at 6% concentration.

Table 4 Sensitivity of pathogens to soap concentrations after 18 hours

|  |  |
| --- | --- |
| **Test organism** |  **Mean Zone of inhibition (mm)** |
|  **MRG Concentration (%)**  |  **NEM Concentration (%)**  |
|  | 0 | 2 | 6 | 12 | 0 | 2 | 6 | 12 |
| *S. aureus* | 13.0 | 17.5 | 17.5 | 18.5 | 15.75 | 15.75 | 17.70 | 29.0 |
| *B. subtilis* | 16.5 | 19.0 | 23.0 | 23.5 | 16.5 | 15.75 | 21.0 | 26.25 |
| *E.coli* | 18.5 | 26.50 | 18.75 | 18.5 | 18.5 | 36.5 | 34.0 | 18.5 |
| *T. rubrum* | 15.5 | 30.25 | 25.0 | 18.25 | 15.5 | 31.25 | 31.25 | 18.75 |
| *P. aeruginosa* | 23.5 | 26.75 | 19.0 | 25.0 | 23.5 | 23.5 | 18.0 | 17.75 |
| *C. albican* | 15.25 | 17.25 | 23.25 | 26.0 | 15.25 | 33.0 | 23.0 | 17.75 |

Table 5. Sensitivity of pathogens to soap concentrations after 48 hours

|  |  |
| --- | --- |
| **Test organism** |  **Mean Zone of inhibition (mm)** |
|  **MRG Concentration (%)**  |  **NEM Concentration (%)**  |
|  | 0 | 2 | 6 | 12 | 0 | 2 | 6 | 12 |
| *S. aureus* | 13.0 | 19.0 | 19.5 | 19.75 | 17.5 | 29.75 | 30.25 | 16.75 |
| *B. subtilis* | 16.5 | 17.5 | 23.5 | 26.50 | 16.5 | 26.25 | 21.0 | 19.5 |
| *E.coli* | 18.5 | 28.5 | 27.0 | 26.25 | 18.5 | 36.5 | 28.5 | 17.0 |
| *T. rubrum* | 15.5 | 28.5 | 27.25 | 36.5 | 15.5 | 31.25 | 32.0 | 19.0 |
| *P. aeruginosa* | 23.5 | 28.25 | 20.0 | 27.0 | 23.5 | 23.5 | 19.0 | 18.75 |
| *C. albican* | 15.25 | 28.0 | 22.75 | 33.5 | 15.25 | 35.25 | 23.25 | 18.25 |

For *B. subtilis,* MRG soap proved to be more effective in inhibiting the pathogen at 12 % concentration as can be seen with a zone diameter of 26.50 mm. On the other hand, the effectiveness of MRG and NEM soaps to inhibit *E. coli* growth decreases with concentrations. While NEM soap showed the highest inhibition at 2 % concentration, MRG does at the same concentration with 26.50 mm zone diameter. Similar results were obtained by Mohammed *et al*., 2022 who investigated the antiseptic properties of soaps with the seed, bark and leaves of Neem. Results also showed that both soap samples exhibited effective inhibition to the fungi *P. aeruginosa* at low concentrations of 2 % with 26.75mm and 23.5mm for MRG and NEM respectively.

Table 5 presents the result of the pathogens sensitivity to the soap samples after 48 hours of contact. There was a similar trend in the inhibition behavior of the soap samples. The implication of this is that inhibition of soap sample is not a function of contact time.

Table 6. MIC Values for MRG and NEM

|  |  |  |  |
| --- | --- | --- | --- |
| Pathogen |  MRG (mg/mL) |  NEM  (mg/mL) | Susceptibility |
| *S. aureus* | 0.0147 | Very high (resistant) | MRG is much more effective. |
| *B. subtilis* | 0.0810 | Very low | NEM soap is much more effective. |
| *E. coli* | 0.00077 | 493,733.18 | MRG is highly effective, but NEM soap is ineffective. |
| *T. rubrum* | 0.1431 | 0.00000099 | NEM soap is significantly more effective. |
| *P. aeruginosa* | Extremely high (resistant) | Very high(Resistant) | Both soaps are largely ineffective. |
| *C. albicans* | 0.0851 | Very high (resistant) | MRG is more effective. |

\*(using a 5 mm inhibition threshold): MRG (Moringa), NEM(Neem)



 Fig 3. Regression analysis of MIC of MRG and NEM Soap on test Pathogens

The regression analysis of Moringa and Neem soaps revealed distinct antimicrobial properties based on their botanical composition. In Figure 3, Moringa soap exhibited strong antibacterial activity against *S. aureus* with 0.0147 mg/mL, whereas Neem soap showed high resistance, supporting Anwar *et al*., 2007 findings that *M. oleifera* extracts contain isothiocyanates and flavonoids with potent effects against Gram-positive bacteria. Conversely, Neem soap was significantly more effective against *B. subtilis* due to the presence of azadirachtin and nimbidin, compounds with broad-spectrum antibacterial properties (Girish & Shankara, 2008). For *E. coli,* Moringa soap exhibited superior efficacy with 0.00077 mg/mL, while Neem soap was largely ineffective (493,733.18 mg/mL), aligning with reports of Okeke et al, 2005 that Moringa extracts contain pteridosperm and benzyl isothiocyanate, which are particularly effective against Gram-negative bacteria. Regarding fungal activity, Neem soap demonstrated higher antifungal effects against *T. rubrum* with 0.00000099 mg/mL compared to Moringa soap (0.1431 mg/mL), corroborating previous studies that highlight Neem's efficacy against dermatophytes (Ospina Salazar *et al.,* 2015). However, both soaps exhibited poor efficacy against *P. aeruginosa*, which is known for its biofilm formation and efflux pump mechanisms that reduce susceptibility to plant-derived antimicrobials (Nguyen *et al.,* 2011). Finally, Moringa soap displayed better activity against *C. albicans* with 0.0851 mg/mL, whereas Neem soap showed high resistance, which aligns with findings that phenolic and flavonoid compounds in Moringa contribute to its antifungal properties (Zanna *et al.,* 2015, Nwosu and Okafor, 1995).

**Conclusion**

This study developed antiseptic soaps from cocoa pod husk using *Moringa oleifera* and *Azadirachta indica* as antimicrobial agents. Moringa soap demonstrated greater antibacterial efficacy against pathogens *Staphylococcus aureus* and *Escherichia coli*, while Neem soap was more effective against the fungus *Trichophyton rubrum*. Both soaps were ineffective against *Pseudomonas aeruginosa*, suggesting a need for enhanced formulations or synergistic combinations to expand their antimicrobial spectrum. The ability of these soaps to inhibit pathogens at low concentrations highlights their potential as natural alternatives to synthetic antiseptics, warranting further research to enhance their formulations and assess their long-term stability and efficacy.

**Conflict of interest**

Authors have declared that no competing interests exist.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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