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Production and Characterization of Soaps from *Croton Megalobotrys* and *Ricinus Communis* seed oils Indigenous to Botswana with High Levels of Antimicrobial and Antioxidant Activity

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Abstract
Crotonmegalabotrys mixed with Ricinnus cummunis and coconut seed oils produced high quality organic soaps with some levels of antioxidant and antimicrobial activities. The pHs of these soaps ranged from 10.01 and 10.30 showing that these soaps are mildly alkaline and hence cannot burn the skin while at the same time killing bacteria and other microorganisms on the skin surface. The low levels of moisture content at 2.78 % to 2.97 % and low levels of free fatty acid at 0.29 % to 0.75 % shows that these soaps have long shelf live hence good candidates for commercialization. Low levels of matter insoluble in alcohol from 42.00 % to 47.18 % is an indication that soaps produced from these seed oils have few impurities. C. megalabotrys and R. cummunis seed oils exhibited strong antioxidant activity while the soap produced from these seed oils also exhibited some level of antioxidant activity proving that both the oils and the soaps can scavenge for free radicals produced on the skin surface reducing the formation of most skin diseases. The C. megalabotrys/R. cummunis seed oils soap exhibited antimicrobial activity against gram-positive bacterium - an indication that this soap can prevent growth of gram-positive bacteria growing on the skin surface such as Staphylococcus aureus and Streptococcus pyogenes which are the most identified causes of skin infections in humans.

Key Words*: Soap Production, Antimicrobial Soap, Antioxidant Activity, Croton megalobotrys, Saponification reaction, Soap Quality*

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I. Introduction

Climate change and population increase have inspired researchers to research on sustainable green feedstocks for industrial production lines including that of soap and cosmetic industries (Nicola *et al*., 2012, Graebel and Klee, 2002). Soaps are surfactants (surface-active agents) which have both the hydrophilic and hydrophobic functional groups. During cleaning of greasy surfaces or oily fabrics, the hydrophobic (lipophilic) end attaches to the oil/grease breaking it up into smaller molecules while the hydrophilic end attaches to water molecules dispersing them and washing away the dirt (Rambabu *et al*., 2020 and Lin *et al*., 2005). Soaps are also defined as water soluble salts of fatty acids which contain more than eight carbon atoms (Shehata, 2020). Natural soaps are alkali salts of fatty acids from vegetable oils and animal fats produced through the process of saponification (Antoni´c et al., 2020 and Vidal et al., 2018), Figure 1.

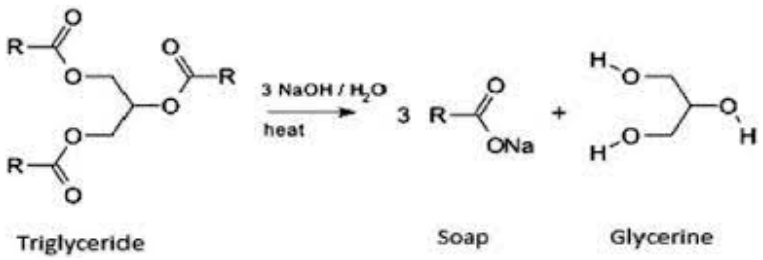

Figure 1: Saponification reaction of plant oil using NaOH (aq) to produce soap and glycerine as a byproduct. (Deb et al., 2017).

There are two broad types of soaps depending on the feedstocks used being, natural and synthetic (syndet) soaps. In natural soaps, oils and fats from plants and animal sources are used as triglyceride sources without the addition of plasticizers, binders, and preservatives (Friedman, 2016; Abbas *et al.,* 2004). Synthetic soaps are made from blends of synthetic surfactants, plasticizers, binders, and other additives. They are formulated from fats, petroleum, and oil-based products using a mixture of sulfonation, ethoxylation, and esterification processes (Hollstein and Spitz, 1982; Abbas *et al*., 2004).

Maintaining a beautiful, clean skin and hair is done daily by all people all over the world including Botswana and Africa as a whole. Therefore, the application of safe cosmetics with fewer to zero environmental effects and less toxicity to users is highly desirable. Cosmetics from natural sources containing bioactive phytochemical compounds have been found to offer these desirable effects (Atolani *et al*., 2016). It has been noted that cosmetics, including soap, from natural feedstocks can improve social, psychological, and clinical impulses of users. The use of cosmetics from synthetic and artificial sources have been found to result in brittle and browning of hair, damaged and dry skin, and itching of the scalp and skin among many other side effects (Joshi and Pawal, 2015). The criteria for the selection of oil for industrial or domestic application in soap-making includes the presence of natural characteristic aroma, clarity, natural color, low moisture content and absence of flat and rancid (unpleasant) odor (Manji *et al.*, 2013).

The year 2019 saw the entire world plagued by Covid-19 pandemic of catastrophic effect. Small countries such as Botswana and the African continent were highly affected. Frequent and correct washing of hands and surfaces with soap and water were encouraged by the World Health Organization (WHO) as a sustainable and cost effect way of preventing the spread of Covid-19 virus (WHO, 2020, Wiktorczyk-Kapischke *et al*., 2021). This frequent handwashing led to significant usage of soaps. There is, therefore, a need to increase production of soaps by developing countries. All these detergents' chemical ingredients most of which are derived from petrochemicals are transferred to the environment after every wash. Therefore, there is a need for eco-friendly cleaning agents for human and environmental safety (Chirani *et al*., 2021).

Atolani *et al*., 2016, formulated natural antiseptic soaps from seed oils of *Daniellia oliveri, Elaeis guineensis* and *Vitellaria paradox* plants with *Moringa oleifera* seed oil as a source of antimicrobial agents. The soaps made from the oil of *Daniellia oliveri* and *Vitellaria paradoxa* inhibited the growth of *Streptococcus aureus,* a gram-positive bacterium and *Klebsiella granulomatis,*a gram-negative bacterium. This shows that this natural soap can protect the skin against most bad bacteria which are prone to affecting the human/animal skin. Of recent, great attention has also been given to the use of natural antioxidants for prevention of skin diseases caused by oxidative stress in the human body (Teow *et al*., 2006). Free radicals that are generated by both endogenous and exogenous factors constantly expose skin cells to the harmful effects of these free radicals. The skin is susceptible to the effects of free radicals when they are produced in excessive amounts even though the skin has natural defense mechanisms against them. Natural compounds present in plants including plant seed oils exhibit antioxidant properties and the ability to scavenge these free radicals (Godic *et al.*, 2014; Nichols and Katiyar, 2010).

Croton megalobotrys is a semideciduous savannah species that grows at medium to low altitudes on alluvial soils and is a component of riverine and swamp fringe woodland or thicket. It is a non-edible plant, and it belongs to the large genus of *Euphorbiaceae*, comprising around 1,300 species of trees, shrubs and herbs distributed in tropical and subtropical regions of both hemispheres. It is a 15-metre-tall tree with a conical to rounded, spreading crown. The bark is pale grey and smooth when young, with vertical lines of lenticels, but becomes rough and fissured as the tree becomes older. Simple, serrated, ovate leaves with three veins showing from the leaf's heart-shaped base (Setshogo and Fenter, 2003; Antonio *et al*. 2007; Abiola *et al*., 2018). The tree grows mostly in Botswana, in the Northwestern region, primarily along the Thamalakane River, Okavango River, and the pristine Okavango Delta and it is known as Motsebi or Letsebi tree in these areas while it is called Moshoole tree in the central district near rivers in the Tswapong region (Setshogo and Fenter, 2003; Paphane *et al*., 2021).

In our earlier work, we reported that *C. megalobotrys* seed oil is made up of five (5) fatty acids, the most abundant being linoleic acid which makes up 58.01 % of the seed oil. The other two significant fatty acids are palmitic and oleic acids at 19.51 % and 18.37 %, respectively (Paphane *et al*., 2021). Linoleic acid helps strengthen the skin's barrier so it can effectively keep water in and irritants out. Oils that are rich in linoleic acid prevent the overproduction of oleic acid, therefore balancing sebum production and oiliness that is associated with acne-prone skin. In some cases, just rubbing a small amount of linoleic acid on mild acne can reduce the size of pimples. On top of that, it is used to treat other skin concerns such as dryness, dehydration, pigmentation, and sensitivity (Liddle, 2021). Palmitic acid on the other hand is used to produce soaps, cosmetics, and industrial mold release agents. These applications use sodium palmitate, which is commonly obtained by saponification of Palmitic acid containing seed oil such as palm oil (May and Nesaretnam, 2014). Oleic acid is a major component of soap as an emulsifying agent. It is also used as an emollient (moisturizer). Small amounts of oleic acid are used as an excipient in pharmaceuticals, and it is used as an emulsifying or solubilizing agent in aerosol products (Susan, 1992).

We also reported that saponification values for *C. megalobotrys* seed-oils from different regions in Botswana ranged from 140.09 ± 1.81 to 153.01 ± 1.67 (Paphane et al., 2021). These relatively high saponification values implies that the seed oil contain high molecular weight triglycerides which confirm their suitability and useful applications for the manufacture of soaps and other cosmetic products (Omari et al., 2015). In this paper, we discuss the production, quality parameters, antioxidant, and antimicrobial abilities of toilet (solid bar) and hand (liquid) soaps produced from *C. megalobotrys* seed-oil and other seed oils.

II. Materials And Methods

Croton megalabotrys seed oil and *Ricinus cummunis* seed oil were extracted with the soxhlet extraction unit using the solvent extraction method in *n*-hexane (Bokhari *et al*., 2015). Quality parameters and characterization of these seed oils have been reported extensively in our earlier work (Paphane *et al*., 2021). Helianthus annuus (Sunflower) and *Cocos nucifera* (coconut) seed oils, commercial solid bar soap (dettol branded, South Africa) and liquid soap (Sunlight branded, South Africa) which were chosen for comparison purposes were bought from the local supermarket and used without further purification.

Soap Production

The soap samples used in this study were produced by mixing a standard prepared quantity of lye (NaOH (aq) for solid bar soaps and KOH (aq) for liquid soaps) with a known quantity of the oil under vigorous mixing with a standard electric food blender. The standard lye solution was prepared by calculating the quantity of NaOH/KOH (caustic soda) that would saponify each sample of the oil. The quantity of caustic soda used in the production of the soaps was determined using the mathematical relation below (Mohammed and Usman, 2018).

$$\text{Quantity of caustic soda} = \frac{200\ x\ Std\ S.V(Oil)}{1} \qquad (1)$$

Where,
200 = the quantity of oil used
StdS.V(Oil) = the standard saponification value of oil.
1 = number of grams of oil saponified by 1g of Caustic Soda

From equation 1, the quantity of caustic soda used to saponify 200 g of different oils is calculated and presented in Table 1 below. The amount of water used to dissolve the caustic soda was set at a caustic soda water ratio of 1:3.

Table 1: Quantities of Caustic Soda used to produce the Oil Soaps.

Name of Oil	Standard Saponification value	Quantity of caustic soda (g)
*Croton megalobotrys*seed oil	0.153	30.6
Sunflower seed oil	0.185	37.0
Ricinus Cummunis seed oil	0.141	28.2

Processing of the Soaps

For solid bar soaps the cold saponification method of production was used to produce the soaps. 200g of 1:10 ratio of olive oil and oil of interest (*C megalabotrys*/sunflower) was slowly poured into the corresponding 25% aqueous sodium hydroxide solution and vigorously stirred repeatedly to form liquid paste. The paste was stirred continuously using an electric blender until the thickness of the paste increases and a trace-mark begins to appear. The paste was then transferred into a plastic mold and allowed to cure at ambient temperature into a solid rectangular soap bar. After about 9 hours the bars were cut into smaller bars and left to dry completely under ambient temperature in a desiccator for 7 days before quality analysis.

For liquid soaps the same procedure was followed but this time using aqueous potassium hydroxide instead of sodium hydroxide. The mixture was stirred vigorously using an electric blender until the mixture has turned into a thick paste. The paste was let to cure at ambient temperature for 24 hours and then dissolved in water at 1:2 soap paste water ratio for quality determination. Figure 1 and 2 shows the soaps produced using different oil combinations.

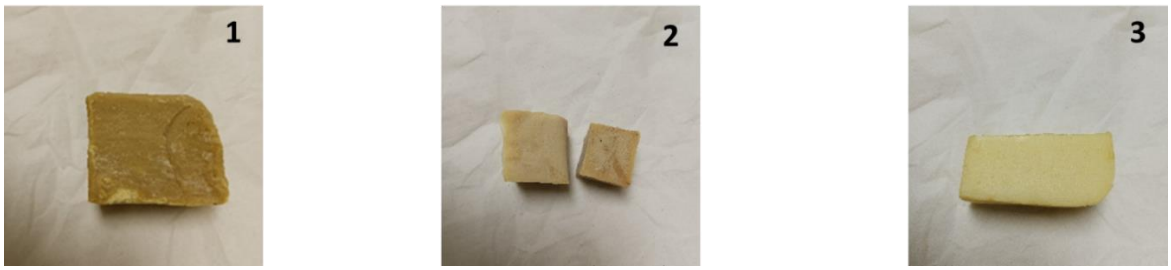

Figure 1: Solid bar soaps produced from 10:1 ratio of *C. megalabotrys* and Coconut seed oils (1), 7:2:1 ratio of *C. megalabotrys, R. cummunis* and Coconut seed oils (2) and 10:1 ratio Sunflower and Coconut seed oils (3 (control)).

Figure 2: Liquid soaps produced from 7:2:1 ratio of *C. megalabotrys, R. cummunis* and Coconut seed oils (4) and 10:1 ratio Sunflower and Coconut seed oils (5 (control)).

Quality assessment of soaps

Analysis of quality parameters of the different soaps produced were performed as per the International Standards Organization (ISO) number 685 of 2020 (ISO 685:2020_Analysis of Soaps).

Determination of pH

2 g of soap sample was added into 20 mL distilled water in a conical flask and vigorously shaken until the soap sample has completely dissolved. The soap suspension was allowed to stand under ambient temperature for at least 12 hours before pH measurements were taken. pH measurements were measured using ino-Lab 7110 WTW Lap-pH meter (UK).

Determination of matter insoluble in Alcohol (MIA)

5 g of soap sample was dissolved in 50 mL of hot ethanol and quantitatively transferred into a pre-weighed filter paper on a conical flask. The residue remaining in the filter paper was dried in an oven set at 105 ˚C for 30 minutes. After 30 minutes the filter paper with the soap residue was cooled then weighed.

$$\text{MIA} = \frac{Ws-FP}{W} \text{ x } 100$$

Where Ws is the weight of soap residue + filter paper, FP is the weight of pre-weighed filter paper and W is the weight of sample.

Determination of Moisture Content/Volatile matter (MC)

Five (5) g of soap sample was weighed into a pre-weighed tarred dry moisture dish and dried in an oven at 101 ˚C for 2 hours then cooled and weighed. The process was repeated until constant weight was reached.

$$\% \text{ Moisture/Volatiles} = \frac{Cs-Cl}{Cs-Cw} \text{ x } 100$$

Where, C_w is weight of crucible, C_s is the weight of crucible + sample and C_L is weight of crucible + sample after drying.

Determination of Free Fatty Matter (FFM)

$$FFM = \frac{100-(MC-MIA)}{1.085}$$

Where, MC is moisture content and MIA is matter insoluble in alcohol.

Determination of foam stability

One (1) % (w/w) of soap sample was prepared in distilled water. Five (5) mL of soap solutions was then taken into a test tube and shaken vigorously for 1 minute and then allowed to stand for 5 minutes. The height of the foam for each sample was observed and recorded.

Determination of washing properties

A small amount of dry soap sample was used to wash hands using deionized water. The lathering properties and feel of the soap was then recorded as either very slippery, greasy, or about normal.

Determination of cleaning properties

A drop of used oil collected from the University refectory was placed on a strip of white cloth. The cloth was then washed using soapy water of the soap sample prepared and then rinsed with distilled water. The cleaning power of each soap sample was observed and recorded.

Determination of Free caustic alkali (NaOH/KOH) and/or Free Fatty Acid

Ten (10) g of soap sample was weighed into a 250 mL conical flask containing 100 mL of ethanol. The soap sample was then dissolved over hot plate with continuous stirring using magnetic stirrer. Then, 5 mL of barium chloride solution was added to remove any traces of carbonates present. Two (2) to three (3) drops of phenolphthalein indicator were then added to the mixture. If the solution turned to pink the solution was titrated with HCl until the pink color disappeared and its alkalinity calculated. If not, the solution was titrated against NaOH (aq) until pink color appears and percentage free acid was calculated as Linoleic acid ($C_{18}H_{32}O_2$) for soaps prepared with *C. megalobotrys* seed oil.

Antioxidant and anti-microbial studies

Radical scavenging ability (DPPH assay)

The basis of the radical scavenging method is reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals by antioxidants which results in the discoloration of the DPPH methanol solution from purple to yellow (Kukic et al., 2008). DPPH solution was prepared according to Shimamura *et al*., 2014, method with modifications. Ten (10) mL of 0.1 mM DPPH solution was transferred in a test tube which was stoppered immediately. The stoppered test tube was immediately wrapped with aluminum foil and kept in the dark for 1 hour 30 minutes for DPPH to stabilize. Sample solutions (2000 µL) were transferred into test tubes then methanol (1000 µL) was added to each sample and mixed by shaking. To each sample mixture, 1000 µL of DPPH solution was added. The solutions were immediately mixed by shaking for 10s. The test tubes (wrapped in aluminum foil) were left in a dark cupboard for 30 minutes and 1 hour respectively. After 30 minutes and 1 hour, the test tubes were observed for color change respectively (Ghazanfari, *et al*., 2020).

Anti-microbial activity

Antimicrobial activity of all the samples were determined using a modified disc diffusion assay (Sautron and Cock, 2014). The microbial strains used in this study were obtained from the Faculty of Science, Department of Biological Sciences, University of Agriculture and Natural Resources, Botswana. These strains included one Gram-positive bacterium, *Escherichia coli* (*E coli*), and one Gram-negative strain; *Pseudomonas aeruginosa* Test bacteria. *Pseudomonas aeruginosa* and *Escherichia coli* were grown with shaking (150 rpm) at 37℃ in 250 mL Erlenmeyer flasks containing 100 mL nutrient broth to a density of MacFarland 0.5. The test strains were inoculated over the entire surface of Muller Hinton agar (Merck, Germany) using a sterile swab (Nazzaro *et al*., 2013).

Sterilized blank filter paper discs (5 mm in diameter) were impregnated with selected dilutions of seed oils and concentrated soap suspensions in water for 10 min. The discs were placed onto the surface of inoculated agar plates. Each plate contained a maximum of three impregnated discs evenly spaced. The plates were incubated at 37℃ for 24 hours. After 24 hours the plates were observed for zones of inhibition. Standard discs of penicillin and tetracycline (Oxoid Ltd, Ireland) served as positive controls for Gram-positive and Gram-negative bacteria respectively, while distilled water was used as negative control for antimicrobial susceptibility (Goni *et al*., 2009).

Statistical analysis

All experiments were performed in triplicate. Results are presented as means and standard deviations (±SD). A statistically significant difference between treatments was found by one-way analysis of variance (ANOVA). Values of $p < 0.05$ are considered significant.

III. Results And Discussion

The experimentally produced soaps were evaluated by chemical parameters, including pH, amount of matter insoluble in alkali (MIA), amount of free fatty matter (FFA), amount of moisture/volatile matter (MC), washing properties, cleaning properties, soap foam stability and free caustic alkali/Free fatty acid. The results were compared against International Standards Organization standard for soaps and detergents of 1986 (ISO 8212:1986) and other related standards to ascertain the quality and safety of the soaps for use.

pH Results

The pHs of the soaps of interest (*C. megalobotrys* soaps) ranged between 10.01 ± 0.02 and 10.30 ± 0.03 for both bar soaps and liquid soaps, see Table 1. This means that the soaps are mildly alkaline and fall within the allowable limits for household soaps of 8.00 to 10.50 (Shehata, 2020). Mildly alkaline soaps are preferred because they will not burn the skin during washing of the skin but is effective enough to kill bacteria and other bad microorganisms found on the surface of human skin. The results agreed with results for control soaps made from commercial sunflower oil (10.03 ± 0.01 to 10.30 ± 0.03). Commercially bought soaps had slightly lower pHs (9.89 ± 0.02 to 9.95 ± 0.02) which is not significantly different from our prepared soaps. This slightly lower pHs might be because they are not purely organic soaps but have artificial compounds added to suppress the pH to make the soaps to have minimal itching effect on the human skin.

Table 1: Color and pH results for the different soaps produced.

Name of Soap	pH of soap solutions	Color
C. Megalabotrys bar soap	10.01 ± 0.02	Golden Brown
C. Megalabotrys/R. Cummunis bar soap	10.12 ± 0.01	Golden Brown
C. Megalabotrys/R. Cummunis liquid soap	10.30 ± 0.03	Golden Brown
Sunflower bar soap	10.30 ± 0.03	Cream White
Sunflower liquid soap	10.03 ± 0.01	Cream White
Commercial bar soap (Dettol) as control	9.89 ± 0.02	Light Green
Commercial liquid soap (Sunlight) as control	9.95 ± 0.02	Dark Green

Moisture Contents

Moisture content is a parameter used in assessing the shelf life of a product. High moisture content in soap would lead to reaction of excess water with unsaponified fat to give free fatty acid and glycerol in a process called hydrolysis of soap on storage (Mahesar *et al*., 2019). Therefore, soaps with low moisture content will have a longer shelf life and hence are preferred. Our bar soaps of interest had very low moisture content ranging from 2.78 ± 0.01 % for *C. Megalabotrys* bar soap to 2.97 ± 0.00 % for *C. Megalabotrys/R. Cummunis* bar soap, respectively, see Table 2. These results agree with results for the control soaps at 2.39 ± 0.01 % for sunflower bar soap and 2.33 ± 0.00 % for Dettol branded commercial bar soap. These results prove that *C. megalabotrys* organic soaps can have a significantly longer shelf life hence preferred for commercialization even though it will be slightly shorter than for other soaps used in this study as controls.

Table 2: Percentage Moisture Content of Bar Soaps.

Name of Soap	% Moisture Content
C. Megalabotrys bar soap	2.78 ± 0.01
C. Megalabotrys/R. Cummunis bar soap	2.97 ± 0.00
Sunflower bar soap	2.39 ± 0.01
Commercial bar soap (Dettol) as control	2.33 ± 0.00

Matter Insoluble in Alcohol (MIA) and Free Fatty Matter (FFM) Content

Matter insoluble in alcohol (MIA) is a parameter that is used to determine the purity of soap. It is the measure of non-soap ingredients known as builders or fillers such as sodium silicate, sodium phosphate, sodium carbonate and minor constituents such as bleachers, whitening agents and fluorescing agents in the finished product. The soap with high MIA values suggests that it contains high level of impurities which may be attributed to the level of impurities of alkali used in producing the soap (Ogunsuyi and Akinnawo, 2012). The MIA values

for *C. megalabotrys oil*-containing soaps are 47.18 ± 0.03 %, 44.00 ± 0.05 % and 42.00 ± 0.03 % for *C. megalabotrys* seed oil bar soap, *C. megalabotrys/R. cummunis*oil bar soap and *C. Megalabotrys/R. Cummunis* seed oil liquid soap, respectively; and these are lower than the maximum standard specification of 70 % (Owoicho, 2021). MIA values for sunflower oil soaps are much lower at 20.14 ± 0.04 % and 25.98 ± 0.05 % for bar and liquid soaps, respectively. This could be because the natural-oil soaps produced in this work do not contain any additives. The MIA values for the commercial bar soap (Dettol) used as control, was higher at 74.16 ± 0.06 % and this could be attributed to the presence of additives. MIA values for commercial liquid soap (sunlight), used as control, was 47.26 ± 0.05 % showing that this commercial soap had few additives compared to the commercial bar soap tested, see Table 3.

The free fatty matter (FFM) of the soaps as presented in Table 3 for *C. megalabotry* seed *s*oil bar soap and *C. megalabotrys/R. cummunis* seed oil bar soap was 46.12 ± 0.04 % and 48.88 ± 0.06 %, respectively. Sunflower oil bar soap had the highest FFM value of 71.40 ± 0.05 % due to low MIA content and the commercial dettol bar soap had the lowest FFM content of 22.59 ± 0.03% due to high MIA content. These parameters further prove that *C. megalabotrys* see oil can produce high quality organic soaps.

Table 3: Matter Insoluble in Alcohol (MIA) and Free Fatty Matter (FFM) Content for soaps produced

Name of Soap	% MIA	% FFM
C. megalabotrys bar soap	47.18 ± 0.03	46.12 ± 0.04
C. megalabotrys/R. cummunis bar soap	44.00 ± 0.05	48.88 ± 0.06
C. megalabotrys/R. cummunis liquid soap	42.00 ± 0.03	N/A
Sunflower bar soap	20.14 ± 0.04	71.40 ± 0.05
Sunflower liquid soap	25.98 ± 0.05	N/A
Commercial bar soap (Dettol) as control	74.16 ± 0.06	22.59 ± 0.03
Commercial liquid soap (Sunlight) as control	47.26 ± 0.05	N/A

Free Caustic Alkali (FCA) and Free Fatty Acid (FFA) Content

Percentage free caustic alkali is a measure of unreacted lye, in this case, sodium hydroxide and potassium hydroxide during the saponification reaction. Free caustic alkali was undetectable in all soap samples assessed as shown in Table 4. This shows that all the lye used to produce soaps reacted with fatty acids in the oils used. Even though care was taken to calculate the exact amount of lye needed to react completely with the fatty acids in the oils used that is not usually the case. Undetectable free caustic alkali normally infers that lye was the limiting reagent during saponification reaction while the free fatty acids were in excess. In our case, percentage free fatty acids for *C. megalobotrys* seed oil soaps ranged from 0.29 ± 0.01 % to 0.75 ± 0.02 % (table 4) which is negligible with liquid soap having the lowest % FFA content. This agreed with soaps used as control (Sunflower seed oil soap (0.27 ± 0.01 % to 0.45 ± 0.02 % FFA) and commercial soaps (0.21 ± 0.01 % to 0.51 ± 0.02 % FFA)). Low levels of FFA are an indication of good quality of the soap and helps the soaps from becoming rancid and odorous, thus, increasing their shelf live (Nchimbi, 2020). Nevertheless, soaps with FFAs have richer moisturizing effect, and has better foaming ability (Berneckė and Maruška, 2013).

Table 4: Free Caustic Alkali (FCA) and Free Fatty Acid Content (FFA)

Name of Soap	% FCA	% FFA
C. Megalabotrys bar soap	Not detectable	0.75 ± 0.02
C. Megalabotrys/R. Cummunis bar soap	Not detectable	0.66 ± 0.01
C. Megalabotrys/R. Cummunis liquid soap	Not detectable	0.29 ± 0.01
Sunflower bar soap	Not detectable	0.45 ± 0.02
Sunflower liquid soap	Not detectable	0.27 ± 0.01
Commercial bar soap (Detol) as control	Not detectable	0.51 ± 0.02
Commercial liquid soap (Sunlight) as control	Not detectable	0.21 ± 0.01

Foam Stability, Washing and Cleaning Properties

All soaps studied exhibited moderate to normal, cleaning and washing properties, respectively. This proved that these soaps can be used on fragile human skin. Foaming stability for *C. megalabotrys* seed oil soaps ranged from 67 ± 3 mm to 81 ± 3 mm after 5 minutes. This is not very far from foam stability of commercial soaps from 85 ± 3 mm to 92 ± 5 mm (Table 5). Liquid soaps were found to have better lathering abilities compared to bar soaps.

Table 5: Foam Stability, Washing and Cleaning Properties of different soaps

Name of Soap	Foaming Stability (mm)	Cleaning Properties	Washing Properties
*C. Megalabotrys*seed oil bar soap	67 ± 3	Moderate	Normal
*C. Megalabotrys/R. Cummunis*seed oil bar soap	78 ± 5	Moderate	Normal
*C. Megalabotrys/R. Cummunis*seed oil liquid soap	81 ± 3	Moderate	Normal
Sunflower seed oil bar soap	67 ± 4	Moderate	Normal
Sunflower seed oil liquid soap	87 ± 2	Moderate	Normal
Commercial bar soap (Dettol) as control	85 ± 3	Moderate	Normal
Commercial liquid soap (Sunlight) as control	92 ± 5	Moderate	Normal

Antioxidant and anti-microbial studies

Radical scavenging ability (DPPH assay)
The seed oils used in this study (*C. megalabotrys, R. cummunis* and sunflower seed oils) exhibited strong DPPH radical scavenging activity. After just 30 minutes the DPPH had lost its strong purple color. Of the three seed oils *C. megalabotrys* exhibited stronger antioxidant scavenging activity followed by sunflower and *R. cummunis* seed oil respectively, Figure 3. The strength of the radical scavenging ability is attributed to the total phenolic content in the oil (Ghazanfari et al., 2020 and Oun et al., 2022). This therefore means that of the three seed oils *C. megalabotrys* seed oil has the highest amount of phenolic compounds followed by sunflower and *R. cummunis* seed oils, respectively.

The soaps produced showed limited antioxidant activity against DPPH, but after 60 minutes some of the soaps exhibited some level of activity being *C. Megalabotrys/R. Cummunis, C. Megalabotrys,* Sunflower seed oil and Commercial bar soaps. *C. Megalabotrys/R. Cummunis and* Sunflower seed oil liquid soaps exhibited little to no activity, Figure 3. It seems like during saponification reaction the phenolic compounds in the oils are broken down hence the loss in DPPH scavenging activity of the soaps. KOH seems to break phenolic compounds better than NaOH hence limited to no antioxidant activity in liquid soaps compered to in bar soaps.

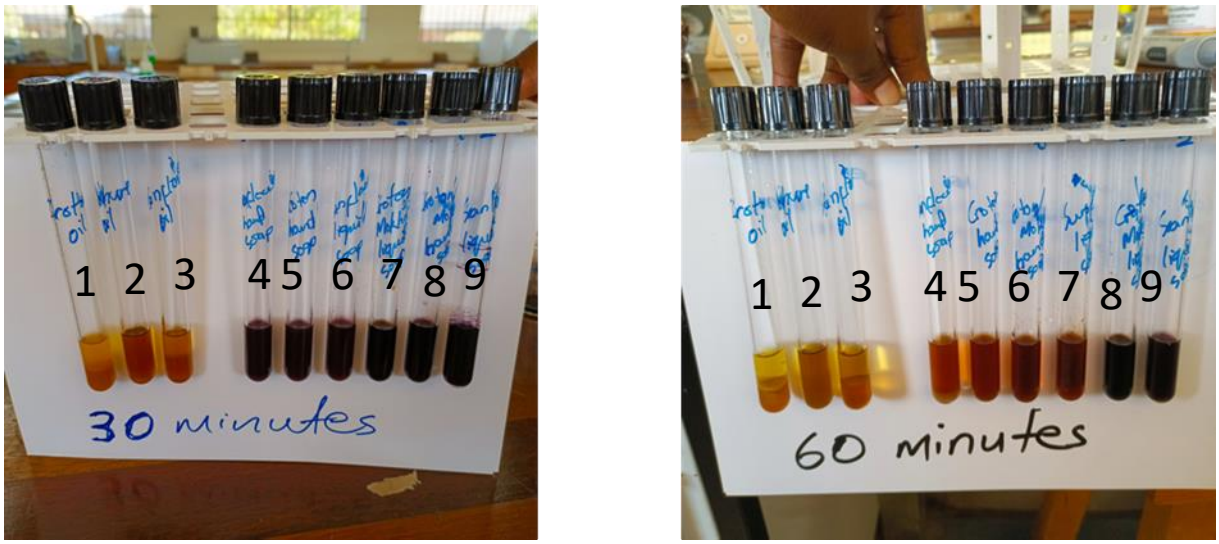

Figure 3: DPPH scavenging activity of oils (1,2 3 being *C. megalabotrys, R. cummunis* and sunflower seed oils respectively) and soaps (4,5,6,7,8 and 9 being *C. Megalabotrys/R. Cummunis, C. Megalabotrys,* Sunflower seed oils and Commercial bar soap, *C. Megalabotrys/R. Cummunis and* Sunflower seed oil liquid soaps respectively) after 30 minutes and 60 minutes respectively.

Anti-microbial activity.
Only two soaps produced, *C. megalabotrys/R. cummunis* and sunflower seed oil bar soaps showed anti-microbial activity against *Escherichia coli* (*E coli*) a gram-positive bacterium though the activity is limited, see Figure 4, while other soaps including the seed oils themselves showed no activity. None of the soaps or seed oils showed any activity against *Pseudomonas aeruginosa* a gram-negative bacterium. *Staphylococcus aureus* and *Streptococcus pyogenes* bacteria are the most identified causes of skin and soft tissue infections (Templer and Brito, 2009). *Staphylococcus aureus* and *Streptococcus pyogenes* bacteria are gram positive bacteria which means they can be prevented by using *C. megalabotrys/R. cummunis* and sunflower seed oil bar soaps.

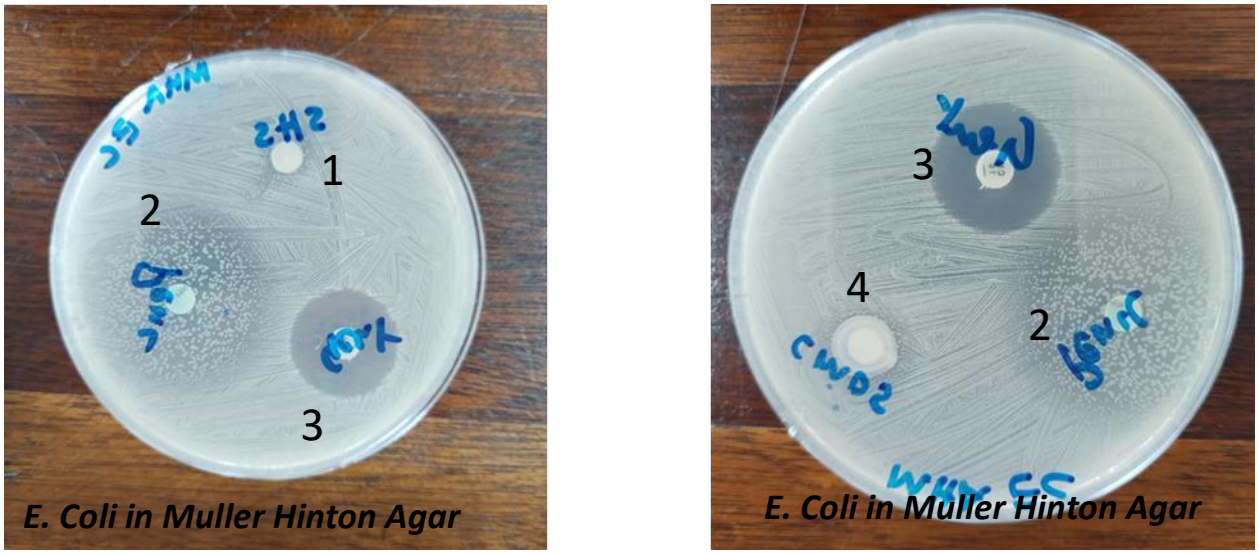

Figure 4:*C. megalabotrys/R. cummunis*(4) and Sunflower (1) seed oil bar soaps, Penicillin (2) and tetracycline (3)

IV. Conclusion

C. megalabotrys seed oil in combination with *R. cummunis* and coconut seed oils have proved to produce high quality organic soaps with good antioxidant and antimicrobial activities. The pH of these soaps ranged from 10.01 ± 0.02 and 10.30 ± 0.03 showing that these soaps are mildly alkaline and hence cannot burn the skin while at the same time killing bacteria and other microorganisms on the skin surface. The low levels of moisture content at 2.78 ± 0.01 % to 2.97 ± 0.00 % and low levels of free fatty matter at 0.29 ± 0.01 % to 0.75 ± 0.02 % is an indication of good quality of the soaps and helps the soaps from becoming rancid and odorous, thus, increasing their shelf live. Low levels of matter insoluble in alcohol from 42.00 ± 0.03 % to 47.18 ± 0.03 is an indication that soaps produced from these seed oils have higher purity.

C. megalabotrys and *R. cummunis* seed oils exhibited strong antioxidant activity while the soaps produced exhibited antioxidant activity proving that both the oils and the soaps can scavenge for free radicals produced on the skin surface reducing the susceptibility of skin diseases such as various types of skin cancers. *C. megalabotrys/R. cummunis* soap showed antimicrobial activity against gram-positive bacterium (*E. coli*) an indication that this soap can prevent growth of gram-positive bacteria growing on the skin surface such as *Staphylococcus aureus* and *Streptococcus pyogenes* which are the most identified causes of skin and soft tissue infections in humans. Notwithstanding these positive results, there is need to do a thorough human/animal dermatological studies on these soaps before commercial production is conceived to ascertain their safety.

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