PRODUCTION OF ANTIMICROBIAL SOAP USING A BLEND OF *MORINGA OLEIFERA* OIL AND *RICINUS COMMUNIS* (CASTOR OIL)

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DEPARTMENT OF CHEMICAL ENGINEERING,
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JANUARY, 2017

DECLARATION

I declare that the work in this Dissertation entitled, "Production of Antimicrobial Soap Using a Blend of *Moringa Oleifera* Oil and Castor Oil" has been carried out by me in Ahmadu Bello University, Zaria, under the supervision of Dr. B. Mukhtar, Department of Chemical Engineering and Dr. M.S. Aliyu, Department of Microbiology, Ahmadu Bello University, Zaria. The information derived in the literature has been duly acknowledged in the text and list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or other institutions.

EJIKE DAVID UGWUANYI

| --- | --- | --- |
| Name of Student | Signature | Date |

CERTIFICATION

This dissertation entitled **"**PRODUCTION OF ANTIMICROBIAL SOAP USING A BLEND OF *MORINGA OLEIFERA* OIL AND CASTOR OIL**"** by EJIKE DAVID UGWUANYI with registration number P13EGCE8048 meets the regulations governing the award of Master of Science (M.Sc) degree in Chemical Engineering of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To God Almighty for his love, guidance and preservation upon my life that made this programme a huge success.

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First of all, I acknowledge the grace, favors and blessings of the Almighty God upon my life. All that has come of this training, including this report, is by His grace. I would like to show my gratitude to the supervisory team, in persons of Dr. B. Mukhtar and Dr. M.S. Aliyu, with their help, patience and guidance, I gained the needed experience and skills to conduct this research work, may Allah bless you with all your heart desires and make the hereafter a better one for you. My profound gratitude goes to my beloved mother Mrs. Elizabeth Ugwuanyi and my wonderful sisters Ogochukwu and her husband Josephat Ojikeya, Nkechi and Miracle for their prayers, affection and financial support throughout my years of studies. Also not forgetting my spiritual director, Pastor Gilbert Alphonsus and his family and the family of Mrs. Josephine Uruawuike who made her home open for me to stay all through my stay in Zaria (God bless you richly for your benevolence towards me). Less I forget my brother from another womb Samuel Uruawuike, you indeed a brother, succorer and a friend indeed.

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ABSTRACT

The studies on oils in general have a significant role to play in the well being of mankind. In this research work, *Moringa oleifera* oil and castor oil were both extracted from their seeds using mechanical press method. *Moringa oleifera* oil and castor oil were blended in various proportions and used in preparing soap samples which were subsequently characterized. The phytochemical screening and chemical properties analysis of the raw oil were conducted. The physical properties of the prepared soap including hardness and pH were also analyzed. The antimicrobial activity of the soap produced was examined against some clinical isolates of pathogenic microorganisms (*Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*) using agar diffusion method. The pattern of inhibition varied with the soap concentration and the organisms tested. The soaps produced from all the blend of oils exhibited antimicrobial activity against *E.coli,* however, soap of 100 % *Moringa Oleifera* oil gave the highest zone of inhibition (19 mm) at a concentration of 400 mg/ml. Soap prepared using 100 % castor oil alone gave the highest zone of inhibition against *S. aureus* (28 mm) and *C. albicans* (25 mm). Addition of castor oil in the soap formulation further improved activity of the soap against *S. aureus* and *C. albicans*. The antimicrobial activities exhibited by the soap in this study could be attributed to the presence of phytochemical constituents in the oils, which signify the potential of the soap as a typical therapeutic agent. These findings therefore, confirmed the medicinal use of soap prepared using *Moringa Oleifera* oil and castor oil.

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LIST OF ABBREVIATIONS

CFU – Colony Forming Unit

MBC – Minimum Bactericidal Concentration

MIC – Minimum Inhibitory Concentration

MHA - Mueller Hinton Agar

SDA - Sabouraud Dextrose Agar

MHB - Mueller Hinton Broth

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Soap is a mixture of sodium or potassium salts of various naturally occurring fatty acids. It is a substance of ancient origin, the manufacture of which according to Gunstone *et al*. (1986) has evolved from primitive beginnings into a sophisticated chemical process. Evidence of soap making dates back to the Egyptian and Babylonians. Several millennia ago crude mixture of animal fats and the alkaline plant ash were found to generate crude soaps which lathered and cleaned effectively (Phansteil *et al*., 1998). Through the centuries, there have been other times when people were able to make soap using sodium or potassium salts as is done nowadays. When the Germans tribesmen of Caesar's time boiled goat tallow with potash leached from the ashes of wood fires, they were carrying out the same chemical reaction as one carried out on a tremendous scales by modern soap manufacture by hydrolysis of glycerides (acylglycerols) (Morrison and Boyd, 2004). The commonest oils used in soap making are lard and tallow from animal sources, coconut, palm and olive oils from vegetable sources (Pavila *et al*., 1982).The commercial manufacturers use 1-7 % excess fat to reduce the harshness of soap, to produce a dense creamy lather, and to leave the skin feeling smooth and soft (Mabrouk, 2005).

The reaction for making soap (saponification) is a base (usually NaOH or KOH) hydrolysis of triglycerides to make three salts (soap) and glycerol. The molecules crystallize differently depending on the base used. NaOH produces a harder bar while KOH is used more frequently for liquid soaps. Soap is a salt of a compound, known as a fatty acid. A soap molecule has a long hydrocarbon chain with a carboxylic acid group on one end, which has ionic bond with metal

ion, usually sodium or potassium. The hydrocarbon end is non polar which is highly soluble in non polar substances and the ionic end is soluble in water.

The cleaning action of soaps is because of their ability to emulsify or disperse water-insoluble materials and hold them in the suspension of water. This ability is seen from the molecular structure of soaps. When soap is added to water that contains oil or other water-insoluble materials, the soap or detergent molecules surround the oil droplets. The oil is, dissolved in the alkyl groups of the soap molecules while the ionic end allows it to be dissolved in water. As a result, the oil droplets are to be dispersed throughout the water and can be washed away (Girgis, 1997).

Several factors affect the soap-making process and the quality of the soap produced such as the quality of oil, and the amounts of the caustic soda and water used to make it. The reaction rate between the oil and the caustic soda is influenced by free fatty acid content of the oil, the heat of the components before mixing, and how vigorously the mixing is to be done. Free fatty acid contents, vigorous mixing, and heat, speed up the given soap-making process. However, a good soap should not contain chemicals that can be harmful to the environment or cause undue destruction to the environment. It should dissolve easily and remove stains from clothes, human skin or any material being cleaned. It should have ability to produce enough suds, give a sparkling kind of cleanliness and pleasant smell. It should not leave sticky traces on the clothes or on the skin. It should have a good color that is even and does not streak and damage the fibers or textiles (Girgis, 1997).

An antimicrobial soap is designed to safely kill germs and cleanse the skin. In the antimicrobial soap formulation, the types of organisms the product should be effective against and how much

time is required for the product to work have to be considered. In addition, factors related to cleansing such as foam quality, speed of foaming, rinse ability, and skin feel have to be considered also. Furthermore, the product's aesthetic qualities (how it looks and smells) must also be evaluated.

Antimicrobial soap is a regular soap in liquid or solid form. According to Osbore and Grube (1982), good antimicrobial soap can remove 65 – 85 % bacteria from human skin. However, contemporary commercial antimicrobial soaps contain synthetic chemicals such as triclosan, trichlorocarbanilide and chloroxylenol, most of which are thought to be carcinogenic, mutagenic and or generate allergic reactions. Triclosan (2, 4, 4 - trichloro-2-hydroxydiphenyl ester) is used in soaps, shampoo and fabrics, as an antimicrobial agent. Though these compounds are considered to 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 highly toxic (Okumura and Nishikawa, 1996). In addition, it is well known that 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 need for new antimicrobial agents is closely linked with the problem of emergence of strains that are resistant to most synthetic antibiotics. This has arisen due to extensive use of antibiotics, which renders most of the current antimicrobial agents inefficient in controlling some bacterial diseases (Gustavo *et al*., 2010). There is increased evidence to proof that medicinal plants may represent an alternative treatment for non severe cases of infectious diseases. They could also serve as possible source of new and cheap antibiotics to which pathogenic strains are not resistant and several works provide scientific bases for the popular use of plants against infectious diseases (Kitula, 2007; Ajibesin *et al*., 2008; Wu *et al*., 2008).

1.2 Problem Statement

Contemporary commercial antimicrobial soaps contain synthetic chemicals such as triclosan and trichlorocarbanilide most of which are said to be carcinogenic, mutagenic and or generate allergic reactions (Okumura and Nishikawa, 1996). Owing to the potential harm posed by these synthetic antimicrobial agents, it becomes necessary that safer alternatives should be made available. Most vegetable oils used in antimicrobial soap formulation have not been able to exhibit antimicrobial effect on some bacteria such as *Escherichia coli,* it therefore becomes necessary to seek out other alternative oils that are active on this bacteria.

1.3 Aim and Objectives

The aim of this work is to produce antimicrobial soap from the blend of *Moringa oleifera* oil and castor oil.

The aim was achieved through the following specific objectives;

i. extraction and characterization of *Moringa oleifera* oil and castor oil from their seeds
ii. production of antimicrobial soap from the blend of *Moringa oleifera* oil and castor oil.
iii. determination of the antimicrobial properties of the soap.
iv. determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the soap.
v. determination of antimicrobial soap inhibition against some clinical isolates of pathogenic microorganisms (*Staphylococcus aureus*, *Escherichia coli* and *Candida albicans)* using agar diffusion method.

1.4 Justification

There is increased evidence to prove that medicinal plants may represent an alternative treatment for infectious diseases (Bennett *et al*., 2003). The phytochemicals present in *Moringa oleifera* oil and castor oil have been reported to have an active bactericide and fungicide effect. Non-comedogenicity is castor oils least understood or appreciated benefit. To overcome this growing concern of comedogens, cosmetic manufacturers are formulating products with non-comedogenic emollients (Mutlu and Meier, 2010). These oils are natural and readily available.

1.5 Scope

The scope is centered on the procurement of castor seeds from the National Research Institute of Chemical Technology (NARICT) and *Moringa oleifera* seeds sourced from television market in Kaduna south for the production of antimicrobial soap and preparation of agar slants which consisted of medium preparation, sterilizing tubes, slanting, storage and inoculation used for the culture of the test organisms.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Fatty Acids in Oils

Soaps are prepared from animal fat (lard) or vegetable oil. Animal fats and vegetable oils are esters of carboxylic acids; they have a high molecular weight and contain alcohol and glycerol. Chemically, these fats and oils are called triglycerides. The principal acids in animal fats and vegetable oils can be prepared from the natural triglycerides by alkaline hydrolysis (saponification). Addition of essential oil gives scent to the soap (Morrison and Boyd, 2004).

Vegetable oil, like all animal and vegetable fats, are made up of a mixture of triglycerides. Triglycerides are esters of glycerol that contain long hydrocarbon chains (**R**, **R'** and **R"**) whose lengths vary. Biologically, their primary purpose is chemical energy storage. When the R groups contain only singly bonded carbons, they are said to be saturated. R groups containing one or more doubly bonded carbons are said to be monounsaturated and polyunsaturated respectively. Because of their double bonds, the unsaturated hydrocarbon chains exhibit "bends" that prevent adjacent chains from approaching too closely. Thus the intermolecular forces between unsaturated chains are limited and a lower melting point temperature observed. Unsaturated fats are usually liquids while saturated fats are found in the solid state at room temperature. The "R" group can be any fatty acid as shown in Table 2.1.

Table 2.1: Different Types of Fatty Acids Contained in Oils

Fatty Acid	Structural formula	Formula
Butyric Acid	$CH_3(CH_2)_2COOH$	$C_4H_8O_2$
Caproic Acid	$CH_3(CH_2)_4COOH$	$C_6H_{12}O_2$
Capric Acid	$CH_3(CH_2)_8COOH$	$C_{10}H_{20}O_2$
Lauric Acid	$CH_3(CH_2)_{10}COOH$	$C_{12}H_{24}O_2$
Myristic Acid	$CH_3(CH_2)_{12}COOH$	$C_{14}H_{28}O_2$
Palmitic Acid	$CH_3(CH_2)_{14}COOH$	$C_{16}H_{32}O_2$
Linoleic Acid	$CH_3\text{-}(CH_2)_4\text{-}(CH{=}CH\text{-}CH_2)_2\text{-}(CH_2)_6\text{-}COOH$	$C_{18}H_{32}O_2$
Oleic Acid	$CH_3\text{-}(CH_2)_7CH{=}CH(CH_2)_7COOH$	$C_{18}H_{34}O_2$
Stearic Acid	$CH_3\text{-}(CH_2)_{16}COOH$	$C_{18}H_{36}O_2$
Ricinoleic Acid	$CH_3(CH_2)_5CH(OH)CH_2CH{=}CH(CH_2)_7COOH$	$C_{18}H_{34}O_3$
Erucic Acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}COOH$	$C_{22}H_{42}O_2$

Retrieved from Ullmann's Encyclopedia of Industrial Chemistry published by Thomas (2005).

2.2 Raw Materials for Soap Formulation

2.2.1 Alkali

Most soap incorporate an alkali as one of the principal ingredients. Sodium hydroxide (caustic soda) is the strongest of the alkalis and is inexpensive. It has excellent dissolving properties, is a very strong saponifier and has added advantage of being strongly bactericidal. It is, however, highly corrosive to metals especially aluminium and extreme care must be taken when handling it because it can cause severe burns to the skin (Oghome *et al*., 2012). Soap formed using soda and potash is soluble in water, unlike those from the other bases. These reagents are always used in sufficient quantity to combine with the whole of the fatty acids contained in an oil or fat. Though doubtless, by the use of considerably smaller quantities, under pressure, complete resolution of the fatty matter into fatty acids and glycerol could be accomplished. They are, by far, the most important saponifying agents employed in the soap manufacturing processes over a long period of time (Oghome *et al*., 2012).

2.2.2 Oils / Fats

The type of oil or fat that is used in soap formulation has a significant and direct influence on the finished characteristics and qualities of the soap product. Some fats and oils are better for bubbles and others are better for cleaners. Examples of fats and oils that are suited for the traditional soap making process are: neem, coconut, tallow fat, palm oil, palm kernel oil, ground pea nut oil, shea butter oil, and cocoa butter oil (Ekpa and Ekpe, 1995). The palm kernel and coconut oils are good for soapy bubbles.

Besides the various physical properties of oils and fats, such as color, specific gravity, melting point, solubility, etc., they may be distinguished chemically by a number of chemical constants. The various oils used in soap making have similarities and differences that make them exhibit unique chemical and physical properties. The low degree of unsaturation in oil gives them a high oxidative stability. The characteristics exhibited by oils used in soap making are due to their high content of saturation. The characteristic behavior of any oil is due to its fatty acid content and the percentage ratios of saturation levels to unsaturation levels (Alfred, 2002).

2.2.2.1 Neem oil

Neem oil is a vegetable oil pressed from the fruits and seeds of the neem (*Azadirachta indica*). Neem is an evergreen tree which is endemic to the Indian subcontinent and has been introduced to many other areas in the tropics. It is the most important of the commercially available products of neem for organic farming and medicines (Kraus, 1995).

Neem oil varies in color; it can be golden yellow, yellowish brown, reddish brown, dark brown, greenish brown or bright red. It has a rather strong odor that is said to combine the odours of peanut and garlic. It is composed mainly of triglycerides and contains many triterpenoid compounds, which are responsible for the bitter taste. It is hydrophobic in nature; in order to emulsify it in water for application purposes, it must be formulated with appropriate surfactants. Neem oil contains the following fatty acids: oleic acid (25 - 54 %), stearic acid (9 - 24 %), linoleic acid (6 - 16 %), palmitic acid (16 - 33 %), linolenic acid (<1 %) (Kraus, 1995).

Neem oil is not used for cooking purposes. In India, it is used for preparing cosmetics (soap, hair products, body hygiene creams, hand creams) and, in the treatment of a wide range of infections (Mirza *et al*., 2000). This oil has been used in the treatment of inflammation, pain and swelling that occur in arthritis (Subapriya *et al*., 2005). When properly used, the oil combats vaginal infection and sexually transmitted diseases and kills lice (Abdel-Ghaffar and Semmler, 2007). It is used in the treatment of skin diseases such as scabies (Heukelbach and Feldmeier, 2006), ringworm and athlete's foot (Khan and Wassilew, 1987). Mak-Mensah and Firempong (2011) prepared toilet soap using neem oil and suggested that due to the phytoconstituents in neem oil and the favorable chemical characteristics of the soap, it can be used as medical and cosmetic toilet soap as also reported by Warra (2012).

2.2.2.2 Shea butter oil

Shea butter oil is a soft paste of melted fat with a milky colour in solid form and brownish when melted and is obtained from shea tree (*Butyrospermum parkii*). It contains fatty acid triglyceride, phytosterol and a high amount of unsaponifiable matter, from 2.5 - 15 % (Eka, 1997). Shea butter oil contains cinnamic acid, a substance that helps protect the skin from harmful ultra-violet rays (Tella, 1979). Shea butter is a triglyceride (fat) derived mainly from stearic acid and oleic acid. It is widely used in cosmetics as a moisturizer, salve or lotion (Alfred, 2002).

Shea butter extract is a complex fat that in addition to many non-saponifiable components contains the following fatty acids: oleic acid (40 - 60 %), stearic acid (20 - 50 %), linoleic acid (3 - 11 %), palmitic acid (2 - 9 %), linolenic acid (<1 %) and arachidic acid (<1 %) (Davrieux *et al*., 2010). Shea butter melts at body temperature. Proponents of its use for skin care maintain that it absorbs rapidly into the skin, acts as a "refatting" agent, and has good water-binding properties

(Hemat, 2003). Shea butter fat finds uses in soap making, in cosmetics and in traditional medicine in rural areas (Alander, 2004; Maranz *et al*., 2004). In Nigeria, a traditional medicated soap is produced from a mixture of vegetable oils (palm kernel oil and shea butter) that make the soap to have antimicrobial properties recognized in the traditional African households (Getradeghana, 2000; Aliyu *et al*., 2012). Due to its richness in food nutrients, the shea butter oil has found use as baking fat (Akhter *et al*., 2008).

2.2.2.3 Coconut oil

Coconut oil or Copra oil is edible oil extracted from the kernel of matured coconuts from the coconut palm (*Cocos nucifera*). It has various applications in food, medicine, and industry. Because of its high saturated fat content it is slow to oxidize and, thus resistant to rancidification, lasting up to two years without spoiling (Fife, 2005).

Many health organizations advised against the consumption of high amounts of coconut oil due to its high levels of saturated fat. The oil contains the following fatty acids: myristic acid (19 %), lauric acid (52 %), caprylic acid (9 %), palmitic acid (11 %), decanoic acid (10 %), oleic acid (8 %) and others (5.3 %). Coconut oil can be used as a skin moisturizer, helping with dry skin (Agero, 2004) and reduces protein loss when used in hair (Rele, 2003). Before the advent of electrical lighting, coconut oil was the primary oil used for illumination in India and was exported as cochin oil (Brady, 2002).

Coconut oil is an important base ingredient for the manufacture of soap. Soap made with coconut oil tends to be hard, although it retains more water than those made with other oils and therefore increases manufacturer yields. It is more soluble in hard water and salt water than other soaps allowing it to lather more easily. Basic coconut oil soap is clear when melted and a bright white

when hardened (Browning, 2003). A repellent made from coconut oil may be effective to prevent pathogens causing diseases from penetrating the skin (Feldmeire, 2009).

2.2.2.4 Moringa oleifera oil

The *Moringa oleifera* tree is native to India but has been planted around the world and is naturalized in many places. In the Philippines, where the leaves of the *Moringa oleifera* are cooked and fed to babies, it is called "mothers best friend" and "malunggay." Other names for it include the benzolive tree (Haiti), horse radish tree (Florida), Nebedey (Senegal) and drumstick tree (India) (Paliwal and Sharma, 2011). In northern Nigeria it is known in Hausa language as "Zogale" (Warra, 2011). There are about thirteen species of *Moringa oleifera* trees in the family *Moringa oleiferaceae*. They are native to India, the Red Sea or parts of Africa including Madagascar. Of these species, *Moringa oleifera* is the most widely known. It is a multipurpose tree known as nature's medicine cabinet (Palimal and Sharma, 2011). Almost all parts of the plant are potentially useful. The seeds are probably the most useful part of the plant, containing a significant percentage of high quality oil. The seeds of *Moringa oleifera* contain about 35- 40 % oil (Ashfaq *et al*., 2012).

The oil is of excellent quality similar to the olive oil, and is slow to become rancid. It gave high oil yield, which has good antioxidant capacity with potential for industrial, nutritional and health applications (Ogbunugafor *et al*., 2011). This oil is rich in essential fatty acids, making it an ideal moisturizer and healing and soothing emollient for rough, dry skin and therapeutic massages. The fatty acids in *Moringa oleifera* oil are oleic acid (70 %), palmitic acid (7.8 %), behenic acid (6.2 %) and stearic acid (7.6 %) (Abdulkarim *et al*., 2005). Perfume manufacturers esteem the oil for its great power of absorbing and retaining even the most fugitive odors and for its stability.

The fatty acid composition is considered to be similar to that for olive. The oil is light and spreads easily on the skin making it good for massage or as carrier oil for aromatherapy. *Moringa oleifera* oil is utilizable in creams, lotions, balms, scrubs, body oils, and hair care formulations. *Moringa oleifera* oil brings occlusive, "cushiony" emolliency to hair and skin formulas. Determination of antioxidant of *Moringa oleifera* seed oil and its use in the production of a body cream was reported (Ojiako and Okeke, 2013). Production of Soap from an Indigenous *Moringa oliefera* seed oil was also reported (Warra, 2011).

Among the several fatty acids in *Moringa oleifera*, the most abundant of the unsaturated fatty acids is oleic acid which was recommended for use in pharmaceutical preparation preferably in skin treatment. Various extraction methods are employed in obtaining oil from *Moringa oleifera* seeds. *Moringa oleifera* oil is non-drying with a pale yellow consistency. It has various cosmetic values and is used in body and hair care as a moisturizer and skin conditioner. It is useful in removing dirt out of the hair and is an efficient natural cleanser. This oil blends easily with essential oils and this combined with its non-drying quality and its ease of application on the skin makes it excellent massage oil. Other uses include soap making and for use in cosmetic preparations such as lip balm and creams. The oil can be considered having relative potential for cosmetics just like the African shea nut butter (Warra, 2011).

2.2.2.5 Castor oil

Castor oil is a vegetable oil obtained by pressing the seeds of the castor oil plant (*Ricinus communis*). The common name "castor oil", from which the plant gets its name, probably comes from its use as a replacement for castoreum, a perfume base made from the dried perineal glands of the beaver (Thomas, 2005). Castor oil is a colorless to very pale yellow liquid with a

distinct taste and odor once first ingested. It is a triglyceride in which approximately ninety percent of fatty acid chains are ricinoleate. Oleate and linoleates are the other significant components. Castor oil and its derivatives are used in the manufacturing of soaps, lubricants, hydraulic and brake fluids, polishes, nylon, pharmaceuticals and perfumes (Mutlu and Meier, 2010).

The fatty acid composition of a typical castor oil contains ricinoleic acid (87 - 90 %), oleic acid (2 - 6 %), linoleic acid (1 - 5 %), stearic acid (0.5 - 1 %), palmitic acid (0.5 - 1 %) (Rial, 1999). Castor plant (*Ricimus communis*) is now grown in tropical and warm temperate regions throughout the world and is becoming an abundant weed in the south western united state (Salunke and Desai, 1992). It grows naturally over a wide range of geographical regions and may be activating under a variety of physical and climatic regions. Salunke and Desai (1992) reported that castor beans contains about 30 - 35 % oil which can be extracted by variety of processes such as pressing, and solvent extraction.

Non-comedogenicity is castor oil least understood or appreciated benefit. Comedogens are defined as cosmetics or cosmetic ingredients that exacerbate or contribute to acne and are an important factor for dermatologists and consumers alike. To overcome this growing concern, cosmetic manufacturers are formulating products with non comedogenic emollients. Castor oil and its derivatives are recognized as non-comedogens and emollients (Mutlu and Meier, 2010).

2.2.2.6 Palm kernel oil

Palm kernel oil is edible plant oil derived from the kernel of the oil palm (Poku, 2002). It should not be confused with the other two edible oils derived from palm fruits, coconut oil, extracted from the kernel of the coconut, and palm oil, extracted from the pulp of the oil palm fruit. Palm

kernel oil, coconut oil, and palm oil are three of the few highly saturated vegetable fats; these oils give the name to the 16-carbon saturated fatty acid palmitic acid that they contain. This oil is semi-solid at room temperature, is more saturated than palm oil and comparable to coconut oil. It is commonly used in commercial cooking because of its relatively low cost, and because it remains stable at high cooking temperatures and can be stored longer than other vegetable oils (Musa, 2009).

Resembling coconut oil, palm kernel oil is packed with myristic and lauric fatty acids and therefore suitable for the manufacture of soaps, washing powders and personal care products. Lauric acid is important in soap making: a good soap must contain at least 15 % laurate for quick lathering, while soap made for use in sea water is based on virtually 100 % laurate (Musa, 2009). The fatty acid composition contains lauric acid (48.2 %), myristic acid (16.2 %), palmitic acid (8.4 %), capric acid (3.4 %), stearic acid (2.5 %), oleic acid (15.3 %), linoleic acid (2.3 %) and others (0.4 %).

2.3 Chemical Properties of Oil

2.3.1 Acid value (Free fatty acid)

The free fatty acids in oil are estimated by titrating against potassium hydroxide (KOH) using phenolphthalein. The acid value is the amount of milligram of KOH required to neutralize the free fatty acids present in one gram of sample. It is expressed as oleic acid equivalent (Shahidi, 1995).

2.3.2 Saponification value

This is the amount of alkali in milligram required to neutralize a definite quantity (1 g) of an oil or fat. This value is useful for a comparative study of the fatty acid chain length in oil or fat. A known quantity of oil is refluxed with an excess amount of alcoholic KOH. After saponification the remaining KOH is estimated by titrating it against a standard acid (Taiwo *et al*., 2008).

2.3.3 Iodine value

It is a measure of the degree of unsaturation in oil. It is constant for particular oil or fat. Iodine value is a useful parameter in studying oxidative rancidity of oils. Oil contains both saturated and unsaturated fatty acids. Iodine gets incorporated into the fatty acid chain wherever the double bonds exist. Hence the measure of iodine absorbed by oil gives the degree of unsaturation. Iodine value or number is defined as the gram of iodine absorbed per 100 g of the oil.

2.3.4 Peroxide value

Rancidity is brought about by the action of air (oxidative) or by micro organisms in oil. In oxidative rancidity oxygen is taken by the oil or fat with the formation of peroxides. Peroxides value is a measure of the peroxides contained in the oil. The peroxides present are determined by titration against thiosulphate in the presence of KI using starch as indicator (Abiodun and Adeleke, 2010).

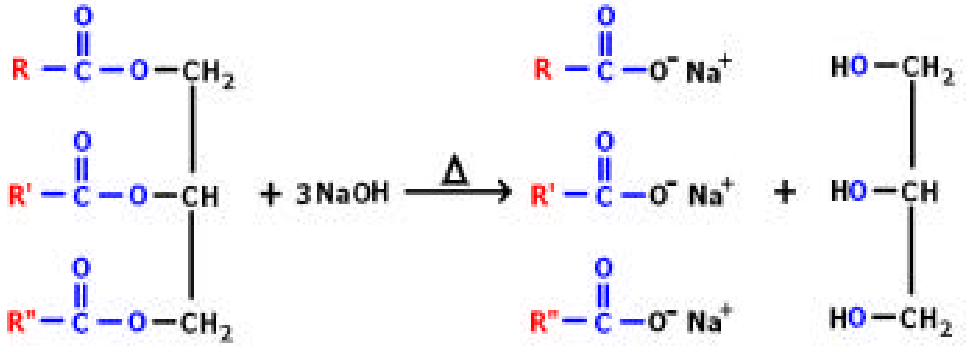
2.4 Phytochemicals

Phytochemicals are plant chemicals that have protective or disease preventing properties. It is a chemical compound that occurs naturally in plants and responsible for color and organoleptic property. Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. It is well-known that plants produce these chemicals to protect themselves but recent research demonstrates that they can also be used to protect human against diseases (Chung *et al.,* 1998). Scientist estimated that there may be as many as 10,000 different phytochemicals having potentials to treat infectious diseases. Although certain phytochemicals are available as diet supplements, some scientists speculate that potential health benefits of phytochemicals may best derive from consumption of whole food (Okwu and Okwu, 2004).

Phytochemical analysis of medicinal plants has shown that numerous compounds in plant traditionally used for medicinal purpose have chemical properties effective at treating illness. Phytochemicals are chemical compound formed during the plant normal metabolic process. These chemicals are often referred to as secondary metabolite of which there are several classes including alkaloids, flavonoids, coumarins, steroids, glycosides, gum, phenol, tannin, terpenes and terpenoids (Harborne, 1973; Okwu and Okwu, 2004). In addition to these substances, plants contain other chemical compounds. These can act as an agent to prevent undesirable side effect of the main active substances or to assist in the assimilation of the main substance. There are some chemicals that are known to have anti-bacterial properties which include; flavonoid and alkaloids. Most of these phytochemical constituents are potent bioactive compounds found in medicinal plant parts of which are precursors for the synthesis of useful drug (Sofowora, 1993).

2.5 Methods of Soap Production

Two conventional methods of soap making are generally used: hot and cold pressed. In soap making, the triglycerides found in vegetable oil are hydrolyzed with concentrated sodium hydroxide to make the soap. The reaction occurs when the d^- side of OH^- is attracted to the d^+ of the carboxylic Carbon atom as shown by the reaction scheme 2.1.



Triglyceride 3 soaps glycerol

Scheme 2.1: The saponification reaction (Warra, 2010)

2.5.1 Hot pressed

Hot-pressed soaps are created by encouraging the saponification reaction by adding heat to speed up the reaction. In contrast with cold-pour soap which is poured into moulds and for the most part only then saponifies, hot-process soaping for the most part saponifies the oils completely and only then is poured into moulds. In the hot process, the hydroxide and the fat are heated and mixed together at 80°C, a little below boiling point, until saponification is complete. In the past, soap makers determined gel stage and full saponification by taste (the sharp, distinctive taste of the hydroxide disappears after it is saponified) or by eye. Tasting soap for readiness is not recommended, as sodium and potassium hydroxides, when not saponified, are highly caustic.

An advantage of the fully boiled hot process in soap making is the exact amount of hydroxide required need not be known with great accuracy. They originated when the purity of the alkali hydroxides were unreliable, as these processes can use even naturally found alkalis, such as wood ashes and potash deposits. In the fully boiled process, the mix is actually boiled (80°C) and after saponification has occurred, the "neat soap" is precipitated from the solution by adding common salt, and the excess liquid is drained off. This excess liquid carries away with it much of the impurities and color compounds in the fat, to leave a purer, whiter soap, and with practically all the glycerine removed. The hot, soft soap is then pumped into a mould. The spent hydroxide solution is processed for recovery of glycerine. The hot press could either be full boiled or semi boiled as the case may be (Warra, 2010).

2.5.2 Cold pressed

Cold process soap bars are made using a combination of oils or fats and lye. The caustic qualities of the lye are removed during the saponification process. When the lye interacts with the oils or fats, it creates glycerine. The type of oils and fats used make a difference in how hard or soft the soap bar ends up being, and how well it lathers. The cold process method does not require or utilize external heat but rather the mixture generates its own internal heat from the exothermic chemical reaction. The cold process method takes the most time, but is undoubtedly the best method for producing the highest quality soaps. Cold-process soap making requires exact measurements of lye and fat mounts and computing their ratio, using saponification charts to ensure the finished product does not contain any excess hydroxide or too much free unreacted fat (Shoge, 2011).

Historically, lye used in the cold process was made from scratch using rainwater and ashes. Soap makers deemed the lye solution ready for use when an egg would float in it. Homemade lye making for this process was unpredictable and therefore eventually led to the discovery of the sodium hydroxide by English chemist Sir Humphry Davy in the early 1800s. A cold-process soap maker first looks up the saponification value for each unique fat on an oil specification sheet. Oil specification sheets contain laboratory test results for each fat, including the precise saponification value of the fat. This value is used to calculate the exact amount of potassium hydroxide to react with the fat to form soap.

The saponification value must be converted into an equivalent sodium hydroxide value for use in cold process soap making. Excess unreacted lye in the soap will result in a very high pH and can burn or irritate skin; not enough lye leaves the soap greasy. Most soap makers formulate their recipes with a 5 % deficit of lye, to account for the unknown deviation of saponification value between their oil batch and laboratory averages. In this process, it is absolutely necessary to use high grade raw materials. Oils and fats should be freed from excess acidity because caustic soda rapidly neutralizes the free fatty acids forming granules of soap which grain out in the presence of strong caustic solution, and since the grainy soap is very difficult to remove without heat increase, the soap tends to become thick and gritty and sometimes discolors. The caustic soda being used should also be pure, it must contain as little carbonate as possible, and the water must be soft and all other materials carefully freed from all particles of dirt (Girgis and Khali, 1997).

2.6 Antimicrobial Soap

An antimicrobial soap is a cleansing product designed to kill microbes on the body or any surface. These soaps are made in either liquid or bar form by blending detergent additives with ingredients, which have antimicrobial properties. An antimicrobial soap is designed to safely kill germs and cleanse the skin. It must therefore consider the types of organisms the product should be effective against and how much time is required for the product to work. It must also consider factors related to cleansing such as foam quality, speed of foaming, rinse ability, and skin feel etc. In addition, the product's aesthetic qualities (how it looks and smells) must also be evaluated (Jungerman, 1996).

According to Carter (2000) an ideal antimicrobial agent is expected to have the following qualities:

a) Very low or no toxicity to human.
b) Non irritant to human tissues.
c) Microbiocidal activity is preferred to Microbiostatic activity to avoid reoccurrence of infection.
d) Cheap and readily available.
e) Active against a wide range of microbes.
f) Resistance not easily developed to its action by target microorganism.
g) Stable to light, room temperature (for storage), and water for easy formation into dosage form and acid for oral administration.
h) Little or no side effects, especially where long period of administration is required.

Unfortunately, no antimicrobial agent has been discovered that has all these qualities. Therefore, research continues towards the discovery of agents with more of the listed qualities than existing ones.

2.7 Test Organisms

2.7.1 *Candida albicans*

Candida albicans is a diploid fungus (a form of yeast). It is a member of the normal microbiota within the gastro intestinal tract, respiratory tract, vaginal area and mouth. In healthy individuals *Candida albicans* does not produce disease. Growth is suppressed by other microbiota. However, if anything upsets the normal micro biota, it may multiply rapidly and produce candidiasis. Recently *Candida* species have become important nocosomial pathogen. In some hospital they represent almost 10% of nocosomial bloodstream infections. Diagnosis of candidiasis is difficult because the fungus is a frequent secondary invader in diseased host and a mixed micro biota which is most often found in the disease tissue. Again, there are no completely specific immunologic procedures for the identification of *Candida* (Lansing *et al*., 2002).

2.7.2 *Escherichia coli*

Escherichia coli is a straight rod, peritrichous or non-motile organism and a facultative anaerobic. The cells are about 2 μm long and 0.5 μm in diameter with a cell volume of 0.6 - 0.7 cm. *E. coli* uses mixed-acid fermentation in anaerobic conditions producing lactate, succinate, ethanol, acetate and carbon dioxide. Since many pathways produce hydrogen gas, these pathways required the level of hydrogen to be low, as is the case when *E. coil* lives together with consuming organism such as methanogenes or sulphate-reducing bacteria. *Escherichia coli* are undoubtedly the best studied bacterium and the experimental organism of choice for many

microbiologists. It is an inhabitant of the colon of humans and other warm blooded animals and it is quite useful in the analysis of water for fecal contamination. Some strain cause enteritis or urinary tract infections. Several enteric genera contain very important human pathogens responsible for the variety of diseases. *Escherichia coli* have the formic hydrogen lyase complex that degrades formic and to hydrogen and CO_2. The optimum growth of *E. coil* occurs at 37^oC, but some laboratory strains can multiply at temperatures of up to 49^oC. Growth can be driven by aerobic or anaerobic respiration using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen and amino acids and the reduction of substrates such as oxygen, nitrate, dimethyl sulfoxide and trimethylamine N-oxide (Hugo and Russell, 2007).

2.7.3 *Staphylococcus aureus*

The genus *Staphylococcus* consist of gram positive cocci, 0.5 – 1.5 μm in diameter, occurring singly, in pairs and in tetrand and characteristically divided into more than one plane to form irregular clusters. The cell wall contains peptidoglycan and teichoic acid. *S. aureus* are normally inhabitants of the upper respiratory tract, skin, intestine and vagina. *Staphylococci, Pneumococci,* and *Streptococci* are members of group of invasive gram positive bacteria known as the pyogenic or pus producing cocci.

These bacteria cause various pus forming disease (example boil, carbuncles, folliculate, impetigo, scalded skin syndrome and abscesses) in humans. *Staphylococci* can be divided into pathogenic and non- pathogenic strains based on the synthesis of the enzymes coagulase. Coagulase positive strains, such as *Staphylococcus aureus,* often produce a yellow carotenoid pigment which has led to their being commonly called golden staph and causes severe chronic infections. Coagulase negative *Staphylococci* such as *S. epidermidis* are non-pigmented and are

generally less invasive but have increasingly been associated (as opportunistic pathogens) with serious nosocomial infections.

2.8 Minimum Inhibitory Concentration

In microbiology, minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents (Andrews, 2001). MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism (Turnidge, 2003). The MIC is usually tested against specific bacteria. This can be done using any of the following methods as outlined;

2.8.1 Broth dilution method

Serial dilution of the agent whose biostatic activity is to be determine are made in an appropriate nutrient medium to get different concentrations. These are then inoculated with the test organism and incubated. The highest dilution (for lowest concentration) of the agent which prevents microbial growth is then taken as the minimum inhibitory concentration (MIC). Optimum temperature and time of incubation (48 h for bacteria and several days for fungi) are used. Organic matter may be added to the medium. Age of the culture and number of variable cell contained can also affect results. An inoculum containing 10^5 viable cell/ml is normally used.

2.8.2 Agar diffusion method

Agar diffusion method is generally preferred to serial dilution because of the ease which quantitative result can be obtained. It cannot be used when the test substance does not readily

diffuse through the gel. About 0.1 ml of the culture is added to the tubes containing the molten nutrient agar (45^{o}C). This is poured into a sterile petri dish and mixed thoroughly. The agar is left for 10 mins to set. The dish is divided into four quadrants and cut is made in the center of each and the disc is removed. Four different dilution of the bacteriostatic solution are prepared and two to four drops of each are added in the cup. The plate is allowed to stand for some time at room temperature for prediffusion and then incubated at 37^{o}C for 24 h for bacteria and 25^{o}C for 48 – 96 h for fungi and yeast (Aliyu *et al*., 2012).

2.9 Minimum Bactericidal Concentration

The Minimum Bactericidal Concentration (MBC) test determines the lowest concentration at which an antimicrobial agent will kill a particular microorganism. The MBC is determined using a series of steps, undertaken after a Minimum Inhibitory Concentration (MIC) test has been completed. MBC testing is useful for comparing the germ-killing activity of several antimicrobial agents at once. The MBC test allows determination of the minimum concentration of an agent necessary to achieve a bactericidal effect. It is worth noting, however, that the duration of time the antimicrobial is in contact with the test organism is quite long for this method, on the order of 18 h. Thus, the test truly does determine the minimum concentration needed to kill the test organism, since all other parameters are conducive to biocidal effect. It can be determined from broth dilution minimum inhibitory concentration (MIC) tests by subculturing to agar plates that do not contain the test agent (Aliyu *et al*., 2012).

2.10 Previous Related Works

Girgis and Khalil (1997) worked on physical characteristics of toilet soap made from apricot kernel oil and palm stearin of which only the physio-chemical properties of the soap were carried out. The antimicrobial properties of the soap were unchecked.

Eke *et al.* (2004) worked on analysis of locally produced soap using shea butter oil blended with palm kernel oil of which only the physico-chemical properties such as moisture content, iodine value, saponification value, foam ability and pH of the soap were tested.

Warra *et al.* (2010) worked on cold process synthesis and properties of soaps prepared from different triacyglycerol sources (shea nut oil, groundnut oil, tallow) of which the physical properties of the soap were analyzed but the antimicrobial properties remained unchecked.

Shoge (2011) worked on quality of soaps using different oil blends of which the physico-chemical properties and cost analysis were evaluated for different soaps but their antimicrobial properties were left unchecked.

Aliyu *et al.* (2012) worked on antimicrobial activity of *Sabulun salo* a local traditional medicated soap of which the soap inhibited *Staphylococcus aureus* and *Candida albicans.*

Garba *et al.* (2012) worked on analysis of the antibacterial activity of Africa black soap on some selected pathogens of which the soap inhibited *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.*

Ameh *et al.* (2013) worked on synthesis and characterization of antiseptic soap from neem oil and shea butter oil of which the soap hardness, foam ability and pH were analyzed. The soap also possessed antibacterial properties inhibiting *Staphylococcus aureus* and *Bacillus subtilis.*

From the above related works it can be deduced that works on antimicrobial property of soap only inhibited the gram positive bacteria and fungi, so it becomes necessary to seek out alternative oils for soap formulation that will exhibit antimicrobial property on both the gram positive bacteria, gram negative bacteria and fungi.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials and Media

The *Moringa oleifera* seeds were obtained from television market in Kaduna South, Kaduna and the Castor beans from the National Research Institute of Chemical Technology (NARICT) Basawa, Zaria. Confirmation of taxonomic identity of the seed was achieved by comparison with voucher specimen (voucher number 225). Other materials include Distilled water, Sodium hydroxide (lye), Saline water, Oils, Hydrogen peroxide, Sodium citrate, Plasma, Tryptone water and Ehrlich reagent. The media includes Nutrient agar, Potato dextrose agar, Muller Hinton agar and Muller Hinton broth.

3.2 List of Equipment / Apparatus

The following are list of equipment used in this study namely, Weighing Balance, Autoclave, Incubator, Thermometer, Soap moulds, Refrigerator, Gas burner, Microscope, Beakers, Petri dishes, Measuring cylinder, Swap sticks, Wire loop and Stirring rod. Table 3.1 gives the description, manufacturer and model of some of these apparatus.

Table 3.1 Descriptions of Apparatus / Equipment Used

Apparatus	Description	Manufacturer	Model
Autoclave	Autoclaves use pressurized steam to destroy microorganisms	Indiamart	HIPL-001B
Incubator	Is a device used to grow and maintain microbiological cultures or cell cultures.	China Wincom	DH-3600II
Refrigerator	A refrigerator has a heat pump driven by an electric motor that keeps items unspoiled.	Berktree	NSBR051W MW/0
Gas burner	Is a device which is used to generate a flame for heating, sterilization and combustion.	Ansun	Burner -06
Microscope	An optical instrument having a magnifying lens for inspecting objects too small to be seen or too small to be seen distinctly and in detail by the unaided eye	Tab kart	HD-7
Beakers	They are glassware for stirring, mixing and heating liquids.	Akshar	1080
Measuring cylinder	Glassware for measuring the volume of liquid.	Akshar	1040
Thermometer	Is an instrument for measuring temperature, often a sealed glass tube that contains a column of liquid, as mercury, that expands and contracts, or rises and falls, with temperature changes.	Narang	DP33
Weighing balance	used to measure *weight* or calculate mass	Telexco	AD10K

3.3 Experimental Procedure

The mechanical press method of extraction was used in this research work to extract the oils from the seeds. A good number of procedures used in the extraction process are highlighted below;

3.3.1 Storage

This was done to ensure that the seeds remained dried. The seeds were stored in an environment void of moist or not humid in nature.

3.3.2 Cleaning

The seeds were cleaned to remove stones, sand, dirt, and spoiled seeds. This was done by screening and washing. The importance of cleaning the seeds in preparation for oil extraction cannot be downplayed hence it becomes very essential in the purity of the oil.

3.3.3 De-husking

This involves removing the husk or seed coat of the seeds and separating them from chaff. This removal was done by grinding because of the difficulty involved using hand removal technique.

3.3.4 Heating

The seeds were subjected to heating in order to increase efficiency of extraction and protein availability. In general, warming seed before processing increases oil yield (Lardy, 2008).

3.3.5 Extraction

The oil was extracted mechanically with an oil press. This method of extraction is accomplished by exerting sufficient force on confined seed to create pressure high enough to rupture the cells and force oil from the seed to escape. The extraction is done by compressing the material in a container that has small perforations, either round or slotted, that allow the liquid component to leave.

3.4 Determination of Chemical Properties of the Oil

3.4.1 Saponification value

One gram (1 g) of oil was weighed into a flask and 50 ml of alcoholic KOH was added to the oil in the flask. A blank was prepared by taking only 50 ml of alcoholic KOH allowing it to drain into another flask. The reflux condenser was connected to the flask and allowed to boil gently for 1 h. After boiling, the flask was then allowed to cool and 1 ml of indicator was added and titrated against 0.5 M HCl until the pink color disappeared (Taiwo *et al*., 2008).

$$\text{Saponification value (mgKOH/g)} = \frac{(B-S) \times M \times 56.1}{\text{weight of sample (g)}} \quad (3.1)$$

where B = blank titre value

S = sample titre value

M = molarity of the acid

Molecular mass of KOH = 56.1

3.4.2 Free fatty acids (Acid number or Acid value)

One gram (1 g) of the oil was dissolved in 50 ml of isopropanol solvent in 250 ml conical flask. 3-4 drops of phenolphthalein indicator was added to the mixture, and the content was titrated against 0.1M KOH, until a pink color which persisted for 15 s was obtained (Shahidi, 1995).

$$\text{Acid value (mgKOH/g)} = \frac{\text{titre value x 0.1M KOH x 56.1}}{\text{weight of sample (g)}} \quad (3.2)$$

while,

$$\text{Free fatty acid (mgKOH/g)} = \frac{\text{acid value}}{2} \quad (3.3)$$

3.4.3 Iodine value

A clean glass stopped bottle containing 0.25 g of oil was weighed. 25 ml of hanus iodine solution was added using a pipette, and drained in a definite time. The solution was allowed to mix well and allowed to stand in the dark for exactly 30 min with occasional shaking. 10 ml of 15 % KI was added and stirred thoroughly. In addition, 100 ml of freshly boiled and cooled water was added to wash down any free iodine on the stopper. The solution was titrated against 0.1M sodium trioxothiosulphate (VI) until the yellow solution turns almost colorless, few drops of starch as indicator was added and titrated until the blue color completely disappeared. A blank titration was also prepared and titrated (Edem *et al.*, 2009).

$$\text{Iodine value (g}I_2\text{/100 g oil)} = \frac{(B-S) \text{ x M x } 126.9}{\text{weight of sample (g)}} \quad (3.4)$$

where B = blank titre value

S = sample titre

M = molarity of thiosulphate

126.9 is the molecular weight of Iodine.

3.4.4 Ester value

This was determined by the difference between the saponification value and the acid value. (Shahidi, 1995).

Ester value (mgKOH/g) = Saponification value – Acid value (3.5)

3.5 Preliminary Phytochemical Screening of the Oil

The preliminary phytochemical studies were carried out using the procedures in Trease and Pharmacology (Evans, 1996). These phytochemicals are bioactive which makes them bactericide and fungicide.

3.5.1 Test for tannins

The oil was weighed (1.0 g) into a beaker and 10 ml of distilled water was added. The mixture was boiled for five minutes and two drops of 5 % $FeCl_3$ was added. The production of a greenish precipitate was an indication of the presence of tannins (Vinoth *et al*., 2012).

3.5.2 Test for flavonoids

Two drops of 10 % sodium hydroxide was added to the portion of the oil (1.0 g). Yellow coloration indicated presence of flavonoid (Vinoth *et al*., 2012).

3.5.3 Test for saponins

Distilled water (2 ml) was added to 2 ml of the oil sample in a test tube and shaken vigorously. The presence of persistent honeycomb froth indicated the presence of saponins (Vinoth *et al*., 2012).

3.5.4 Test for alkaloids

To a portion of the oil sample (2 ml), few drops of Mayer‘s reagent (1.3 g of mercuric chloride and 5.0 g of potassium iodide were dissolved in distilled water in a 100 ml volumetric flask to produce the Mayer’s reagent) were added. A cream precipitate indicated the presence of alkaloids (Vinoth *et al*., 2012).

3.5.5 Test for glycosides

The test oil (2 ml) was mixed with 2 ml of chloroform in a test tube, after which two drops of concentrated H_2SO_4 and 2 ml of acetic anhydride (containing traces of ferric chloride) were added to the solution from the side of the test tube. A green coloration indicated the presence of glycosides (Vinoth *et al*., 2012).

3.5.6 Test for phenolics

Ethanol (2 ml) was added to the test oil and few drops of ferric chloride solution were added. A blue coloration indicated the presence of phenolics (Harborne, 1992).

3.5.7 Test for steroids

A portion of the test oil (1 ml) was dissolved in 2 ml of acetic anhydride and two concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids (Harborne, 1992).

3.6 Soap making (cold process)

Eleven samples containing blend of *Moringa oleifera* oil and castor oil in different proportions were prepared by mixing them in the ration 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100, respectively. Each combination weighed 100 g. Soap samples were prepared from each combination using the cold process. Using castor oil of weight 100 g, a calculated amount of NaOH (15. 13 g) was weighed (this was obtained from the saponification value of the oil) and 35 g distilled water was added to it. The caustic soda was stirred well using a pestle until it blends with the oil. The caustic soda was poured very gradually into the oil and stirred gently in one direction to enhance thorough mixing of the solution until the mixture traces. The plastic container was insulated with pieces of cloths to maintain heat generated from the caustic soda solution to allow the soap mix properly. The soap mixture was poured into the mould and it was left for 5 days for solidification and proper hardening up. This was repeated for each of the other samples (Mabrouk, 2005).

3.7 Physical Characterization of Soap

The physical properties of the produced soap, which include hardness test and pH were determined.

3.7.1 Hardness test

The hardness of the soap was determined by using a needle (6.4 cm in length; 1 mm in diameter) to which a lead fishing weight (130 g) was attached and was lowered into the soap, the distance into which the needle penetrates the soap, after 30 s, was recorded as a measure of its hardness. This was repeated thrice for each soap sample and the mean and standard deviation computed. (Ameh *et al*., 2013).

3.7.2 pH test

The pH values of the soaps produced were analyzed using a pH meter. The produced soaps (2 g) 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 computed.

3.8 Antimicrobial Activities of the Soap

3.8.1 Test organisms

The test organisms used for this analysis were clinical isolates of bacteria and fungus obtained from the Department of Microbiology, Ahmadu Bello University, Zaria. The isolates were *Staphylococcus aureus, Escherichia coli* and *Candida albicans*. The isolates were confirmed using different biochemical tests such as catalase test, coagulase test, indole test, methyl red test, voges proskauer test and citrate test (Chesseborugh, 2006). The *Candida albicans* appears as large and round colonies when grown in the laboratory, which emit a yeasty odor on agar plates at room temperature.

3.8.2 Preparation of the standard inocula of the organisms

The test organisms were prepared by streaking on a freshly prepared nutrient agar plates to obtain discrete colonies. A colony was picked with a sterile wire loop and transferred aseptically into bijou bottle containing sterile normal saline, it was then shaken to dissolve completely and the turbidity was compared with that of a McFarland turbidity standard scale 0.5 which is equivalent to a bacterial cell density of 1.5×10^8 CFU/ML.

3.8.3 Culture media

The culture media used for the analysis include Mueller Hinton agar (MHA), Sabouraud Dextrose agar (SDA), Mueller Hinton broth (MHB) and nutrients agar. The mentioned media were used for sensitivity test, determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC). All the media were prepared according to manufacturer‘s instructions and sterilized by autoclaving at 121^{o}C for 15 min.

3.8.4 Determination of inhibitory activity of the soaps using agar well diffusion method.

Freshly prepared Muller-Hinton agar (20 ml) was poured into sterile Petri-dishes and the agar was allowed to solidify (gel). The bacterial strains and fungus were swabbed on the Nutrient agar plates. Four wells each of diameter 6 mm were made into each agar plate using a cork borer and the plate were labeled. Unto each plate, 0.2 ml of appropriate soap dilution was placed in appropriate wells, that is, 400, 200, 100 and 50 mg/ml, respectively. The plates were left for about 1 h for the soap to diffuse into the agar and then were incubated at 37^{o}C for 24 h for the bacteria and 25^{o}C for 48 h for the fungi. After incubation, the plates were observed for evidence of inhibition, which will appear as a clear zone completely devoid of growth around the well (zone of inhibition). The diameters of the wells were measured using a calibrated ruler in millimeters. The experiment was performed in duplicates and the mean of the zone of inhibition computed.

3.8.5 Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined using broth dilution method (Aliyu *et al*., 2012). The lowest concentrations of the fractions showing inhibition for each organism were serially diluted in the test tube containing Mueller Hinton broth. The bacterial strains and fungi strain were inoculated in tubes with equal volume of nutrient broth and fractions. The tubes were incubated at 37°C for (24 h for the bacteria and 48 h for the fungi). Three control tubes were maintained for each strain (media control, organism control and soap control). The lowest concentration (highest dilution) of the fractions that produced no visible growth (no turbidity) when compared with the control tubes were considered as the MIC.

The Plate 3.1 depicts the experimental setup for the MIC assay for the various soap formulations against the test microbial strains.



Plate 3.1: Experimental set up for the MIC assay

3.8.6 Determination of minimum bactericidal concentration

The minimum bactericidal concentration (MBC) value was determined by sub culturing the test dilution (which showed no visible turbidity) on to freshly prepared nutrient agar media. The plates were incubated further for 24 h at 37°C. The highest dilution that yielded no single bacterial colony on the nutrient agar plates was taken as MBC (Rubina, 2011). For the fungi, the plates were incubated for 42 h at 25°C. The highest dilution that yielded no single fungi colony on the nutrient agar plates was taken as MFC.

The Plate 3.2 depicts the experimental setup for the MBC assay for the various soap formulations against the test microbial strains.

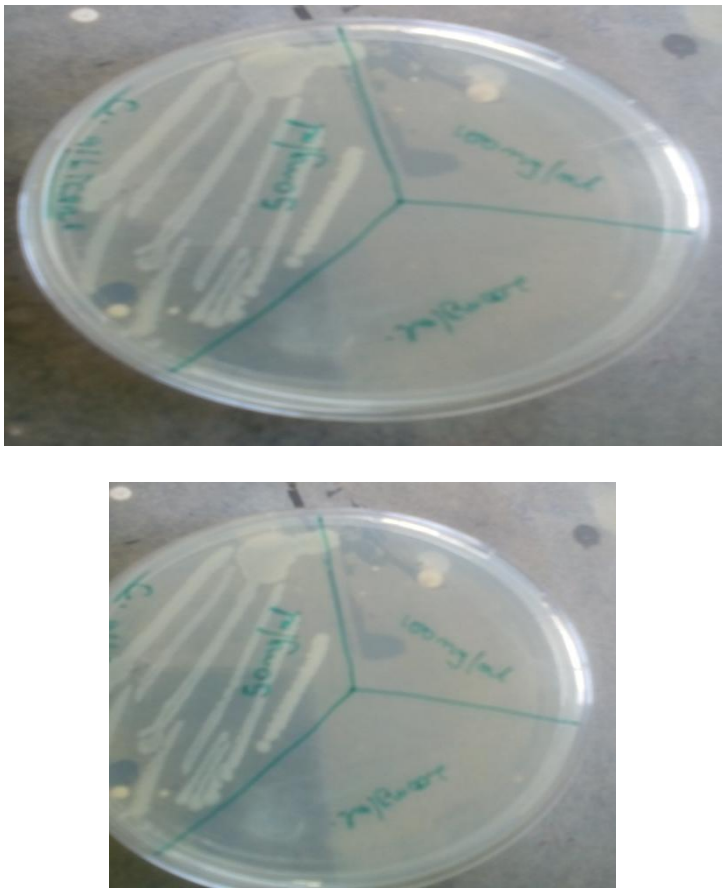


Plate 3.2: Experimental set up of MBC assay

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results of the Chemical Properties of Extracted Oils

The chemical properties of the extracted oils from both *Moringa oleifera* and castor seeds were determined. These properties include the saponification value, iodine value, acid value, free fatty acid and the ester value as shown in Table 4.1

Table 4.1: Chemical Properties of the Extracted Oils

Properties	Oils	
	Moringa oleifera	Castor
Saponification value (mgKOH/g)	166.9	211.8
Iodine value (gI_2/ 100 g)	27.28	12.69
Acid value (mgKOH/g)	7.29	11.22
Free Fatty Acid (mgKOH/g)	3.65	5.61
Ester value (mgKOH/g)	159.61	200.58

As shown in the Table 4.1, Castor oil and *Moringa oleifera* oil have saponification value of 211.8 mgKOH/g and 166.9 mgKOH/g respectively, which are low compared to some oils used in soap making with high saponification values such as coconut oil (257.0) and palm oil (199.1) which have gained high usage in soap making (Abayeh *et al*., 1998). The Saponification value measures the bonded and unbounded acids present in fats and oils. It also gives an indication of the molecular weights of triglycerides in oil. The significantly high proportion of saponification value in the castor oil suggests that the oil is a good raw material for soap production. Higher Saponification value indicates high proportion of lower fatty acids since saponification value is inversely proportional to the average molecular weight or chain length of the fatty acids (Mohammad *et al*., 2011). Therefore, the shorter the average chain length the higher is the saponification number (Tamzid *et al*., 2007). The saponification values give the soap maker an estimate of lye that would be used to saponify a unit gram of fat and oil to avoid the soap from being too caustic (oil x saponification value = quantity of lye).

The Iodine value of Castor oil and *Moringa oleifera* oil were 12.69 $gI_2/100g$ and 27.28 $gI_2/100g$, respectively. Iodine number which indicates the level of unsaturation in oil is one of the chemical characteristics usually used to established identity. The higher the iodine value the higher the level of unsaturated fatty acid (degree of unsaturation) in the oil. Oils with unsaturated fatty acids is easily assimilated and broken down to produce calorific energy than oil with saturated fatty acids however when the level of unsaturation becomes high as indicated by high iodine number, the stability of the oil reduces because unsaturated fatty acids are highly susceptible to oxidation. Thus, oil having high iodine value and invariably higher level of unsaturation may be an indication that it will be susceptible to oxidative rancidity than the other oil samples. According to Kochhar (1998) oils having iodine value less than 100 are classified as non-drying oils and

they have the advantage of not undergoing oxidation to form a film, hence are useful in the formation of soaps. The fact that castor oil and *Moringa oleifera* oil fall under the non-drying oils accounts for their wide range of application in the cosmetics industry due to the non-drying effect they give to the skin.

The Acid value of castor oil and *Moringa oleifera* oil were 11.22 mgKOH/g and 7.29 mgKOH/g, respectively. Acid value is a measure of the free fatty acids in oil. Normally, fatty acids are found in the triglyceride form, however, during processing the fatty acids may get hydrolyzed into free fatty acid. The higher the acid value, the higher the amount of free fatty acids (FFA) present, which translates into decreased oil quality. Acceptable levels for all oil samples should be below 0.6 mg KOH/g (measured in potassium hydroxide per gram) (AOCS Official Method Cd 8-53, 2003). A high acid value indicates stale oil. The high acid value of castor oil and *Moringa oleifera* oil can be attributed to the hydrolysis probably caused by a variety of agents such as presence of moisture in the oil, elevated temperature (above room temperature) and most important of all the lipases (enzyme) coming from the source or contaminating microorganisms. This observation supports previous study that unrefined vegetable oils had higher acid value than refined oils (Rajko *et al*., 2010). Long storage of the oil seeds before or after processing may also have been responsible.

4.2 Phytochemical Constituents Result

The phytochemical constituents, which are secondary metabolites of the extracted oils, were determined. These constituents include alkaloids, flavonoids, tannins, saponins, glycosides and phenolics as shown in Table 4.2

Table 4.2: Phytochemical constituent of the extracted oils

Phytochemical Constituent	Oils	
	Moringa oleifera	Castor
Tannins	-	-
Flavonoids	-	-
Saponins	+	+
Glycosides	+	+
Phenolics	+	+
Alkaloids	+	+

The + sign indicates the presence of the phytochemicals constituent

The results for phytochemical screening shown in Table 4.2 revealed the absence of flavonoids and tannins but affirmed the presence of alkaloids, steroids, glycosides and saponins. Similar components except tannins and steroids were found to be present in honey (Mizrahi, 2008). These classes of compounds are known to possess therapeutic properties against several pathogens and are therefore supporting its traditional use in curing diseases. Saponins detected in both oils have been found to be an antibacterial substance on cell wall of many organisms (Harborne, 1992) and are considered a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effect (Liu and Henkel, 2002). Alkaloids, the most revered of all the phytochemicals have bactericidal effects (Okwu and Okwu, 2004). These alkaloids are said to be pharmacologically active and their actions are felt in the autonomic nervous system, blood vessels, promotion of dieresis, respiratory system, gastrointestinal tract, uterus, malignant diseases, infections and malaria (Trease and Evans, 1989). In addition, flavonoids, glycosides, and tannins are reported to be important antimicrobial component (Chung *et al*., 1998; Karou *et al*., 2005).

4.3 Soap Formulation Results

The soap was formulated using varying blends of both the *Moringa oleifera* and castor oils in different ratio. Eleven soap samples were formulated with the blend of *Moringa oleifera* oil to castor oil in the ratio of 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100. The reason behind the blending of the different oils is on the basis that no individual oil possesses all the properties required to produce a good soap. The samples nomenclature is shown in Table 4.3.

Table 4.3: Nomenclature of the prepared soap samples

Parameter	Ratio (M:C) %
S1	100:0
S2	90:10
S3	80:20
S4	70:30
S5	60:40
S6	50:50
S7	40:60
S8	30:70
S9	20:80
S10	10:90
S11	0:100

M:C, Ratio of *Moringa oleifera* to castor oil;

4.4 Soap Hardness Result

The hardness of each of the formulated soaps was tested. Table 4.4 shows the hardness test result for each of the soap sample.

Table 4.4 Hardness of the soap samples

Soap sample	Depth of needle penetration (cm)
S1	0.40
S2	0.43
S3	0.60
S4	1.27
S5	1.30
S6	1.40
S7	1.45
S8	1.50
S9	1.55
S10	1.60
S11	1.63
Dettol	0.50

As evident from the results the depth of needle penetration increased steadily with increase in the castor oil content, which accounts for the decrease in the hardness of the soap bar. The decrease in hardness of the soap bar can be is traceable to the effect of addition of more castor oil in the blend which gives the soap the softness. Castor oil belongs to the group of soft oils for this reason it is blended with other hard oils like *Moringa oleifera* oil to produce a relatively hard soap. Excess of castor oil in any soap formulation gives rise to a relatively soft soap (Mutlu and Meier, 2010).

4.5 Results of pH Measurement

The pH which is a measure of the acidity or alkalinity was determined. The pH of the soap is an important property that must be checked first before application on the skin. A right balance of the pH of the soap must be maintained because a soap that is highly acidic or alkaline irritates or burns the skin. The pH of all the soap samples fell within the recommended range (9 – 11) for bathing soap according to MakMensah and Firempong (2011). Table 4.5 shows the pH values of the soaps samples

Table 4.5: pH of the soap samples

Sample	pH
S1	9.00
S2	9.10
S3	9.00
S4	9.00
S5	9.10
S6	9.10
S7	9.10
S8	9.20
S9	9.00
S10	9.00
S11	9.10
Dettol	9.10

4.6 Biochemical Tests Result

The identification of antimicrobial strains was done to further analyze the characteristics of the clinical isolates. The biochemical tests carried out were more of confirmatory test for all the microbial strains collected from the laboratory as shown in Table 4.6.

Table 4.6: Characteristics of the microbial strains

Test	Microbial strains		
	E.coli	*S.aureus*	*C.albicans*
Catalase	+ve	+ve	+ve
Coagulase	NA	+ve	-ve
Voges-proskauer	-ve	NA	NA
Citrate	-ve	NA	NA
Methyl red	+ve	NA	NA
Indole	+ve	NA	NA
Microscopy	Rod shape	Round cocci	Yeast

NA- Not Applicable

4.7 Susceptibility Results of Microorganisms

The susceptibility of the three microorganisms (*S. aureus, C. albicans* and *E. coli*) to various concentrations of the soaps is shown in Table 4.7.

Table 4.7: Susceptibility of microorganism to various concentrations of soap

Test Organism	Zone of inhibition (mm)											
	Concentration of S1 (mg/ml)				Concentration of S2 (mg/ml)				Concentration of S3 (mg/ml)			
	400	200	100	50	400	200	100	50	400	200	100	50
E. coli	19	17	10	9	18	15	10	9	17	16	11	8
C. albicans	19	16	14	10	19	17	13	11	21	19	17	15
S. aureus	20	17	16	11	21	19	18	15	23	22	19	17
Test Organism	Concentration of S4 (mg/ml)				Concentration of S5 (mg/ml)				Concentration of S6 (mg/ml)			
	400	200	100	50	400	200	100	50	400	200	100	50
E. coli	17	15	11	8	16	14	10	8	15	12	10	8
C. albicans	21	18	16	12	22	20	17	15	23	21	20	16
S. aureus	23	22	21	19	24	22	19	17	25	22	21	17
Test Organism	Concentration of S7 (mg/ml)				Concentration of S8 (mg/ml)				Concentration of S9 (mg/ml)			
	400	200	100	50	400	200	100	50	400	200	100	50
E. coli	15	13	12	11	15	12	11	8	15	11	9	8
C. albicans	23	20	19	16	24	21	19	17	24	22	20	17
S. aureus	25	22	20	18	27	24	21	19	27	25	22	19
Test Organism	Concentration of S10 (mg/ml)				Concentration of S11 (mg/ml)				Concentration of dettol (mg/ml)			
	400	200	100	50	400	200	100	50	400	200	100	50
E. coli	15	11	9	8	15	10	9	9	17	15	12	11
C. albicans	25	23	20	18	25	23	20	17	24	21	20	17
S. aureus	28	24	23	19	28	25	22	19	27	24	21	19

From Table 4.7 for the test organism (*E.coli*) soap S1 gave a higher zone of inhibition (clear zone) with the highest concentration of 400 mg/ml. Further formulation of soap samples with inclusion of castor oil, a decrease in clear zone was observed for all the soap samples (S2, S3, S4, S5, S6, S7, S8, S9, S10 and S11). In comparison with the standard soap (dettol) the clear zone as observed in S1 is slightly higher by a difference of 1 mm. For the test organism *C.albicans* and *S.aureus,* soap S11 gave the highest zone of inhibition when compared to other formulations from S1 - S10. This result affirmed that the addition of castor oil to the blend played a significant role in the increase as seen for all soaps.

Antimicrobial agent if in contact with any organism that is susceptible to it at a concentration cidal or static to the organism should make the population of the organism to reduce gradually until such a time that the medium may become sterile. The microorganisms (*S. aureus* and *C. albicans*) were similarly susceptible to all prepared *Moringa oleifera* oil and castor oil soaps (S1 to S11). The bacteria (*E. coli*) was also susceptible to the prepared soap fractions but showed a higher resistance inhibition when compared with the other microorganisms used in this study. It may also be observed that the zone of inhibition decreased with decreasing soap concentration of which we can affirmatively claim that the sensitivity of microorganisms was concentration dependent. On the other hand, 50 mg/ml soap concentration showed higher colony growth of *E. coli, C.albicans* and *Staphylococcus aureus*, whereas 400 mg/ml soap concentration inhibited the growth of all the microorganisms. It was seen clearly that gram positive bacteria (*Staphylococcus aureus*) were killed at low concentration of soaps than gram negative bacteria (*Escherichia coli*).

The findings of this study showed that the soaps have antimicrobial effect against *S. aureus, E .coli* and *C. albicans* with a maximum zone of inhibition of 28 mm and 27 mm, respectively. *S. aureus* and *C. albicans* have been incriminated in causing skin infections including boils, thrush

and impetigo etc. The susceptibilities of these organisms to the soap indicate the therapeutic potentials of the soap in the treatment of such diseases. Secondary metabolites phytochemicals present in the oils used in this study may play a role in defence through cytotoxicity towards pathogenic microorganisms (Briskin, 2000).

The cell wall of *S. aureus* which is a gram positive bacterium is made up of mainly peptidoglycan. Peptidoglycan is found to be distorted by long chain fatty acids that are found in vegetable oils of which is present in both *Moringa oleifera* oil and castor oil an active ingredient in the soap (Ugbogu, 2006). The effect of long chain fatty acid may be the disruption of the fungal membrane leading to leakage of macromolecules such as nucleotide, inorganic acid or phosphorylated ammonium compound (Arora, 2004). This explains the inhibitory effect exerted by the soap against the fungus (*C. albicans*). *E. coli* being gram negative organism has little peptidoglycan in its cell wall and this may hinder the activity of the active components of the soap (fatty acids and phytochemicals). The resistance of *E. coli* to antimicrobial agents is usually due to chromosomal mutation which lowers the permeability of the bacteria to the agents or acquisition of resistance (R) plasmids and transponsoms (Arora, 2004). Therefore, the resistance showed by *E. coli* to the soap may be due to chromosomal mutation which may have resulted to lower permeability of the bacterial cell.

The Plate 4.1 show the susceptibility assay of the microorganisms to various concentration of soap samples as indicated by the clear zones observed.



Plate 4.1: Susceptibility assay showing diameter of zone of inhibition

4.8 Minimum Inhibitory Concentration Result

The Minimum Inhibitory Concentration (MIC) test demonstrates the lowest level of antimicrobial agent that inhibits microbial growth. The Table 4.8 gives us an overview of the MIC for all the soap samples against the microbial strain.

Table 4.8: Minimum inhibitory concentrations (MIC) of the soap samples against the microorganisms.

Test Organism	Observation of Turbidity											
	Concentration of S1 (mg/ml)				Concentration of S2 (mg/ml)				Concentration of S3 (mg/ml)			
	200	100	50	25	200	100	50	25	200	100	50	25
E.coli	-	MIC	+	+	-	MIC	+	+	-	MIC	+	+
C.albicans	-	MIC	+	+	-	-	MIC	+	-	-	MIC	+
S.aureus	-	-	MIC	+	-	-	MIC	+	-	-	MIC	+
Test Organism	Concentration of S4 (mg/ml)				Concentration of S5 (mg/ml)				Concentration of S6 (mg/ml)			
	200	100	50	25	200	100	50	25	200	100	50	25
E. coli	-	MIC	+	+	-	MIC	+	+	-	MIC	+	+
C.albicans	-	-	MIC	+	-	-	MIC	+	-	-	MIC	+
S. aureus	-	-	MIC	+	-	-	MIC	+	-	-	MIC	+
Test Organism	Concentration of S7 (mg/ml)				Concentration of S8 (mg/ml)				Concentration of S9 (mg/ml)			
	200	100	50	25	200	100	50	25	200	100	50	25
E. coli	-	MIC	+	+	-	MIC	+	+	-	MIC	+	+
C.albicans	-	-	MIC	+	-	-	MIC	+	-	-	MIC	+
S. aureus	-	-	MIC	+	-	-	MIC	+	-	-	MIC	+
Test Organism	Concentration of S10 (mg/ml)				Concentration of S11 (mg/ml)				Concentration of dettol (mg/ml)			
	200	100	50	25	200	100	50	25	200	100	50	25
E. coli	-	MIC	+	+	-	MIC	+	+	-	MIC	+	+
C.albicans	-	-	MIC	+	-	-	MIC	+	-	-	MIC	+
S. aureus	-	-	MIC	+	-	-	MIC	+	-	-	MIC	+

+ represents the presence of turbidity at the given concentration which connotes the presence or the activity on the microbial strain.

Table 4.8 gives the MIC result of the antimicrobial soap against the test organisms. From the results obtained the MIC of all the soaps (S1 – S11) for the test organism (*E.coli*) was the same all through, by inference the organism in question was not dependent on the different formulations of the soap hence no change in MIC results. The same applied to *S.aureus* that maintained a constant value for the MIC. The same trend followed for *C.albicans* with exception of soap S1. It is safe to say at this point that the various blending ratio of both oils had little or no effect on the minimum inhibitory concentration of the antimicrobial soap. The positive sign as seen on the table above depicts the presence of turbidity at the given concentration which connotes the presence or the activity on the microbial strain.

The method employed was the tube dilution test which is the standard method for determining levels of microbial resistance to an antimicrobial agent. Serial dilutions of the test agent were made in a liquid microbial growth medium which was inoculated with a standardized number of organisms and incubated for 24 h. The lowest concentration (highest dilution) of the test agent preventing appearance of turbidity (growth) was considered to be the MIC. At this dilution the test agent is bacteriostatic. The low MIC value (50 mg/ml) observed for *S. aureus* and *C. albicans* for most of the soap formulated is a good indication of high efficacy against this bacterium and fungus. On the other hand, higher MIC value (100 mg/ml) was obtained for the bacterium *E. coli* which may be an indication of low efficacy or that the organisms have the potential for developing resistance to the bioactive compounds (Doughari *et al.,* 2007). The MIC and the zone of inhibition were inversely correlated. In other words, the more susceptible the microorganism is to the antimicrobial agent, the lower the MIC and the larger the zone of inhibition. Conversely, the more resistant the microorganism, the higher the MIC and the smaller the zone of inhibition.

4.9 Minimum Bactericidal Concentrations Results

The minimum bactericidal concentration (MBC) demonstrates the lowest level of antimicrobial agent that results in microbial death. This means that even if a particular MIC shows inhibition, plating the bacteria onto agar might still result in organism proliferation because the antimicrobial did not cause death. The Table 4.9 show the MBC of various soap samples.

Table 4.9: Minimum bactericidal concentrations (MBC) of the soap samples against the test microorganisms.

Test Organism	Observation of Growth								
	Concentration of S1 (mg/ml)			Concentration of S2 (mg/ml)			Concentration of S3 (mg/ml)		
	200	100	50	200	100	50	200	100	50
E.coli	MBC	+	+	MBC	+	+	MBC	+	+
C.albicans	-	MBC	+	-	MBC	+	-	MBC	+
S.aureus	-	MBC	+	-	MBC	+	-	MBC	+

Test Organism	Concentration of S4 (mg/ml)			Concentration of S5 (mg/ml)			Concentration of S6 (mg/ml)		
	200	100	50	200	100	50	200	100	50
E. coli	MBC	+	+	MBC	+	+	MBC	+	+
C. albicans	-	MBC	+	-	MBC	+	-	MBC	+
S. aureus	-	MBC	+	-	MBC	+	-	MBC	+

Test Organism	Concentration of S7 (mg/ml)			Concentration of S8 (mg/ml)			Concentration of S9 (mg/ml)		
	200	100	50	200	100	50	200	100	50
E. coli	MBC	+	+	MBC	+	+	MBC	+	+
C. albicans	-	MBC	+	-	MBC	+	-	MBC	+
S. aureus	-	MBC	+	-	MBC	+	-	MBC	+

Test Organism	Concentration of S10 (mg/ml)			Concentration of S11 (mg/ml)			Concentration of dettol (mg/ml)		
	200	100	50	200	100	50	200	100	50
E. coli	MBC	+	+	MBC	+	+	MBC	+	+
C. albicans	-	MBC	+	-	MBC	+	-	MBC	+
S. aureus	-	MBC	+	-	MBC	+	-	MBC	+

Table 4.9 gives the MBC result of the antimicrobial soap against the test organisms. From the results obtained the MBC of all the soaps (S1 – S11) for the test organism (*E.coli*) was the same all through, which suggest that the organism in question was independent on the different formulations of the soap, hence no change in MBC results. The same applied to *S.aureus* that maintained a constant value for the MBC. The same trend followed for *C.albicans* without exception in any of the soaps. It can be deduced that blending ratio of both oils played little or no significant effect in affecting the minimum bactericidal concentration of the antimicrobial soap. The positive sign as seen on the table above depicts the presence of growth of the test organisms at the given concentration.

The MBC value for both the *S. aureus* and *C. albicans* was 100 mg/ml by implication the formulated soap with this minimum level of concentration is capable of causing death of both microbes. For the *E. coli* the MBC was somewhat higher with a value of 200 mg/ml which suggests that at this concentration of the formulated soap, the microbe will certainly not survive. Also, it showed that the lower the MBC value the higher the efficacy of the antimicrobial agent and the higher the MBC value the lower the efficacy of the antimicrobial agent. The result confirms the high efficacy of the antimicrobial soap against *S.aureus* and *C.albicans* in comparison to the *E.coli* strain.

CHAPTER FIVE

5.0 SUMMARY, CONCLUSION AND RECOMMENDATION

5.1 Summary

This study evaluated the chemical properties of the extracted *Moringa oleifera* oil and castor oil. The chemical properties include the saponification value, iodine value, acid value, free fatty acid value and ester value. The extracted oils were used in the formulation of soap in varying ratios of both oils. The preliminary qualitative phytochemical screening of the oils revealed the presence of alkaloids, glycosides, phenols, saponin and steroids most of which were reported by previous literatures to possess antimicrobial properties. The formulated soaps were tested against three microbial strains (*S. aureus, C. albicans* and *E.coli*) for their antimicrobial properties. Sensitivity assay were carried out on the various soap samples formulated to determine the zone of inhibition alongside the MIC and MBC test. This study also confirmed the susceptibility of gram positive bacteria (*S. aureus*) and fungus (*C. albicans*) to antimicrobial agent in relation to gram negative bacteria (*E. coli*). The results obtained also showed that addition of castor oil to the soap formulation process further improved the efficacy of the soap against the gram positive bacteria and fungi. The antimicrobial property exhibited by the prepared soap samples in this study can be attributed to the presence of the phytochemical constituents in the oils, which signifies the potential of the soap as a typical therapeutic agent.

5.2 Conclusion

The following conclusions were reached at the end of the study and they are highlighted as follows:

a) The saponification values of the castor oil and *Moringa oleifera* oil were 211 and 166.9 mgKOH/g, the iodine values for the castor oil and *Moringa oleifera* were 12.69 and 27.28 gI_2/100 g and the free fatty acid for the castor oil and *Moringa oleifera* oil are 5.61 and 3.65 mgKOH/g respectively.

b) The soaps produced from all the blend of oils exhibited antimicrobial activity against *E.coli*, however soap of 100 % *Moringa oleifera* oil gave the highest zone of inhibition (19 mm).

c) Soap prepared using castor oil alone gave the highest zone of inhibition against *S. aureus* (28 mm) and *C. albicans* (25 mm). Addition of castor oil in the soap formulation further improved activity of the soap against *S. aureus* and *C. albicans.*

d) Gram positive bacteria (*S. aureus*) are more susceptible to antimicrobial agents than the gram negative bacteria (*E. coli*).

e) The antimicrobial effect exhibited by the soap in this study signifies the potential of the soap as a typical therapeutic agent.

5.3 Recommendation

a) Solvent extraction method should be used for the extraction of the oils for the use in the soap production to determine the effect of extractive solvents on the antimicrobial properties of formulated soap.

b) Economic analysis of the produced soap samples should be done to check their viability against the commercial antimicrobial soaps.

c) Further studies should be carried out on other non- edible vegetable oils for their antimicrobial activity.

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