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JIMMA UNIVERSITY

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DEPARTMENT OF BIOLOGY

MICROBIAL QUALITY OF SELECTED BRANDS OF BABY SKIN LOTIONS AND ANTIMICROBIAL ACTIVITY OF SOME PLANT EXTRACTS/ PURE COMPOUNDS AGAINST POTENTIAL PATHOGENIC ISOLATES

BY

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Abstract

Cosmetic are products which are intended to be applied on external part of human body mostly to encourage attractiveness, without affecting the body's structure. Most cosmetics contain many components that favor the growth of microorganisms and the production of cosmetics cannot be completely hygienic process. The current study was conducted to determine the microbial quality of some selected brands of baby lotions and anti-microbial activity of extracts of Erythrina brucei, Calpurnia aurea and Millettia ferruginea. Questionnaires were used to collect preliminary data from pharmacies, boutiques, supermarkets and cosmetic shops to assess the risk factors for contamination of cosmetic products. A total of 90 cosmetic samples representative of three brands of baby lotions and oil were purchased from boutiques, supermarket and pharmacies for microbiological analysis. Based on the findings, 69.77 % of respondents reported that the Ethiopian government's quality controlling system of cosmetics focus mainly on monitoring of expire date of products. About 39.53% of pharmacists, 20.93 % and 2.32% of respondents who have been working in boutique and supermarkets, respectively, reported that they did not have special time preference to transport the products from site of distribution (Addis Ababa and different site of Jimma town) to their own shop. However, 4.65% of respondents from boutique s reported that they transport the products in afternoon. In addition, 20.93% and 2.32% of boutique and cosmetics shop workers, respectively, reported that they did not consider the hostile effect of high temperature on the quality of cosmetics. In relation to the physicochemical properties of the assessed cosmetics, pH of the products was found ranging between 6.5-6.8. Microbiologically, the mean count of aerobic mesophilic bacterial and yeasts were 3.38 x 10^{7} cfu/ml and 2.1x10^{7} cfu/ml, respectively. Among the most prevalent microbial groups were coagulase negative Staphylococcus spp, Bacillus spp. Coliforms, Penicillin spp and Saccharomyces spp. Bacillus was more resistance isolate for tested antibiotics. Antimicrobial activity evaluation of extracts of the three selected plants and pure compounds isolated from the seeds of Millettia ferruginea showed poor activity on isolated bacterial species, A.The present study also showed that the preservatives used in all brands of baby lotions and oil were not active enough to protect the product from microbial spoilage as revealed by growth of different microbial groups upon storage of the cosmetics.

Keywords:cosmetics,microbialquality,preservatives

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CHAPTER ONE

1. INTRODUCTION

A cosmetic product is defined as any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odors and/or protecting them or keeping them in a better condition (Angela andAlina, 2014). Cosmetics may contain surfactants, proteins, oils, emulsifiers, vitamins, minerals and botanical extracts mixed together with abundant amount of water that could attract variety of microorganisms (Kevin, 2010). Many of these products exhibit a pH near neutrality and are usually kept at a temperature close to or above ambient, particularly when they are used in countries with warm climatic conditions (Qasem and Rania, 2012).

The field of cosmetics and microbiology had not come into contact much before the 1930s and cosmetic microbiology became more important in 1940s (Curry *et al.,* 2006; Gamal *et al.*, 2015). The first contamination of cosmetics was reported in1946 by several cases of neonatal death from talcum powder containing *Clostridium tetani* (Baird, 1998; Gamal *et al.*, 2015).

Industry guidance recommends a Total Viable Counts (TVC) of <100 cfu/g for higher risk products (eye, baby products, and others) and <1000 cfu /g for all other products. However, all cosmetic products must be devoid of pathogenic microorganisms, such as, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* and *E. coli* (CTFA, 1996). Since 1960s, opportunist organisms, such as *Klebsiella pneumoniae*, *P. aeruginosa*, *Pseudomonas sp.*, *Serratia sp*., and *Enterobacter sp.*, have been isolated from cosmetic products to a certain extent (Gamal *et al.*, 2015). Certain yeast and mold have been reported to degrade the microbiological quality of such product (Kamal *et al.,* 2013).

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Contamination of cosmetic products by micro-organisms such as *Clostridium tetani, S. aureus,* molds and yeast may cause serious disease of the skin and mucous membrane which are difficult to cure in several cases (Pollack, 2000).Impact of microbial contamination of cosmetics may include serious microbial skin infections and even blindness, if the product is applied around theeyes. A link between outbreaks of *P. aeruginosa* in a neonatal intensive care unit was reported in the USA with contaminated hand washing lotion. Hydrocortisone ointment contaminated by *Pseudomonas sp* resulted in severe eye infections when used in the treatment of an ophthalmic condition (Michael and Mary, 2014). In Jeddah, at King Abdulaziz University Hospital in Saudi Arabia, three babies in the nursery were found infected with *Serratia marcescen,* purulent conjunctivitis and omphalitis. The death of one baby was also reported in the same study from *S. marcescens* meningitis and septicaemia. All infections were traced to intrinsically contaminated baby shampoo introduced to the units five days before the first reported case (Samiah, 2013).In African continent, Nigeria in particular, studies have been carried out to evaluate the microbiological quality of many brands of commercial cosmetic products. Accordingly, creams and lotions in Benin City were found contaminated with *Staphylococci, Penicillium, Aspergillus fumigates* and *Microsporium* species (Hugbo *et al.,* 2003). Similarly, Okorie (1992) reported the presence of *Klebsiella, P. aeruginosa, Bacillus sp, S. aureus and Penicillium sp* from some brands of commercially hawked cosmetic products. Whereas, in Egypt reports revealed that tooth pastes were generally more heavily contaminated than mouth washes with respect to both bacterial and fungal counts (Ashour, 1986).

In Ethiopia, Drug Administration and Control Authority spend either little or no time to protect the public against the harmful effects emanating from cosmetics as in many parts of the world. Though, it is the companies' responsibility to check the safety of the cosmetic products, these firms are business oriented and give little or no attention to the cosmetics safety because of many reasons (Wayessa *et al*., 2012). In Ethiopia, different brands of local and international baby skin lotions are available and being used for kids. Despite, the availability and wide usage of these products in all corners of the country, there is no comprehensive reports on their microbial quality and being used without information regarding their microbial quality. The main focus of this study is, therefore, to determine the microbial quality of some selected brands of baby lotions marketed in Jimma town, southwest of Ethiopia.

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1.2 Statement of the problem

Darmstadt*et al.* (2002) mentioned that daily infant massage with oils is a common practice in many societies. A study conducted on family attitudes in Bangladesh showed that baby massage begins within the first 3 days in premature and full term infants and is believed to prevent infections and loss of heat. Darmstadt and Saha (2002) also explained that Mustard oil is commonly used for massaging purposes. In rural areas of Ethiopia and other parts of Africa, many people commonly use animal products like butter in combination with different plant to massage children. Nowadays due to probably economic transition and cultural changes in the country especially around cities, different brands of baby skin lotions are introduced in the market. However, people use these products without being aware and conscious of their safety level. They do not even consider that these products could be contaminated with pathogenic microorganisms. In line with this, the present study is intended to answer the following question.

- ✓ What are the possible risk factors for cosmetics contamination?
- ✓ Is there any microbial quality difference among cosmetics products which are sold at different sources?
- ✓ Which species of bacteria has high prevalence in cosmetics products?
- ✓ Which brands of baby lotion has active preservative?
- ✓ Which isolated species of bacteria develops resistance for antibiotics?
- ✓ Which selected plant has antimicrobial activity on isolated bacteria species?

1.3 Objective of the study

1.3.1 General objective:

- ✓ To determine the microbial quality of selected brands of baby lotions and evaluate anti-microbial activities of plant extracts
- ✓ **1.3.2 Specific objectives**

1.3.2 The specific objectives of this study are:

- To assess the prevalence of pathogenic microorganism in baby lotion and preservative capacity of selected brands of baby lotion marketed in Jimma town.
- To determine the drug resistance patterns of pathogen isolated from baby lotions.

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- To compare microbial quality of selected baby lotion marketed in pharmacies, supermarkets and boutiques
- To determine the risk factors for contamination of baby lotions.
- To evaluate antibacterial activities of the crude extracts of *Erythrina brucei*, *Calpurniaaurea* and *Millettia ferruginea* and pure compounds isolated from the seeds of *Millettia ferruginea*

1.4 Significance of the study

The findings of the study will redound to the benefit of the society considering that cosmetics prepared and sold for children need high caution. It will help concerned bodies to know the status of microbial quality of the product. It also reveals how the quality of storage and distribution possibly affects the quality of product. Nowadays, there is a great need to identify medicinal plants for preserving cosmetics due to skin irritation and allergenic reactions provoked by chemical preservatives. So this study has contribution as it gives clue for further exploration of medicinal plants that can be used as natural preservatives in cosmetics industry. This in turn has a substantial input to solve health problem of children caused by using of inadequately preserved baby lotion and using of lotion which is preserved with dangerous chemicals, the findings of this study will also be used as base line data for further investigation on related issue.

1.5 Definition

- Preservative capacity**:** is the ability to inhibit spoilage organisms and prevent growth of pathogens.
- Lotion**:** a thick, smooth liquid preparation designed to be applied to the skin for medicinal or cosmetic purposes.
- Natural preservatives**:** these are the chemical constituents extracted from natural sources that offer intrinsic ability to protect products against microbial growth.
- Microbiological criteria: the acceptability of a product based on the presence/absence or number of microorganisms (and/or their toxins) per unit(s) of mass, volume, area or lot.
- An antimicrobial agent**:** is an agent that kills microorganisms or inhibits their growth.

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CHAPTER TWO

2. LITERATURE REVIEW

2.1 Microbiology of Cosmetics

The majority of cosmetic products contain a lot of ingredients which are convenient for microbial growth, in that their production is not a sterile process with at least the storage temperature is nearly optimal for microbial growth (Siegert *et al.*, 2005). Cosmetics might contain microbes, either due to impurity of raw materials or being contaminated during usage. Microbial spoilage cannot only alter physical properties of the product including color, taste, odor and viscosity, but also appears to deactivate crucial constituents depriving cosmetic of its feature. Microbiological contaminants possibly produce endotoxins and metabolites causing irritation and allergic reaction of the skin. They can be also pathogens causing hazard to the human health (Angela and Alina, 2014**)**.

The different needs for microbiological examinations of cosmetic products are established from the microbiological risk analysis, which is carried out in order to determine the type of cosmetic product to be made. The microbiological risk analysis include consideration of the type of user, site of application, potential alteration of cosmetic products as well as the pathogenicity of microorganisms (Danish Minstry of Enviroment, 2010). Specified microorganisms are aerobic mesophilic bacteria or yeasts whose presence are undesirable in a cosmetic product and recognized as a skin pathogens that may be harmful for human health or as an indication of hygienic failure in the manufacturing process. Microorganisms considered as specified microorganisms are *S. aureus*, *P. aeruginosa*, C. *albicans* and *E. coli* (Danish Minstry of Enviroment, 2010).

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Table 1 Microbial limits for cosmetic finished products (Bureau of Indian standards, 2011)

Product	*Microbial Limit (CFU/g or CFU/ml)*
Shampoos, powders	Total Microbial Count 1000,*Max* Yeast and Mould Count: 100,*Max*
Skin creams and lotions	Total Microbial Count: 1000,*Max* Yeast and Mould Count: 100,*Max* Absence of Gram negative pathogens: *E. coli* and *Pseudomonas aeruginosa/g* *S. aureus*: absent/g *C. albicans*: absent/g
Baby products	Total Microbial Count: 200, *Max* Yeast and Mould Count: 100, *Max* absence of Gram negative pathogens: *E.coli* and *Pseudomonas aeruginosa/g* *S.aureus* :absent/g *C. albicans*: absent/g
Eye products (products to be used in and around the eyes)	Total Microbial Count: 100, *Max* Yeast and Mould Count: 100, *Max* Absence of Gram negative pathogens: *E. coli* and *P. aeruginosa/g* *S. aureus*: absent/g *C.albicans*: absent/g
Lip care	Total Microbial Count: 1 000, *Max* Yeast and Mould Count: 100, *Max* Absence of Gram negative pathogens :*E. coli* and *P. aeruginosa/g* *S. aureus*: absent/g *C. albicans*: absent/g
Tooth paste and tooth powders	Total Microbial Count: 1 000,*Max* Yeast and Mould Count: 100,*Max* Absence of Gram negative pathogens :*E. coli* and *P. aeruginosa/g* *S. aureus* : absent/g *C. albicans*: absent/g
All other products requiring microbiological assessment	Total Microbial Count: 1 000, *Max* Yeast and Mould Count: 100, *Max*

CFU/g=for solids. *CFU/ml* = for liquids

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2.2 Contamination of Cosmetics Product

Contamination during production and filling in cosmetic products may occur. Raw materials can contribute to a significant level of microbial contamination to the finished product. Testing of raw materials before use, especially those of natural origin is important. The specifications of the raw materials must include microbiological purity (Danish Ministry of Environment, 2010). Water is the most common ingredient and it must be tested continuously for microbial growth. Many other conditions of production may influence the contamination during manufacturing, such as contaminated areas, insufficient manufacturing hygiene, personnel hygiene and insufficient preservation (Daniel, 1995). Effective cleaning is very important (Danish Ministry of Environment, 2010). During use, cosmetics can also be contaminated with a variety of spoilage organisms found in the household environment. A few of these may invade and create disease. With more and more immune compromised individuals in the population from the pandemic of AIDS, even spoilage organisms are considered to be opportunistic pathogens (Daniel, 1995).

2.3 Preservatives

According Siegert (2012), preservatives are used for the protection of consumers, and minimize the spoilage of products during the intended and predictable use. However, they should not replace good manufacturing. Contamination by microorganisms during production has to be avoided by recognizing and eliminating its sources. Recently, Narges*et al.*(2015) suggested thatpreservatives can be used to prevent growing of microorganisms in water containing product during use. They must be free of pathogenic microorganisms and total number of aerobic microorganism per gram or milliliter must be as per the pharmacopeia criteria. To control microbial growth and to stabilize any cosmetic product, some form of preservative needs to be used. However, in many cosmetics no expiry date has been reported and may lose the preservative activity and became a potential risk for microbial contamination (Fatma *et al.*, 2010).Minor pH changes also inactivate other preservatives. Minor shifts in ionic strength or changes in the buffering system in a product can also alter a bacterium's susceptibility to a biocide or affect how a preservative partition between the water matrix and the microbial cell (Daniel, 1995). Biofilm can form on unprotected surfaces in storage tanks. Biofilm provide protection for microorganisms making it more difficult for the preservative in a product to

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eradicate the microorganisms. The more often large microbial populations are exposed to inadequate levels of preservatives, the greater the chance that some microorganisms may respond to the preservatives and after their susceptibility to it (Warwick, 1993).

Qasem *et al.* (2014) reported several bacterial species from contaminated cosmetics and found that many of the isolates exhibited resistance to more than one preservative category; but there were no correlation between preservatives and antibiotic resistance.On the other hand, there is an indication that bacterial contaminants of cosmetics were resistant to many antibiotics, but again this resistance was not correlated to that of preservatives. In a few instances, bacterial cross resistance between biocides and antibiotics were noted, while in others, no direct link between biocide exposure and antibiotic resistance could be established.

2.3.1 Natural Preservatives

According to Sanjay and Rupashree (2014), natural preservatives are chemical constituents extracted from natural sources that offer intrinsic ability to protect products against microbial growth. These include essential oil constituents, flavonoids, and other phenolic compounds. Nowadays, more and more consumers have questioned on the safety of chemical preservatives. Mariola and Karolina, (2013) also mentioned that traditionally used chemical preservatives often cause skin irritation and provoke allergenic reactions. Growing demand for natural and preservative-free cosmetic products prompted an idea of the replacement of synthetic preservatives with natural compounds of antimicrobial properties.

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2.3.2. Plant based preservatives

Plant derived compounds are readily available and environment-friendly. Nowadays, much attention has been focused on essential oils that demonstrate antimicrobial activities and have been proposed preservatives; these include tea tree (*Melaleuca alternifolia*), thyme (*Thymus vulgaris*), lemon grass (*Cymbopogon citratus*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), calamint (*Calamintha officinalis)* or lavender (*lavendula officinalis*) and many others (Mariola and Karolina, 2013).Essential oils are the subject of intensive scientific research and also attract attention of cosmetic and pharmaceutical industries due to their potential active pharmacological compounds or natural preservatives. They have been considered as promising candidates. Although excellent antimicrobial properties have been confirmed for some of them, the studies on application of essential oils in cosmetic formulations as preservatives have not brought satisfactory results. The most promising candidates for preservatives are: tea tree, lemongrass, oregano and clove, which show activity against a wide range of gram positive and gram negative bacteria (with exception of *P. aeruginosa*), with MIC lower than 1% (Mariola and Karolina, 2013). Among plants (Shirisha *et al.*, 2014), ginger (*Gingiber officinale*) extract is highly effective preservative as it could inhibit the growth of *Aspergillus niger, Penicillium* sp. and *C.albicans.* Likewise,Babool was found effective against *Penicillium* sp. as Green tea inhibit growth of *A. niger, S. aureus* and *P. aeruginosa.* Henna (*Lawsoniainermis*) is effective against *C. albicans*. Similarly, Liquorice (*Glycyrrhizaglabra)* extract is highly active against the growth of *Paeruginosa, Bacillus subtilis* and *Enterobacter aerogenes.* Beet root was found inhibiting *P. aeruginosa and B. subtilis*. Amla extract was able to inhibit the growth of *S. aureus* and *P. aeruginosa* (Shirisha *et al.*, 2014).

It is estimated that more than 3000 essential oils (EOs) are of commercial importance and used in flavor and cosmetic industries (Bakkali *et al.*, 2008). Trade of the most popular oils such as that of eucalyptus or lemon are calculated at over 1000 metric tons a year with estimated value of several hundred million Euros (Brud,2010).Antimicrobial activities of EOs are well established and are effective against both saprophytic bacteria and fungi, the main source of contamination for cosmetics (*Bacillus* sp., *Micrococcus* sp., *aereomonas sp., acinetobacter* sp., *aspergillus* sp. or *Penicillium* sp.) and causes of human pathogens

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(*Staphylococcus* sp., *Streptococcus* sp., *Salmonella* sp., *Candida* sp. and others) (Mariola and Karolina, 2013).

In general, antimicrobial activity of essential oils is determined by their composition and concentration of components (Blitzke *et al*., 2009) he number of constituents in essential oils varies with figure as high as 100. In addition, due to thyme's purifying, tonic and protecting properties mainly supported by ethnobotanical sources, essential oil is often incorporated into hygiene and skin care products such as soaps, toothpastes, shower gels, shampoos, deodorants and body lotions. The essential oil of thyme succeeded in satisfying for preservation efficacy against bacteria. The fungal toxic activity of carvacrol has also been demonstrated and found to exhibit the highest activity against different strains of *A. flavus* and *A. parasiticus* at a minimum of 10 mL carvacrol per paper disk (Manou *et al*., 1998). Other report also suggested that every part of neem tree (bark, fruit, seed, flowers leaves gum and sap) is used for medicinal and cosmetic purposes. Cosmetically, the chemical constituents of neem are considered to be antiseptic and naturally preservative (Joshiand Pawar, 2015).

2.4 Medicinal Plant

Traditional, complementary and alternative medicines are commonly used to treat or prevent disease and chronic illness to improve quality of life (WHO, 2003). Global traditional medicine usage is widespread and growing in various parts of the developing world such as Africa, India, and Latin America in which about 80, 65, and 40-71%, respectively, of their population use traditional medicine to meet their primary healthcare needs (WHO, 2003).

The medicinal value of plant is attributed to its chemical constituents which could be under class of alkaloids, flavonoids, tannins and other phenolic compounds (Edeoga *et al.,* 2005).In Africa and elsewhere in the world, plant extracts are still widely used in the treatment of malaria (Cátia *et al.*, 2008).For instance, in Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicines at home(Sanjay and Rupashree, 2014). In addition, some *Millettia* species are traditionally used for the treatment of bacterial and malarial infection (Khalid and Waterman, 1986; Anderson, 1986; Desta, 1993).Traditional medicine has also been used in the treatment and care of such life-threatening illnesses like AIDS. Studies in Africa and North America have shown that up to 75% of people

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living with HIV/AIDS use traditional medicine alone or in combination with other medicines for various symptoms or conditions (WHO, 2003).

Similarly, in Ethiopia, traditional medicine plays an important role in the health care system. It is estimated that more than 80% of the population relies upon traditional medicine due to cultural acceptance, relatively low cost and lack of access of modern health facilities (Heidi and Girma, 2013).

2.5 Medicinal importance of *Erythrina abyssinica, Millettia ferruginea and Calpurnia aurea*

According to Charles and Kayanja (2012) *Erythrina abyssinica* (Leguminosae) is a species of leguminous tree distributed in Congo Republic, the Sudanese Republic, Ethiopia, Eritrea,Uganda, Kenya, Tanzania and Zimbabwe. In northern and western Ethiopia, it is found at elevations between 1600 and 2100m. *E. abyssinica* is a deciduous savannah legume. It grows in open woodland and grassland. It has characteristic red overflowing flowers. It can be propagated through seedlings, cuttings and truncheons. It has various traditional medicinal applications in livestock. It is also used in traditional human medicine.The antimicrobial activity of crude extracts of root barks and stem barks of *E. abyssinica* had activity against *S. aureus.* Benedicta *et al*. (2013) also explain that *Erythrina abyssinica* is a common plant in sub-Saharan Africa where it is used to treat inflammation, gonorrhea, wounds, stomach problems, diarrhea and viral infections.

Calpurnia aurea, a member of the subfamily Papilionoideae of the family Fabaceae (Coates, 1983), is a plant commonly used in traditional medicine to treat diverse medical conditions and parasitic infestation, both in humans and animals (Hutchings, 1996). It is a small, multi-stemmed tree, 3–4 m tall, occurring widespread in bush land and grassland in sub-Saharan Africa and India(Germishuizen and Meyer, 2003 ; Pooley, 1993).In Western Ethiopia, the juice of crushed leaves and barks of *C. aurea* is used for tick control (Regassa, 2000). The Borana people of Northern Kenya and Southern Ethiopia soak leaves of *C. aurea* in cold water to treat louse infestations in humans and calves (Heine and Brenzinger, 1988).The plant is also used in Ethiopia to treat stomach disorders, amoebic dysentery and eye diseases (Abate, 1989). In Southwestern Ethiopia, the leaves of C. *aurea*, mixed with other plant species, are crushed and squeezed to obtain a juice, which is applied through the auricular route for 2 days to treat earache

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in humans (Yineger and Yewhalaw, 2007). In the same area, the plant is traditionally used to treat rheumatism (Yineger *et al.*, 2008), for antibacterial and antioxidant activity (Adedapo *et al.*, 2008), to treat bacterial dermatitis (Tadeg *et al.*, 2005), as a natural pesticide to improve grain storage (Blum and Bekele, 2002), for the treatment of syphilis, malaria, rabies, diabetes, hypertension, diarrhea, leishmaniasis, trachoma, elephantiasis, fungal diseases, different swellings, stomach-ache, bowel, and bladder disorders (Natesan *et al.*, 2015.).

Millettia ferruginea, locally called *birbira*- in Amharic, *sotallo*- in Affan Oromo, belongs to the family Fabaceae and a sub family Papilionoideae. It is an indigenous plant species found only in Ethiopia. There are two sub-species known to be found in this country. These are: *Millettia ferruginea* sub species *ferruginea* which is confined to the Northern part of the country and *Millettia ferruginea* sub species *darasana* which is found in Southern provinces, particularly Sidama zone. The seed extract of *Millettia ferruginea* showed promising larvicidal activities against *Aedes agypti*, *Aedes africanus* and *Culex quinquefasciatus* (Debella *et al*., 2007). Traditionally, bark, mature fruit and seeds of *M. ferruginea* are used as fishing poison. The fruits, leaves, seeds and stem decoction of *M. ferruginea* are used respectively for the treatments of pain, earache and bacterial infection of nails, insecticidal properties and toothaches (Banzouzi *et al*., 2008). Furthermore, *M. ferruginea* leaf has activities against wound causing bacteria (Taye *et al*., 2011).

The seed of *Millettia ferruginea* has high oil and protein content (Andualem and Gessesse, 2014). In addition to its high protein content, birbira seed contains high concentration of minerals, especially phosphorus, potassium, magnesium, sodium and calcium (Andualem and Gessesse, 2010). Rotenone is one of the dominant constituents found in the seed, stem and bark of *M. ferruginea* (Jembere *et al*., 2007) although a variety of chemical compounds, particularly rotenoids and isoflavones have been isolated from the bark, seedpods, seeds and root bark of *M. ferruginea* (Dagne *et al*., 1990). It can be used as insect pesticide if production and application method is developed. Glutamic acid was the most predominant amino acid followed by aspartic acid, leucine, and lysine while the values of cysteine and methionine were in the lowest(Andualem and Gessesse, 2014).

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2.6. Some Compounds of *Erythrina abyssinica, Millettia ferruginea and Calpurnia aurea*

According to Manash*et a*l. (2016) phytochemical analysis of seeds and bark of *M. ferruginea* revealed a number of rotenoids, flavonoids and chalcones. Most of the published literature on Millettia involved phytochemical analysis of the plant samples and this genus was found to contain a rich variety of structurally distinct isoflavonoids. The water-insoluble isoflavonoid constituents of *M. ferruginea*, viz., barbigerone, calopogonium isoflavone-A and durmillone (fig 1) contributed to the antibacterial activity of water and methanol extracts of *M. ferruginea* against Gram-negative strains.

Fig 1 compounds of *M. ferruginea* seed

The Isoflavones compounds are common metabolites to *C. aurea.* For example, 4′,5,7-trihydroxyisoflavone (B-1), 7,3′-dihydroxy-5′-methoxyisoflavone (B-2), 7-hydroxy-4′,8-dimethoxyisoflavone (B-3), 7-acetoxy-4′,8-dimethoxyisoflavone (B-4) and 3',7-dihydroxy-4′,8-dimethoxyisoflavone (B-5), (fig 2) were isolated from its stem bark together with a pterocarpan (3-acetoxy-9-methoxypterocarpan) (B-6) and a quinolizidine alkaloid (calpurnine) (B-7). Evaluation of the compounds against some cancer cell lines indicated that 3',7-dihydroxy-4′,8-dimethoxyisoflavone (B-5) displayed the highest anticancer activity against breast (MCF7), renal (TK10) and melanoma (UACC62) (Erick, 2012) followed by 3',7-dihydroxy-5'-methoxyisoflavone (B-2). The genus *Calpurnia* has not been extensively studied for phytochemical compounds and only two species, *C. aurea* and *C. subdecandra* have been studied phytochemically. These two species have yielded bicyclic and tetracyclic quinolizidine alkaloids (Erick, 2012). Alkaloids are the major class of compounds possessing antimalarial activity as exemplified by quinine, one of the oldest antimalarial agents from plant Cinchona and

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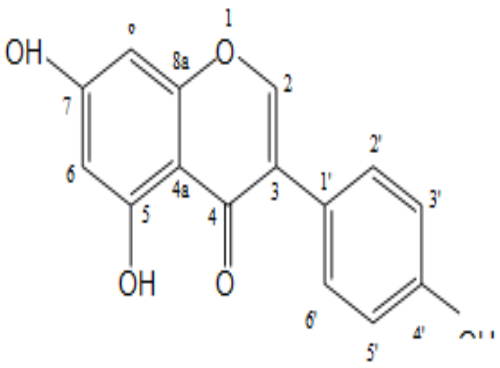
are still in use. There are also a number of antiplasmodial secondary plant metabolites that have shown antimalarial activities belonging to the classes of terpenes, limonides, flavonoids, hromones, xanthones, anthraquinones, phenolic compounds, quassinoids, sesquiterpenelactones (artemisinin) and other related compounds Accordingly, antimalarial activity of *C. aurea* could be attributed to the presence of plant secondary metabolites like alkaloids, terpenoids, flavonoids, and phenolic compounds contained in the leaf of the plant. These may have exerted their antimalarial activity in synergy or any one of them alone (Birhanu, 2015).



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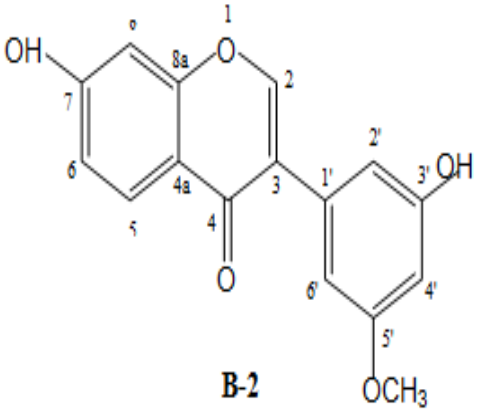
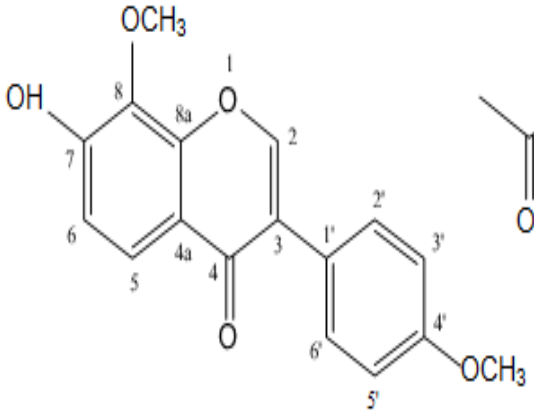
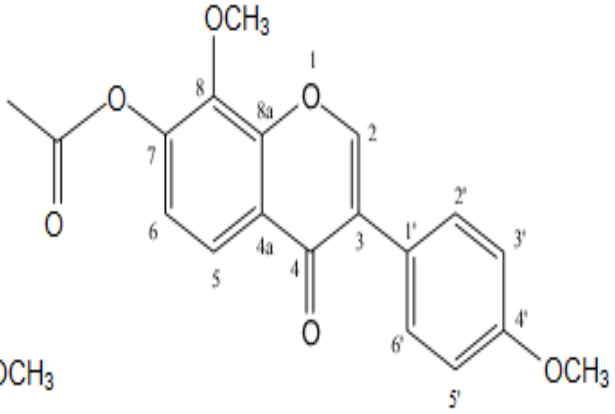


B-2
$C_{16}H_{12}O_5$
Exact Mass: 284.0685



B-3
$C_{17}H_{14}O_5$
Exact Mass: 298.0841



B-4
$C_{19}H_{16}O_6$
Exact Mass: 340.0947

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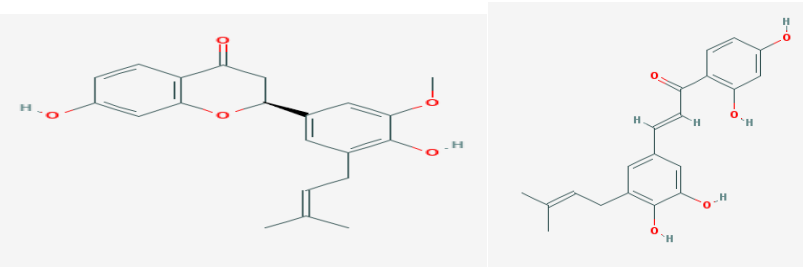
Fig.2 compounds of *C. aurea*

Report shows that the acetone extract of the roots and ethyl acetate extract of the stem bark of *E. abyssinica* showed potent anti-plasmodial activity against both chloroquine sensitive and chloroquine resistant strains of *Plasmodium falciparum (*Derese et al., 2003*)*. Chromatography separation of acetone extract yielded a pterocarpene [3-hydroxy-9-methoxy-10-(3,3-dimethy allyl) and an isoflav-3-ene [7,4′-dihydroxy-2′,5′-dimethoxyisoflav-3-ene] along other known compounds.From ethyl acetate extract of the stem bark, a new chalcone,2′,3,4,4′-tetra hydroxy-5-prenylchalcone (trivial name 5-prenylbutein) and a new flavanone, 4′,7-dihydr oxy-3′-methoxy-5-prenylflavanone (trivial name, 5-deoxyabyssinin II have been isolated (Derese *et al.*, 2003) (Fig. 3)



5-prenylbutein 5-deoxyabyssininII

Fig. 3 The structure of new chalcone (5-prenylbutein) and a new flavanone (5-deoxyabyssinin II) isolated from the stem bark of *E. abyssinica*

2.7Control of contamination of baby lotions

The safety of baby lotion calls for implementation of good hygiene practices. The practice encompasses, but not limited to, the following:

Good selection and handling of raw material According to FDA (1997) the raw materials must be identified, stored, examined, tested, inventoried, handled, and controlled to ensure they conform to appropriate standards and specifications. In particular, raw materials should be:

- Stored and handled to prevent mistakes (i.e., mix-ups or selection errors), contamination with microorganisms or other chemicals, and degradation from

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exposure to excessive environmental conditions (e.g., heat, cold, sunlight, moisture, etc.).

- Held in closed containers
- Maintained in containers that are labeled with the identity, lot number, and control status (release or quarantine).
- Sampled and tested for conformance with specifications and to ensure the absence of filth, microorganisms, and other adulterants prior to processing or usage (Animal and vegetable origin materials and those produced by cold processing methods should be reviewed for filth and/or microorganism contamination.); and
- Properly identified and controlled to prevent the use of materials that fail to meet acceptance specifications (FDA, 1997).

Water: The water used as a cosmetic ingredient is used as-is (i.e., directly from the tap) or if it has been treated before being used (i.e., has it been treated by such means as deionization, distillation, or reverse osmosis).If water is stored in tanks prior to use it should be treated with a biocide or stored at >75°C to control microbial growth (FDA, 2013).Although radiation treatment of stored deionized water is not widely practiced in the cosmetic industry, it is potentially a valuable means for controlling water quality (Stanley, 1967).

Cleaning and sanitization: Cleaning and sanitization is essential to ensure microbial quality during manufacturing of cosmetics and personal care products. These procedures should be validated in order to consistently meet hygienic manufacturing requirements (Public Review Draft, 1999). Cleaning is the process of removing product residue and contaminants such as dirt, dust, and grease from surfaces. Cleaning is an essential step that needs to be performed before the performance of a sanitization procedure. It is important that personnel involved in cleaning have a working understanding of the nature of different types of soils and the chemicals. Sanitization is the process utilized to reduce viable microbial contaminants to an acceptable level. All surfaces must be clean for the sanitization procedure to be effective (Public Review Draft, 1999).

Sterilization: Periodic sterilization of the physical equipmentS: tanks, pipes and pumps are required and desirable. Keeping a 2% solution of hydrogen peroxide in contact with the equipment for a two-hour period has been reported effective (Stanley, 1967).

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Storage and handling: Storage areas should be designed or adapted to ensure good storage conditions. They should be clean, dry and well maintained. Where special storage conditions are required (temperature and humidity) these should be provided, checked and monitored (HSA, 2013).Finally, microbiological monitoring programs are a very important component in any program designed to ensure that contamination problems in manufacturing are controlled and minimized. Raw materials, water, products and equipment must be sampled for microbiological analysis on a regular basis (DanishMinistry of Environment, 2010).

2.8Quality management

Cosmetic products must be manufactured in such a way as to ensure that they are fit for their intended use and are not expected to put consumers at risk due to inadequate safety or quality (HPRA, 2015). It is also important to note that Quality Control Laboratories must be designed appropriately to control the quality of the product, and adequate space must be provided to avoid mix-ups and cross-contamination. Sufficient and suitable storage space should also be provided for test samples, retained samples, reference standards, reagents and records (Ministry of Health and Family Welfare of India, 2005). The laboratory must also establish and maintain procedures to control all documents related to the quality of the analyses *(*Danish Ministry of Environment, 2010). There should be an adequate number of personnel having knowledge, experience, skill and capabilities relevant to their assigned function. They should be in good health and capable of handling the duties assigned to them (HSA, 2013).

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CHAPTER THREE

3. METHOD AND MATERIALS

3.1 Study Design

Cross section study was carried out to survey the microbial quality of selected brands of baby lotion being sold in Jimma town. Experimentally, antimicrobial activities of extracts of *Erythrina brucei*, *Millettia ferruginea* and *Calpurnia aurea* were evaluated against potential contaminants of baby lotions. Accordingly, the study involved data collection using pre-designed questionnaire, observation of the vending environment, laboratory activities.

3.2 Study area

The current study was carried out in Jimma town, Oromia Regional State (Fig.4 Jimma is located 353km southwest of Addis Ababa. Its geographical coordinates are approximately 7^{o} 41'N latitude and 36 ^{o} 50'E longitude. The town is found in an area of average altitude of about 5400ft (1780 m) above sea level. It lies in the climatic zone locally known as semi temperate which is considered ideal for agriculture as well as human settlement.The town lies on a low hill on the left side of the wide alluvial plain of the river Gibe, an affluent of the Omo River. It is crossed by two small streams, the Aweyitu and the Kitto, which subsequently join the Gibe via the Gilgal (small) Gibe (Seifu, 2002). Based on the 2005 national census, the total population of the town was 151,679 (CSA, 2005). Majority of the people in the city was engaged in more than 4500 business entities/trade and commerce which created jobs for 20,000 people. About 280 people engaged in transportation sector and 7500 employed on agricultural sectors. Whereas the industry sector hold small share, majority of the peoples employed in government and private sectors (JCASP, 2006).

Now a day, Jimma has many shops and private pharmacies in which cosmetics could be sold. According to the town Trade and Development office, even though there are many shops in which cosmetics are being sold, legally registered shops are only 16. According to the Health Bureau of the town, there are 53 private pharmacies, and 21 whole sellers under the supervision of branch office Food Drug and Health Care Administration and Control authority of Southwest

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Ethiopia branch office. Microbiological laboratory activities were conducted at Department of Biology while extraction of plant materials was carried out at Department of Chemistry of Jimma University.

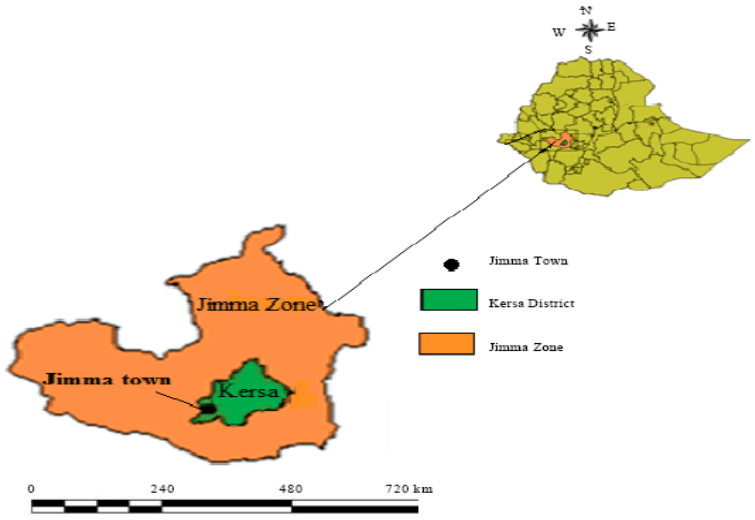

Fig 4. Map of the study site

3.3 Sampling technique:

Simple random sampling technique was employed for collection of sample from private pharmacies. Stratified random sampling that involved the division of a population into smaller groups, known as strata, was used to select shops where baby lotion are being sold.

3.4 Sample size

Sample size of pharmacies was determined by the following two formula (Cochran, 1977) and (Cochran, 1963).The first equation was used to determine sample size (n) and the second equation was correctional formula used to determine the final sample size of the study.

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$$n = \frac{N}{1 + N(e)^2}$$

$$n\prime = \frac{n}{(1+(n-1)/N}$$

N: the population size (53), n: sample size, n': corrected sample size and e: the level of precision (95%; e = 0.05)

In order to determine the corrected sample size, the sample size obtained with the first formula was reduced with the above second formula; consequently, 28 pharmacies were selected. The most widely used three brands of baby lotion were purchased from each shop and pharmacies that are included in the study. So, the total number of the baby lotions required for the study was 129.

3.4.1 Sample collection

A total of ninety cosmetic products representing, 3 brands of baby skin lotion and oil with appropriate date of manufacturing and expiry were purchased from different boutiques, supermarket and private pharmacies outlets in Jimma town and transported to microbiology Laboratory for analysis. The collected samples were stored at room temperature and surface of sample container disinfected with aqueous mixture of 70% ethanol (v/v) and dried with sterile gauze before opening. A code was given for each brand**.** The plant materials; *Erythrina brucei*, *Millettia ferruginea* and *Calpurnia aurea* were collected from different part of Oromia region and processed for extraction following standard procedures.

3.5 PreliminaryData Collection

3.5.1 Questionnaire

Questionnaire were distributed to shopkeepers and pharmacists to get preliminary information concerning widely used brands of baby skin lotion, the criteria used by users to prioritize the lotion, any health problems associated with the use of these cosmetics, and information on how they store the lotions and the issue of expiry dates (Annex 1).

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3.5.2 Observation

Expire date of the products were strictly inspected. Available information on the container of each baby lotion and oil, such as production date, batch number, ingredients used, presence of preservatives (additives) and precaution that the users must know were checked.

3.5.3 Microbiological analysis

3.5.3.1 Viable microbial count

Tryptone Soya Broth (TSB) +4% Tween 80 was used as an enrichment media in accordance with procedure recommended in the U.S. Food and Drug Administration's Bacteriological Analytical Manual [FDA BAM] (Denise *et al.,* 1999). This enrichment media contain inactivating agents to overcome the effect of preservatives and have been recommended for use in pharmaceutical and cosmetic testing. In addition, the methods of Hitchings *et al* (2001), Qasem and Rania, (2012) and Gamal *et al.* (2015) were adopted to evaluate the count of viable bacterial and fungal colonies. Briefly, after thorough mixing and serial dilutions, 0.1 ml aliquot was taken from each dilution (10^{-3}, 10^{-4} and 10^{-5}) and spread onto surfaces of solid Soya Bean Casein Digest (SBCD) agar media and incubated at 37°C for 48 h followed by count of the gown colonies. Molds were isolated on Sabouraud Dextrose agar and results were considered positive if, after incubation at 25^0C for 5 days fungal growth appeared on the inoculated plates. Certain bacteria can grow on SDA.Yeasts were counted on Sabouraud dextrose agar (SDA) after the plates were incubated at 25^0C for three days.

3.5.3.2 Enumeration of specific bacterial groups

From 10^{-2} dilution of each sample, 0.1 ml aliquot was spread on to the following medium: VRBA agar, Mannitol salt agar, *Pseudomonas* isolation agar base, and phenol red egg yolk polymyxin (MYP) agar base for detection of coliforms, *Staphylococcus* sp, *Pseudomonas* sp, *and Bacillus* sp, respectively (Kanta *et al.* 2013**;** Elmorsy and Hafez, 2016). All the plates were incubated at 37 °C for 48 hours. Presence of *E. coli* was confirmed by the appearance of bluish black colonies with green metallic sheen on Eosine-Methylene Blue (EMB) agar. Confirmatory biochemical tests were carried out for further identification.

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3.5.3.3 Microscopic identification

3.5.3.3.1 Gram reaction

Gram reaction was carried out according to WHO (2003). Briefly, from 48hr old culture, two or more colonies of the test organism was picked by inoculating loop and emulsified in drop of distilled water on sterilized slide. The smear is allowed to air dry and then rapidly passed three times over the flame of Bunsen burner. It was allowed to cool and covered with crystal violet stain for 30-60 seconds. Then, stain was rapidly washed/rinsed with clean water. After tipping off all the water, the smear was covered with Lugol's iodine for 30-60 seconds and the extra iodine on the slide washed with clean water. The smear was decolorized rapidly for 30-60 seconds with acetone-alcohol and washed immediately with clean water. Then, the smear was covered with safranin for 2 minutes. The stain was washed off with clean water. The back of the slide was wiped clean, and placed in a draining rack for the smear to air dry. Finally, the smear was examined under microscope. Only those slides with cells that retained the primary dye were considered positive for gram reaction.

3.5.3.3.2 Detection of the fungal isolates

The detection of fungi was based on the morphology of colonies on Sabouraud dextrose agar and the direct microscopic examination by lacto phenol stained method (Huda, 2011).Lacto phenol Blue Solution is a mounting medium and staining agent used in the preparation of slides for microscopic examination of fungi. a drop of Lacto phenol was paced Blue Solution on a slide. Then Using an inoculating needle, the fungal culture was carefully spread into a thin preparation. Finally a coverslip edge was placed on the drop and it was observed under microscope.

3.5.3.4Biochemical identification

3.5.3.4.1 Coagulase test

Coagulase causes plasma to clot by converting fibrinogen to fibrin. Coagulase was detected by clotting in the slide. Bound coagulase (clumping factor) convert fibrinogen directly to fibrin without requiring a coagulase reacting factor. It can be detected by the clumping of bacterial cells in the rapid slide test (Cheesbrough, 2006).

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3.5.3.4.2 Catalase test

Catalase test was carried out according to Cheesbrough (2006). Briefly, 2-3ml of 3% H_2O_2 was added to a slide and then several colonies of the test organism from 18-24 hours old culture from plate was removed using sterile glass rod and immersed into the H_2O_2 solution. Production of bubbles of oxygen was an indication for catalase production.

3.5.3.4.3 KOH test

One to two drops of 3% KOH solution was placed on aclean microscopic slide. A colony was picked with a sterile bacteria logical wire loop and inoculating loop was then resided slowly from the mass.Viscosity of KOH solution was taken as a positive result for the cells being Gram -negative. This test was performed for further confirmation of result obtained with gram reaction test.

3.5.3.4.4 Fermentation of carbohydrate

Phenol Red Broth was prepared with a final concentration of one percent carbohydrate. The end product of carbohydrate fermentation is organic acids which, in the presence of phenol red, produce a color change in the medium from red to yellow. The gas produced during the fermentation reaction was collected in inverted Durham tube. Change of color and gas production was considered positive for fermentation of specific carbohydrate.

3.5.3.4.5 Indole test

Bacterium to be tested was inoculated in peptone water, which contains amino acid tryptophan, and incubated overnight at 37 ^{o} C. Following incubation, few drops of Kovac's reagent were added. Kovac's reagent consists of Para-dimethyl aminobenzaldehyde, isoamyl alcohol and con. HCl. Ehrlich's reagent is more sensitive in detecting indole production. Formation of a red or pink coloured ring at the top was considered positive result for iodole production.

3.5.3.4.6 Citrate test

Bacterial colonies were inoculated into Simmon's citrate agar slant and incubated overnight at 37^{o}C. The formation of color change of media from green to blue was taken as a positive.

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3.5.3.5 Challenge test for preservative efficacy

The efficacy of antimicrobial preservation of cosmetic products was investigated as suggested by USP. *P. aeruginosa, E. coli,* and *S. aureus* were used in this study. Freshly grown (activated) bacteria were harvested in sterile solution containing 0.9% of sodium chloride and 0.1% peptone in order to prepare $1x10^8$ cfu/ml inoculums. About 20 ml of each sample was aseptically inoculated with 0.2 ml of each standardized inoculum suspensions. All inoculated samples were shaken and incubated at 25°C for 28 days. About 0.2 ml samples were aseptic drawn at intervals on days 0, 7, 14 and 28. Appropriate dilutions were plated on pre-prepared sterile and dry neutralizing medium [Dey-Engley Neutralizing Agar (Difco)].Plateswere incubated at 37°C 24-48h. At the end of the incubation period, the numbers of viable cells (colonies) were recorded for each plate and counts were expressed as colony forming units per liter (cfu/l) (USP, 2003).

3.5.3.6 Antibiotic susceptibilitytesting

3.5.3.6.1 Turbidity standard for inoculum preparation

A 0.5 McFarland standard was prepared according to WHO (2003). Briefly, the standard was prepared by mixing two solution: solution A and solution B. Solution A was 1% (10ml/l) solution of sulfuric acid and solution B was 1%(10g/l) solution of barium chloride. An amount of 0.6 ml of barium chloride (solution B) was added to 99.4 ml of sulfuric acid solution (solution A). A small solution of turbid solution was transferred to a screw – cupped bottle of the same type as used for preparing the test and control inoculum. Later it was stored in a well-sealed container in a dark place at room temperature until used.

3.5.3.6.2 Growth ofbacterial culture

At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the culture ~~growth~~ is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth. The broth culture is incubated at 35°C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours).The turbidity of the actively growing broth culture is adjusted with

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sterile saline or broth to obtain turbidity optically comparable to that of the 0.5 McFarland standards.

3.3.6.3 Preparation of antibiotic stock solutions

According to Jenifer (2006), suitable range of antibiotic concentrations was chosen for the organisms to be tested.

Stock solutions was prepared using the formula

$$\frac{1000}{P} \times V \times C = W$$

Where P = potency given by the manufacturer (µg/mg), V = volume Required (mL), C = final concentration of solution (multiples of 1000) (mg/L), and W = weight of antibiotic in mg to be dissolved in volume V (mL).

Further stock solutions were prepared from the initial 10,000 mg/L solution.

1 mL of 10,000 mg/L solution + 9 mL diluent* = 1000 mg/L,

100 microliter of 10,000 mg/L solution + 9.9 mL diluent* = 100 mg/L

3.5.3.6.4 Preparation of antibiotic dilution range

Dilutions prepared in the current study ranged between 0.25 - 128 mg/L, Accordingly, 11 labeled universal bottle with varying concentration of antibiotics were prepared as follows: 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 and 0 mg/L. No antibiotic is added to the bottle labeled 0 mg/L (antibiotic free growth control). The detailed procedure for preparation of the above dilutions was presented in Annex 2.

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3.5.3.6.5 Broth dilution for MIC Determination

1. Dilution range of 0, 0.5, 1, 2, 4, 8, 16, 32, 64,128 mg/L of the antibiotics were prepared for addition of an equal volume of inoculum.
2. The broth was prepared and 20ml of broth was added to each tube.
3. 75 x 12 mm sterile capped tubes were arranged in two rows for each antibiotic to cover the range of antibiotic dilutions chosen in duplicate.
4. 1mL volumes of each antibiotic dilution were transferred to the tubes.
5. 1mL aliquots of test organism inocula that have equivalent turbidity with 0.5 McFarland standard was added to one set of tubes. The contents of the tubes thoroughly mixed.
6. The test tubes were incubated at 35-37^{0}C for 18-20 h in air.

3.5.3.6.4 Reading and Interpretation

The lowest concentration of antibiotic at which there is no visible growth was recorded as MIC value of that particular antibiotic.

3.5.3.7 Extraction of the plant

The collected partsof the three plant materials were shade dried and ground using mortar and pestle for extraction. Approximately 100 g of each ground plant material was separately extracted by maceration with chloroform and methanol (1:1, v/v) for 24 hours. This process was repeated by the same solvent system at least three times. Each step is followed by decanting and concentration. Concentration was done using Rotary evaporator operating at 50°C over water bath. The dried crude extracts were stored in the refrigerator at 4°C until use (Akinsulire *et al*., 2007).

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3.5.3.7.1Antimicrobial activity evaluation

The methods of Saha*et al*. (1995), Rajesh*et al*. (2010) and Akinyemi*et al*.(2006) were adopted to evaluate antimicrobial activity of extracts and some compounds on isolated pathogens. About 0.1 mL of the standardized inoculums (0.5 McFarland standards) of the test organisms were separately spread on Muller-Hinton agar plates. A sterile filter paper disc (6mm) previously soaked in 250 mg/ml of crude extract and 2mg/ml of compound of *Millettia ferruginea* were separately placed on freshly inoculated Muller-Hinton agar plates; The plates were incubated for 24 h at 37°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Gentamycin was used as a reference standard and dimethyl sulphoxide (DMSO) was used as a control.

3.6 Data quality management

The sterility of the media used was checked by incubating the media overnight before its use. And regular checking of incubation temperature, careful measurement of inhibition zone diameters were done as part of the data quality management.

3.7 Statistical data analysis

Questionnaire based preliminary data were analyzed using descriptive statistics and percentages used for comparison. Mean differences in the microbial safety levels of the different baby lotions and efficacy of different plant extracts were analyzed using One Way ANOVA.

3.8 Ethical consideration

Ethical permission was obtained from the Research and Ethical Review Board of College of Natural Sciences, Jimma University. The objective and nature of the study was explained to the pharmacist and shop keepers prior to data collection.

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CHAPTER FOUR

4. RESULT

Table 1.Public awareness on safety regulation of cosmetic products, including baby lotions

Source of respondents	Government has a system to control quality and safety of cosmetic product		
	Yes, N (%)	No N (%)	Total N (%)
Pharmacies	25 (58.13)	3 (6.98)	28 (65.10)
Boutique shops	2 (4.65)	9 (20.93)	11(25.58)
Supermarkets	2 (4.65)	0	2 (4.65)
Cosmetics shops	1(2.32)	1 (2.32)	2 (4.65)
Total	30 (69.77)	13 (30.23)	43 (100)

As indicated in Table1, a total of 30 (69.77 %) of the respondents including pharmacists (58.13%), workers in cosmetic shops (2.32%) and supermarkets (4.65%) believed that Ethiopian government has a system of controlling quality and safety of cosmetic products. However, more than a quarter (30.23 %) of the respondents consider that there is no strict safety regulation, and had concern that the public health at risk of using potentially dangerous cosmetics.

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Table 2 Practice of checking for expire date before buying and preference time for transportation of cosmetics from source of distribution

Respondents	Frequency of checking of expire dates of cosmetics (while buying from main source)				Preferred time for transportation of cosmetics			
	Always	Often	Sometimes	**Total**	Any time	Afternoon	Night	Total
Pharmacies	25 (58.13)	3 (6.98)	0 (0)	28 (65.12)	17 (39.53)	0 (0)	11 (25.58)	28 (65.12)
Boutique	8 (16.60)	2 (4.65)	1 (2.32)	11 (25.58)	9 (20.93)	2 (4.65)	0 (0)	11 (25.58)
Cosmetics shop	1 (2.33)	1 (2.33)	0 (0)	2 (4.65)	1 (2.32)	0 (0)	1 (2.32)	2 (4.65)
Supermarket	2 (4.65)	0 (0)	0 (0)	2 (4.65)	1 (2.32)	0 (0)	1 (2.32)	2 (4.65)
Total	36 (83.72)	6 (13.95)	**1** (2.32)	43 (100)	28 (65.12)	2 (4.65)	13 (30.23)	43 (100)

``

The majority of the respondents, mainly pharmacists (58.13 %) and workers in boutique shops (16.28%) always check expiry date of cosmetics during purchasing of same products from distributors (Table 2). Likewise, 55.81% of pharmacists, 4.65% and 16.27% of the respondents serving in supermarkets and boutique, respectively, responded that most of their customers have a regular trend of checking expiry date of cosmetic products before purchase (Annex 3). Similarly, 9.3% of pharmacists, and 2.32% each of respondents working in boutique and cosmetic shops, stated that all of their customers do not buy products without prior checking of expiry date although 6.98% and 2.32% of respondents from the same sources replied that their customers have a practice of reading expiry date only some before buying the products (Annex 3).

With regards to time preference for transportation of cosmetics from site of distribution to vending shops, most pharmacist (39.5%), workers in boutiques (20.9%) and supermarkets (2.32%) responded that they do not have special time preference to transport products from Addis Ababa and different sources of Jimma town to their shops (Table 2). However, 25.58% of pharmacists and workers in supermarket and cosmetics shop (2.32% each) had preference to transportation during night time (cold weather) with due consideration the adverse effect of high temperature on the quality of cosmetics.

Table 3 Storage practice of cosmetic products as observed in Jimma town, 2016/17

Sources of cosmetics	**Methods that protect the products safety in the store**					
	Keeping them away from sun light and providing dry place	Keeping them away from sun light	Providing different shelf	Providing aerations	Cleaning the store	**Total**
Pharmacies	9(20.93)	19(44.19)	0 (0)	**0**(0)	**0**(0)	28(65.12)
Boutique	0 (0)	2(4.65)	4(9.30)	**1**(2.33)	4(9.30)	11(25.58)
Cosmetics shop	0(0)	1(2.33)	0 (0)	**0**(0)	1(2.33)	2(4.65)
Supermarket	1(2.33)	1(2.33)	0(0)	**0**(0)	0(0)	2(4.65)
Total	10(30.23)	23(53.49)	4(9.30)	**1**(2.33)	5(11.63)	43(100)

``

As shown above (Table 3),most of the respondents from pharmacies (65.12%) and few from supermarkets and boutiques (4.65%, each) and cosmetic shops (2.32%) responded that the products have been kept away from direct sunlight during storage. However, relatively high numbers of respondents (20.93%) handling cosmetics in boutique shops store the products carelessly and did not consider the adverse effect of high temperature on the quality of cosmetics.

Table 4 criteria used for product selection

Criteria	Quality	Price	Brand	Manufactured	Media	Popularity
Number of respondents	14(32.56%)	12(27.91%)	4(9.3%)	1(2.33%)	9(20.93%)	3(6.97%)

Preference to different brands of baby lotions appears to depend on different criteria. With quality, price, brand, date manufactured, media promotion and popularity in the market, many of the 43 respondents based there selection mainly on quality (32.56%), price (27.91%), and media promotion (20.93%).However, the packaging qualities of the four brands of baby lotions assessed in the current study varies as some of them lack labeling with regards to batch no, pH, and other safety precaution to users (Table 5). Accordingly, it was not common practice writing batch number and pH value on the container for Johnson baby lotion and marking of the package with special precaution that the consumer must be aware of on Nunu and Just for baby lotions.

``

Table 5 Completeness of labeling of container for cosmetic products, the case of baby lotions, Jimma town,2016/17

Samples/ Brands	Manufacturing date	Expire date	Batch no	Preservative indicated	pH	Special precaution
Johnson	A	A	NA	A	NA	A
Nunu	A	A	A	A	A	N/A
Just for baby lotion	A	A	A	A	A	NA
Just for baby oil	A	A	A	A	A	A

Where, A= available**; NA=**not available

As exhibited in Table 4, it is not a common practice to write a batch number and product's pH on package of *Johnson baby lotion*. In addition, special precaution that the consumer must be aware of with regard to the product quality is not indicated on the *Nunu* and *Just for baby lotions*.

``

Table 6 Mean Total of aerobic mesophilic bacterial and yeast count (cfu/ml) of selected baby lotions, Jimma town, 2016/17

Brand	Total count of aerobic mesophilic bacteria	Bacterial count per brand			Yeast Count	Yeast count per brand		
		Ph	Bu	Su		Ph	Bu	Su
Johnson baby lotion	4.8×10^7	3.3×10^7	6.7×10^7	3.6×10^7	2.4×10^7	1.8×10^7	3.2×10^7	1.7×10^7
Just for baby lotion	2.9×10^7	2.9×10^7	ND	ND	1.96×10^7	1.96×10^7	ND	ND
Nunu baby lotion	3.6×10^7	3.5×10^7	3.93×10^7	ND	2.3×10^7	1.96×0^7	3×10^7	ND
Just for baby oil	2.2×10^7	2.2×10^7	NA	NA	1.75×10^7	1.75×10^7	ND	ND

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TVC, Total Viable Count; Ph: Pharmacy, Bu: Boutique, Su: supermarket, ND =Not Detected

Based on the finding presented in Table 6 above, all samples of each brands of baby lotions exhibited high load of both bacteria and yeast. The mean TVC of bacteria in Johnson lotion was as high as 4.8 x 10^7 cfu/g ranging between 3.3x10^7 (samples from pharmacy) to 6.7x$10^{7.}$(Butique samples). Likewise, the highest mean yeast count (2.4x10^7 cfu/g) was recorded in Johnson lotion samples with average count of different sample sources ranging between 1.7x10^7 (samples from supermarket) to 3.2x10^7 (boutique samples). The same pattern was observed in nunu lotions although the mean count of bacteria (3.6x10^7cfu/g) and yeasts (2.3x10^7cfu/g) were relatively low. In general, the highest bacterial and yeast loads were encountered in Johnson lotion followed by nunu lotion sold in boutiques.

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Table 7 Dominant microbial groups/genera/ isolated from baby lotions, Jimma town, 2016/17.

Brands	Sample size	Isolated bacterial genera/group	Positive sample	Source of sample		
				Ph	Bu	Su
Johnson	30 (16,12,2 from ph, bu, and su, respectively)	*Staphylococcus*	7 (23.33%)	(2)(12.5%)	(5)(41.67%)	0(0%)
		Bacillus spp	4(13.33%)	(2)(12.5%)	(2)(16.67%)	0(0%)
		Coliforms	9(30%)	(3)(18.75%)	(5)(41.67%)	1(50%)
Just for baby lotion	10 (all from Ph)	*Staphylococcus*	3(30%)	(3)(30%)	ND	ND
		Coliforms	4(30)	(3)(30%)	ND	ND
Just for baby oil	20 (all from ph)	*Staphylococcus*	2(10%)	(2) (10%)	ND	ND
		Coliforms	4(20%)	(4)(20%)	ND	ND
Nunu baby lotion	30 (25, 5 from ph and Bu respectively)	*Staphylococcus*	4(13.33%)	(1)(4%)	(3)(60%)	ND
		Coliform	8(26.67%)	(4)(16%)	(4)(80%)	ND

Ph: Pharmacy, Bu: Boutique, Su: supermarket, ND: not determined

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Staphylococcus species, *Bacillus* and Coliforms were among the dominant microbial genera/groups frequently identified from most of the baby lotions evaluated in the current study. As summarized above (Table8)**,** out of 30 samples of Johnson baby lotion, 7 (23.33%) were found contaminated with coagulase negative *Staphylococcus* spp, 5(41.67%) of which were purchased from boutique. Furthermore, *Bacillus* spp was also isolated from 4(13.33%) samples, 2(12.5% each) purchased from pharmacies and boutiques. About a quarter, 9(30%) of Johnson samples most of which were purchased from boutique, 5 (41.67%) were found harboring *coliforms.* Likewise, of 30 Nunu baby lotion, 4(13.33%) were found to contain coagulase negative *Staphylococcus* while *coliform* were isolated from 8(26.67%) samples.

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Table 8.Prevalence of each target bacteria species within each brand of baby lotion and oil.

Brands of baby lotion and oil		Isolated bacteria species			
		Coagulase negative *Staphylococcus*	*Coliform*	*Bacillus*	total
Jonson	Count	$1.6X10^4$	$1.7\ X10^4$	$1.4\ X10^4$	$4.7X10^4$
	% of Total	12.1%	12.8%	10.6%	35.6%
Just for Baby Lotion	Count	$1.2\ X10^4$	$1.5\ X10^4$	0	$2.7X10^4$
	% of Total	9%	11.3%	0	20.5%
Nunu	Count	$1.4\ X10^4$	$1.9\ X10^4$	0	$3.3X10^4$
	% of Total	10.6%	14.4%	0	$3.3X10^4$
Just for Baby oil	Count	$1.1\ X10^4$	$1.4\ X10^4$	0	$2.5X10^4$
	% of Total	2.9%	10.6%	0	18.9
	Count	$5.3\ X10^4$	$6.5\ X10^4$	$1.4\ X10^4$	$1.32x10^5$
	% of Total	40.2	49.2	10.6	100%

Based on the above Table 9, the prevalence of *Coliform* spp was appeared to be the highest. And the total count of coagulase negative *staphylococcus spp* was found to be higher than *bacillus spp* with in selected brands of baby lotion. The prevalence of isolated bacteria spp and groups in Johnson baby lotion is also the highest. Besides the total count of isolated bacteria spp within nunu lotion comes next followed by their prevalence within just for baby lotion. Likewise the total count of isolated bacteria spp within just for baby oil is the lowest. The highest prevalence of coagulase negative *Staphylococcus* spp and *Coliform* spp was observed in Johnson and Nunu

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baby lotion respectively. Since *Bacillus* spp was only isolated from Johnson baby lotion, and hence, its incidence is the lowest.

Antibiotics susceptibility patterns of dominant bacterial isolates

Table 9Antibiotic susceptibility patterns of *Staphylococcus* and *Bacillus* species isolated from baby lotions, Jimma town, 2016/17

Antibiotics	Conc. of antibiotics (mg/L)	Groups tested and proportion found sensitive to the specific conc. of antibiotics	
		CNS(N= 16)	*Bacillus* spp. N=4
Teteracycline	8	2 (12.50%)	ND
	16	9 (56.25%)	1(25%)
	32	5 (31.25%)	2(50%)
	64	ND	1(25%)
Amoxacillin	8	3(18.75%)	ND
	16	9(56.25%)	1(25%)
	32	4(25%)	2(50%)
	64	ND	1(25%)
Cephalexin	8	2(12.50%)	ND
	16	10(62.50%)	2(50%)
	32	4(25%)	2(50%)
Clindamyxin	8	9(56.25%)	ND
	16	5(31.25%)	2(50%)
	32	2(12.50%)	2(50%)
Doxycycline	8	6 (37.50%)	1(25%)
	16	9(56.25%)	1(25%)
	32	1(6.25%)	2(50%)
Eryrthromycine	8	0	0
	16	4 (25%)	2(50%)
	32	8(50%)	2(50%)
	64	4(25%)	ND

CNS = Coagulase negative *Stapylococcus , ND= Not Determined*

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According to Table 10, coagulase negative *staphylococcus* spp were the more sensitive to almost all antimicrobials tested than *Bacillus* species with MIC values as low as 8mg/L. To the contrary, *Bacillus* species revealed better tolerance (hence resistant) to most antibiotics with MIC values as high as 64mg/L for Tetracycline, Amoxacillin, and Erythromycin.

Yield of extracts from selected plants of Medicinal Importance

Table 10.Yield efficiency of extracts from selected plants

Plant (vernacular-and scientific name)	Solvents	plant part used	Weight of plant sample	Dry weight of crude extract	Yield (%)
Digit*a (Calparina aurea*)	$CHCl_3$/MeOH	Immature seed	100g	14.66 gram	14.66
Birbira (*Millettia ferruginea)*	$CHCl_3$/MeOH	Seed	100g	31.73 g	31.73
digita (*Calparina aurea)*	$CHCl_3$/MeOH	Immature pod	100g	14.69g	14.69
digita (*Calparina aurea)*	$CHCl_3$/MeOH	Mature pod	100g	9.73 g	9.73
Birbira (*Millettia ferruginea)*	$CHCl_3$/MeOH	Pod	100g	5.32 g	5.32
Birbira (*Millettia ferruginea)*	$CHCl_3$/MeOH	Bark	100g	6.14g	6.14
Korch (*Erythrina abyssinicas)*	$CHCl_3$/MeOH	Bark	100g	15.5g	15.5
Korch (*Erythrina abyssinicas)*	$CHCl_3$/MeOH	Leaf	100g	31.31 g	31.31

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Maximum (31.73%) and minimum (5.32%) yields were obtained from the seed and pod of *Millettia ferruginea*. The higher yield from seed of *Millettia ferruginea* was followed by yield from Leaf of *Erythrina abyssinica* (31.31%), bark of *Erythrina abyssinica* (15.5 %) and pod of *Calparina aurea*(14.69%).

Antimicrobial activity of plant extracts and pure compounds

Table 11 Antimicrobial assay of extracts against potentially pathogenic isolates from baby lotions

Source of extract	Inhibition zone diameter (mm)	
	Staphylococcus spp	*Bacillus* spp
Immature *Calparina aurea* seed	1.5	0
Millettia ferruginea seed	0	1.5
Immature *calparina aurea* pod	0	0
Mature *calparinaaurea* pod	0	0
Millettia ferruginea pod	2	0
Millettia ferruginea bark	0	0
Erythrina abyssinica bark	0	0
Erythrina abyssinica leaf	0	1

As showed in Table 11 above, all bacterial isolates did not show any susceptibility for immature and mature *Calpurnia* seed pod, *Erythrina abyssinica* bark as well as *Millettia ferruginea* bark. Extract of *Millettia ferruginea* seed pod had very low activity on coagulase negative *Staphylococcus* spp. In addition, extract of *Erythrina abyssinica* leaf had insignificant activity on *Bacillus* Spp. Besides, the immature *Calpurnia* seed had minor activity on coagulase negative *staphylococcus*. *Millettia ferruginea* seed had also very low activity on *Bacillus* spp.

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Table 12 antimicrobial activity of pure compounds of *Millettia ferruginea*

Name of extract	Inhibition zone diameter (mm)	
	Staphylococcus spp	*Bacillus* spp
TSK-2B(Calopogonium isoflavone-A)	0	0
TSK-2G (Durmillones)	0	0
TSK-2J (Prebarbigerone)	0	0
TSK-2I (Barbigerone)	0	0

All pure compounds isolated from *Millettia ferruginea* seed did not show any activity on both Coagulase negative *Staphylococcus* spp and *Bacillus* spp. This observation is in contrast to the mild activity recorded for extract of *Millettia ferruginea* pod (Table 9).

Preservative efficiency of preservatives in selected baby lotions

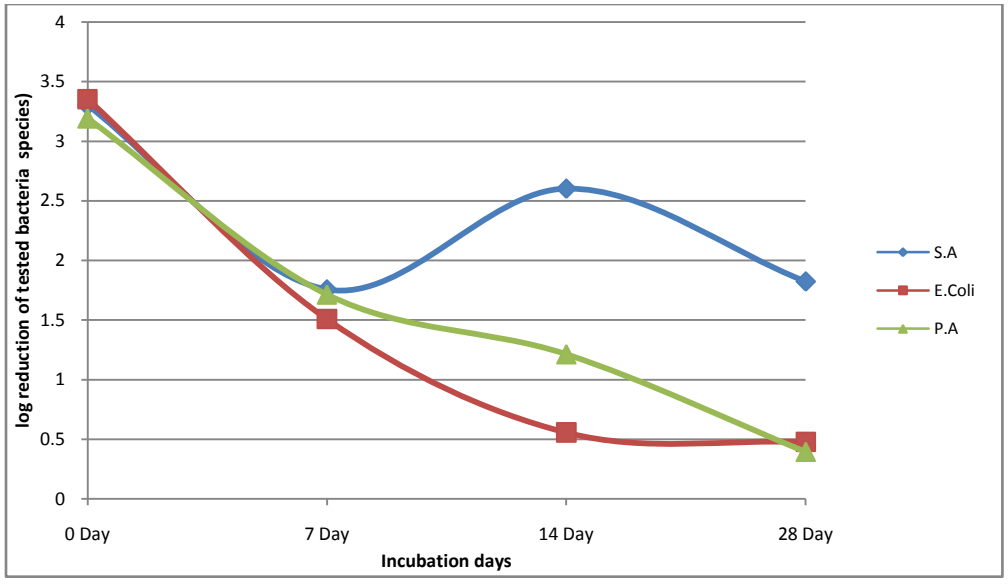

Fig. 1 Preservative capacity of nunu baby lotion against different bacteria species.

According to the above fig only *Staphylococcus aureus* showed significant reduction on day 14 but its reduction was below log 2.and it exhibited increment on day 28.*E.coli* and *Pseudomonasaeruginosa* raised on day 14 and 28.

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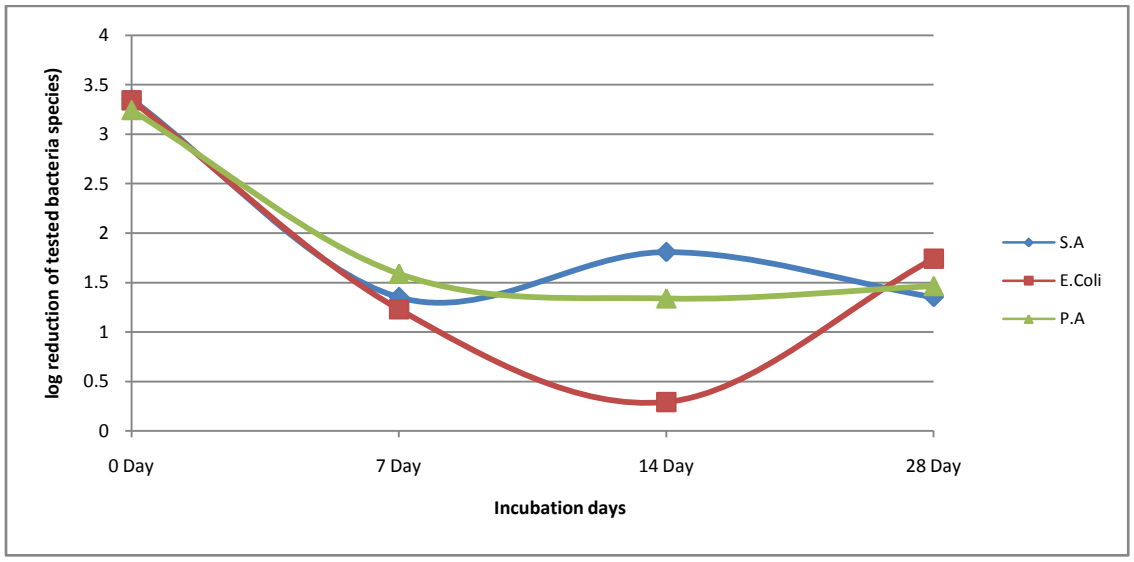
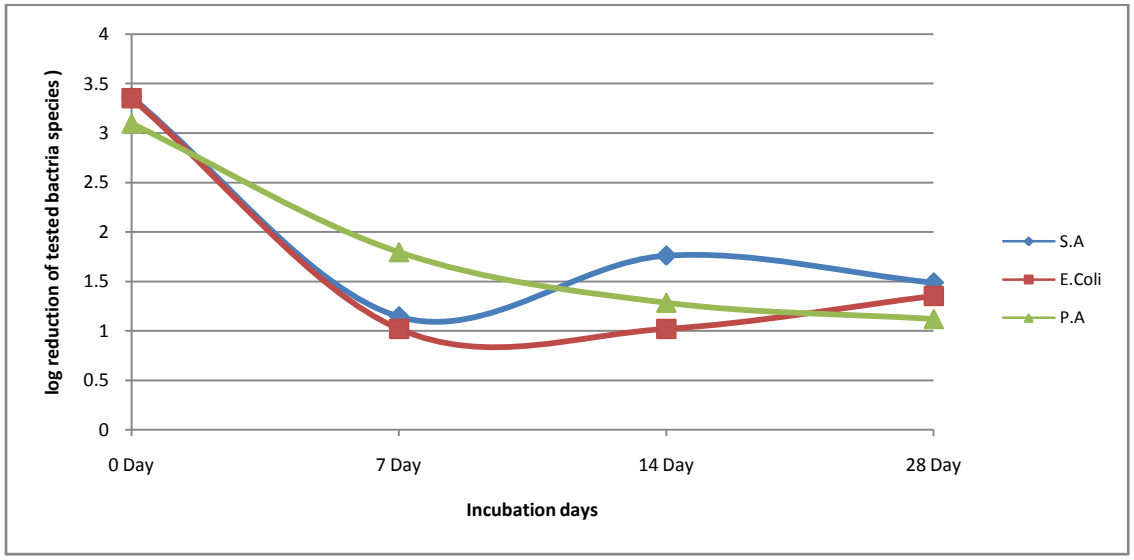
Fig 2 Preservative capacity of Johnson baby lotion on different bacteria species



Based on the above figure, only *Staphylococus aureus* showed continuous reduction up to day 14 but its reduction was below log two. And it showed increment on day 28.*Pseudomonas aeruginosa* and *E.coli*showed increment on day 14.and *E.coli* exhibited significant reduction on day 28.

Fig 3 Preservative capacity of Just for baby lotion on different bacteria species



The above fig 3 revealed that only *staphylococcus aureus* showed reduction on day 14 but the extent of its reduction was below log 2.besides it showed increment on day 28.The degree of

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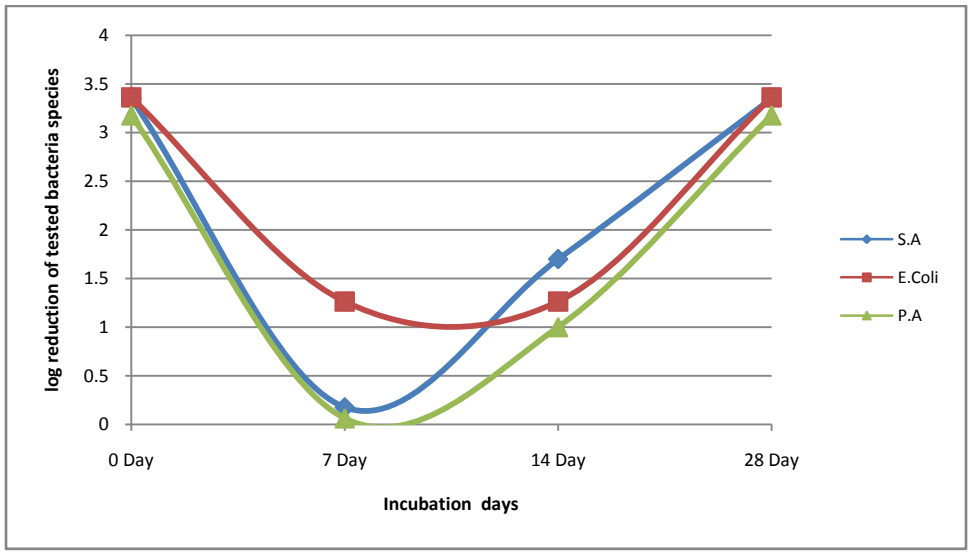
reduction of *E.coli*on day 7 is similar with that of day 14.and it showed significant reduction on day 28.colonies of*pseudomonas aeruginosa*hadincreased on day 14 and day 28.

Fig 4 Preservative capacity of Just for babyoil on different bacteria species



According to the above fig all tested species of bacteria except *E.coli*showed reduction on day 14.and *Staphylococcus aureus* showed greatest reduction.but the reduction of the two species of bacteria was less than log 2.and the reduction of E.coli on day 7 is similar with that of day14.in addition all tested species of bacteria exhibited significant reduction on day 28.

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CHAPTER FIVE

5. DISSCUSTION

Cosmetics products are not expected to be completely sterile as they contains nutrient which support the growth of variety of microorganism but it must be free from pathogenic and the total aerobic bacteria load should be below number that could not impair skin and membrane defense mechanism (Kamal, 2013). In the current study, a total of 90 samples representing three brands of baby lotion and oil were evaluated for microbial quality and safety against potential pathogens (total of bacterial and fungal counts, presence of pathogenic species).

The information disclosed on the labels of all the three brands of the products had production date, and expire dates. In addition, the name of the cosmetic product and its function, full list of ingredients, country of manufacture, the name and address of the producing company are displayed clearly. But, batch number and pH values were not written on Johnson baby lotion. Moreover, special precaution that the consumers should know was not written on Nunu and just for baby lotion. This is against the consumers' right of knowing the contents in the product as the product's information is important for decision making before buying and using the product. Wayessa *et al.* (2012) also report the same gap the necessary information on the label of cosmetics evaluated elsewhere.

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Among the fourteen cosmetic products commonly used by consumers in Jimma town, four of them had no expiry date. It was also stated that list of ingredients was absent on the label of two products. Likewise, Hugbo *et al.* (2003) from Nigeria reported that no product shown the date of manufacture and only three indicated the dates of expiry of their products. According to Hugbo *et al.* (2003), most manufacturers are reluctant to indicate inclusion and type of preservatives used, batch no and expiry dates of their cosmetic products. But, Health Sciences Authority (2014) insists that all the above missed information have to appear on the container or package of the cosmetic products. Similarly, Ethiopian Food, Medicine and Healthcare Administration and Control Authority (2014) stated that the immediate container of an ordinary cosmetic should be affixed or written on a label bearing the above overlooked particulars. To the contrary, some facts that the authority urges to appear on the package (such as the list of ingredients present in the final product and storage condition of the products) are not written on the tag of all the three brands of cosmetics assessed in the current study.

Users of cosmetics appear to have better awareness on the importance of products expiry date. On average 83.7% of the respondents replied that they always read expire dates of products before purchasing, and 76.73% of the product sellers confirmed that their customers are checking the expired date before they buy the same products. Wayessa et *al*. (2012) also reported that about 98% of the consumers in Jimma town were aware of and curious about the expiry date of products. Similarly Bial *et al.*(2016) from Somalia region of Ethiopia described figures of similar practice. Tejal *et al*.(2013) from Bangladesh also stated that. 92% of respondents check expire date of the product. In contrast, lesser proportions of consumers have been reported practicing referring to the products expiry date (Meharie *et al.*, 2014; Dibaba *et al*., 2013). Accordingly, only 39% of university female students read expiry dates of the product beforebuying the products.

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Assessment made to evaluate the consumers' perception on government's monitory scheme on safety of cosmetics on market indicated that 69.77% of respondents have the opinion that Ethiopian government has a system to control quality of cosmetics although the strategy mainly focused on checking of expiry date of the products. Based on the consumers opinion, one can deduce that practically there may be less consideration from the side of Ethiopian government in regulating the safety of ingredients and preservatives being added to cosmetics and related products. In support of our argument, Wayessa *et al*. (2012) also reported that the non-medicated cosmetics in Ethiopia are used without passing through structured safety evaluation and laboratory assessments. However, US Commercial Service (2015) reported different experience which was performed in China with respect to controlling of the safety of cosmetics products: according to the SFDA (State Food and Drug Administration) of People's Republic of China, all foreign cosmetic products manufacturers must fulfill a safety and health quality test, and obtain a hygiene permit before they are allowed to sell in the Chinese market. To this end, the test is checked by organizations appointed by the SFDA.

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Moreover, findings of the present study clearly show that 65.1% of respondents have no special choice of time while they transport cosmetics from Addis Ababa to ensure safety of the product. Few of the respondents do not completely consider the hostile effect of sun light during transportation. Eventually, such sentiment and practice could increase the products' unnecessary exposure to high temperature that can challenge efficacy of the added preservatives besides possibly modifying the chemical composition of main ingredient originally intended for use. Such practice might have contributed to most products' unacceptable level of microbial loads recorded in the present study. In agreement with our observation, Stephane (2015) also stated that cosmetics are subjected to various factors influencing their stability, such as exposure to varying degrees of light, temperature, humidity, and packaging material from the day of production till the time of their use. All these factors have an impact on organoleptic, physico chemical properties, and microbiological aspects, in general. It is clearly identified that temperature accelerates the degradation of preservatives (Thorat *et al*., 2014). According to Thorat *et al*. (2014), high temperature accelerates oxidation, reduction and hydrolysis reaction which lead to pharmaceutical product degradation. Decrease in concentration of preservative, in turn, leads to microbiological risk which can result in a modification of the original formula (acidity increased due to the proliferation of bacteria, microbial load increase).

Even though Ethiopian Food, Medicine and Healthcare Administration and Control Authority (2014) stated that If the person wants to engage in trade in ordinary cosmetics alone or sanitary items it shall have a person who completed at least grade 10 in accordance with the new education policy or grade 12 in accordance with the prior education policy as technical personnel. In addition he/she shall be familiar with basic knowledge regarding the handling, storage, transportation, use, nature, content, side effects and other related character of the product, the findings of this work shows that on average 23.24% of respondents did not consider high temperature as a factor that affect the quality of productwhile it is in the store (table four).

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According to the present study quality was a criteria for 32.5% of customer to choose the product brand. Other criteria such as price, manufactured country, brand, media ,popularity take 27%,9,3%,2.3%.20.93% and 6.9% respectively. Bial *et al*.(2016) and Tejal *et al*.(2013) also reported that. Besides, cosmetics use may be influenced by comparisons made both with real people and with media images. Young adults are more susceptible to the effects of media and they often compare themselves with the idealized images presented in advertising. Around 41% females and 37% of males purchase as per use by their friends & some of them consider price factor before their purchase. Based on the opinion by which they use to purchase the product, like their previous experience, suggested by their doctors, their popularity etc.

Statutory Cosmetic Industry standards stipulated a total viable count of aerobic bacteria, yeasts and molds of less than 100 cfu/g (or ml) for eye and baby products and at completion of manufacture, while potentially pathogenic microorganisms such as *Pseudomonas*, *Candida* and *Staphylococcus* should not be present in the product (Michael and Mary, 2014)

Based on the above restrictions, the aerobic plate count of all cosmetic lotion including the oil analyzed were above the tolerable limit with a total mean count of bacteria was 3.38 X 10^{7}cfu/ml.also the total mean count of yeast was $2.1X10^{7}$. Contamination of cosmetic lotion and oil may come from a variety of sources such as raw materials, manufacturing, storage, packaging and distribution. Similarly Michael *et al*. (2011) reported that the aerobic platecount of all cosmetic babypowder analyzed was above the acceptable limit. But opposed to the findings of the present study, selected powders analyzed were more contaminated with fungi than with bacteria.Regarding to types of contaminant microorganisms Raghad *et al* (2009) reported similar findings with the present study. According to their report since cosmetics products are found with neutral pH bacterial contamination is more likely to occur than yeast and mold contamination.Kamal*et al*. (2013) from Bangladesh also reported that total and pathogenic bacteria with in the commonly used cosmetics were found was higher.In addition Gama *et al.*(2015)stated that 6.7% of the tested samples of cream were heavily contaminated (contained more than 1000 CFU/ g.)

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Furthermore, the results obtained revealed that higher total load of viable bacteria and fungus were exhibited on Jonson baby lotion purchased from boutiques and the lowest amount of bacteria and fungus were found on just for baby oil (Table 5). To this end, it appears that the above variation might be due to ample numbers of ingredients (which are highly made of carbon) concentration on Johnson baby lotion compared to the rest types of lotions and oils under investigation. In addition, Johnson product is packed with a container that could be easily opened. On the contrary, since baby oil is made up of aqua and paraffin; which believed to contains less nutrient, and consequently, the growth of microorganisms are not supported as such., The level of awareness on the effect of handling, storage, and transportation on the quality of cosmetics by most of the people working in the in boutiques is not adequate as revealed in the study. This could be the factor for total microbial load and the prevalence of pathogenic bacterial species to be high within cosmetics products, which are sold from this source.

In this research, twenty six samples (28.89%) were found to be positive at least for one of the three bacteria speciesas contaminants. Accordingly *Bacillus spp,* coagulase-negative *staphylococci spp* and *Coliform* groupswere isolated from the products. Out of the total numbers of products that were analyzed,coagulase negative *Staphylococcus* was isolated from 16 (17.78 %) samples,*Coliform* was isolated from 24 (26.67 %) samples, and *Bacillus* was isolated from 4 (4.4%) samples (Table 6). It could be observed that *Coliform*spp is the most predominant contaminant followed by coagulase negative *Staphylococcus*. The presence of *Coliform* usually meansthat the treatment given for water or raw material in production is not satisfactory .or it shows that there is environmental contamination of raw material. However,*E.coli* and *Pseudomonas* ssp. were totally absent from all samples.This observation is similar with the earlier report by Hugbo *et al*. (2003) that demonstrated the two bacterial spp, namely; Staphylococci *spp*. and *Bacillus* spp werethe most common bacterial contaminants of cosmetics in Benin. Similarly, Qasem*et al*. (2012) also demonstrated that *Bacillus* spp, coagulate negative*Staphylococci* and *Enterobacter* spp were isolated from body lotion. Furthermore study by Raghad *et al.*(2009)from Iraq reported that *Staphylococcus* is one of major contaminant of cosmetics products such as shampoo, hand and body lotion, facial cleanser, and liquid soaps.

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The findings of Kamal *et al*. (2013) also showed that *Staphylococci s*pp and *Bacillus* spp were found in a baby lotion and *E. coli* was absent from all samples. However, in contrary to findings of the present study, Kamal *et al*., (2013) and Qasem *et al*. (2012) reported that *Pseudomonas* was isolated from baby lotion products. Moreover, *Saccharomyces* spp of yeast and *Penicillin* spp mold were detected and isolated from the products. The numbers of products contaminated with mold were very low (5%). This might be attributed to the neutral pH, high water activity and preservative activity of the product. With regard to fungal contamination Gamal *et al*. (2015) also reported that high fungal contamination of some cosmetic to be attributed to high concentrations of solutes and lowered water activity. They also reported the isolation of two molds, *Rhizopus* spp. and *Aspergillus* in foundation creams, different from the species detected by the present study. However, the finding of the current study is closely related with the report Gamal *et al* (2015) conducted on bleaching creams in which5.5% of the evaluated samples would contain *Penicillin.* Similarly Michael and Mary (2015) stated that Penicillin spp was one of fungal genus isolated from the products however, they reported that high number of mold species (which were 7) detected in the baby lotion.

In line with searching for antimicrobial agents from plant sources, different parts of three species (*Erythrina brucei*, *Calpurnia aurea* and *Millettiaferruginea*) of plants were extracted with chloroform/methanol (1:1) using maceration technique, and the resulting extracts were subjected to antimicrobial screening .The antibacterial activities of eight plant extracts were evaluated against isolated bacterial based on inhibition zone. In some species of plants tested for antimicrobial activity, the zone of inhibitions increased with increasing concentration. The study revealed that all isolates did not show any susceptibility against aqueous extract of Immature *Calpurnia* pod, *Erythrina abyssinica*, Mature *Calpurnia* pod and *Millettia ferruginea* bark. The other five extracts showed poor activity on isolated bacteria species (table 9).

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The extracts of *Millettia ferruginea* pod and seed had also very low activity on coagulase negative *staphylococcus* spp. And *bacillus*spp respectively. Some compounds isolated and identified from the seeds of *Millettia ferruginea* had no activity on isolatedspecies of bacteria. Manash *et al*.(2016) also reported that the water-insoluble isoflavonoids constituents of *M. ferruginea*, viz., barbigerone, calopogonium isoflavone-A and durmillone contributed to the antibacterial activity of water and methanol extracts of *M. ferruginea* against Gram-negative strains.

Moreover Immature *Calpurnia aurea* seed shows poor activity on *Staphylococcus* spp but Shemsu *et al.* (2013) reported that the methanol extract of the leaves of *C. aurea* exhibited broad spectrum of antibacterial activity. The findings of the above study showed that the methanol extract inhibited the growth of *Salmonella paratyphi*, *Salmonella typhimurium*, *Shigella*species, *Pseudomonas aeruginosa, and Staphylococcusaureus*moderately. And it is also demonstrated that the extract of motioned plant showed highest activity against *S.typhi* and *E. coli* among the tested microorganisms.

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Extracts of *Erythrina abyssinica* leaf had also insignificant activity against on *bacillus* spp. But opposed to the findings Lagu and Frederick, (2012) extracts of the stem bark of the above mentioned plant did not show any activity on *staphylococcus* spp. Ayalew and Addisu(2015) also reported contradictory findings with the present study. According to their conclusions the extract of *E. abbyssinica* compound showed a very good antibacterial properties against the bacterial pathogen *S.aureus* (gram positive).

Minimal challenge test criteria are suggested to aid manufacturers in evaluating the adequacy of preservation in personal care products; and if the preservative is effective there should be not less than a 2.0 log reduction from the initial count at 14 days, and no increase from the 14 day count at 28 days (USP, 2014).According to the findings of the present research *Staphylococcus aureus* exhibited reduction in all brands of lotion and oil on the 14^{th} day but except in nunu baby lotion the extent of its reduction in other selected brands of lotion and oil on the above mention day was below log 2. In addition except in just for baby oil *Staphylococcus aureus* exhibited increment on day 28.on the other hand except in just for baby oil the other two tested species of bacteria demonstrated increment on day 14 or they showed similar reduction with that of day7. This shows that all used preservative could not kill 99% of tested pathogenic bacteria on the day 14. *Pseudomonas aeruginosa* also showed incrementin just for baby and nunu baby lotion on day twenty eight. In addition E.coli exhibited increment in nunu lotion on day 28.

All these findings shows that preservatives used in all brands of baby lotions and oil were not active enough to protect the product from microbial spoilage.This result is similar with the findings of Hugbo *et al.* (2003). They reported that the preservatives that were used in creams were not sufficiently potent and persistent in their antibacterial activities as to be able to maintain the preparation at the original level of post–production bacterial numbers or even less. In addition Shaqra *et al*. (2012) reported that nine out of 32 hair and skin care products investigated were poorly preserved. This study use onlyP. *aeruginosa* for Preservative efficacy evaluation. Probably if more than one organism had been used, the preservation failure rate in the products investigated would have been higher.

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Regarding to the drug resistance nature of isolated bacterial spp, coagulase negative *staphylococcus*spp was the most sensitive for antibiotics tested in the experiment. Shaqra *et al*. (20l2) also reported that isolates of coagulase negative *Staphylococci* were the most susceptible .The findings of the present study also revealed that clindamycin and doxycycline were the most effective antibiotics against coagulase negative *staphylococcus* spp and *Bacillus* spp, respectively.

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CHAPTER SIX

6. Conclusion

The findings of this research work indicated that all the selected brands of baby lotion analyzed, had poor microbiological quality since their bacterial and yeast load are beyond standards. The study also revealed that coagulase negative *Staphylococcus, Bacillus* and *Coliform* were isolated from the products, based on the microbial load of the products, it was indicated that cosmetics sold in pharmacies and supermarket have better quality than sold in boutiques. The study had also demonstrated that the safety regulation and control of government on cosmetics products is not strict even some information that must be appears on the labels of the product are not written so that this may probably has led to the incidence of risky cosmetic products in shops. In addition, the knowledge of most cosmetic sellers is not satisfactory so as to keep the products safety during transportation and handling them in store. The results of the study had also shown that, the four extract that belongs to *Calpurnia aurea,Erythrina abyssinica*and *Millettia ferruginea* had no any antimicrobial activity on isolated bacteria; and the other 4 extracts that belongs the three plants(Immature *calparina aurea* seed, *Millettia ferruginea* seed, *Millettia ferruginea* pod, and *Erythrina abyssinica* leaf)had poor antimicrobial activity on the isolated bacteria. Compound of *Millettia ferruginea* seed also was not effective on isolated bacteria species. The results obtained in this study also indicate that the preservatives employed in all selected brands of baby lotion did not possibly possess satisfactory preservative capacity to be able to bring about tolerable levels of microbial contamination as demanded byregulatory bodies. Moreover based on the outcomes of the research *bacillus* spp are more resistance isolate against selected antibiotics.

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CHAPTER SEVEN

7. Recommendation

- The controlling system of the regulatory bodies must be strictly deal with microbiological maintenance during raw material specification, manufacturing, packaging storage and distribution of the cosmetics product.
- Cosmetic industries, importers, distributors, and retailers must consider whether the product they sale to the consumer meets appropriate label demanded by the responsible governmental body.
- A strategy must be designed to ensure quality of imported cosmetics product with in the laboratory before they are allowed to sell in the market.
- Great effort must be done to search natural preservatives that can maintain the product with acceptable level of microorganism without causing any harm on the consumer.
- Awareness of cosmetics seller regarding on methods used for keeping the quality of the product while it is in transportation and in store must be enhanced.
- Because many strains have been shown to be resistant to antibiotics, culture and susceptibility testing are recommended before the drug prescribed for patient.

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Annex 1: Questionnaire (filled by pharmacist and shop owner/keeper)

JIMMA UNIVERSITY

COLLAGE OF NATURAL SCIENCE

DEPARTMENT OF BIOLOGY (APPLIED MICRO BIOLOGY)

This questionnaire is designed for the study entitled '**Microbial Quality of Selected Baby Skin Lotions and Antimicrobial Activity of Extracts of *Erythrina brucei*a, *Millettia ferruginea* and *Calpurnia aurea* against Pathogenic Isolates in Jimma town , Southwest Ethiopia** ''

Hence, you are kindly requested to answer the following question

Direction: make the symbol ''X'' on the space provided for close ended question

- How many brands of baby skin lotion are available in your shop/pharmacy?

A. 1 B.2 C 3 D. more than three

- Would you prioritize the product from one up to three based on the need of your customers?

  1.

  2.

  3.

3. How many people use these products?

A. few B. average C. many

4. What criteria people use to choose different brands of the product?

---

5. since when children start to take massage with lotion?

---

6. Where do you bring the product and what kinds of transportation do you use to bring the product to your shop?

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7. What kinds of method do you use to protect them from contamination during transportation and while you are putting them in your shop?

7,1 When do you import the product to you shop

A/night B/afternoon C/at any time

---

8. Do you strictly observe the expire date of each product before you put it on your shop?

A. Yes always BB. yes sometimes C. yes often D. no never

9. Do your customers or users strictly observe the expire date of the product before they buy it?

A. Yes many of them B. a few of them C. No one of them

10. Are you aware of that these products can be contaminated with pathogenic bacteria and fungi?

A. Yes B. No

11. Is there any governmental organization that control the quality, expire date of the product that you sold

A. Yes there is B. No there is not

Please write the name of the organization (if any)

---

12. Do you have any information concerning any health damage caused by baby skin lotion? If yes, please explain it

.---

THANK YOU A LOT!!!!!!!!!!

``

Annex 2. Procedure for preparation of dilutions of antibiotics from stock solution (10,000mg/L)

From the 10,000 mg/L stock, dispense the following amounts with a micropipette:

256 microliter into the container labeled 128

128 microliter into the container labeled 64

64 microliter into the container labeled 32

32 microliter into the container labeled 16

From the 1000 mg/L stock, dispense the following amounts:

160 microliter into the container labeled 8

80 microliter into the container labeled 4

40 microliter into the container labeled 2

From the stock 100 mg/L dispense the following amounts:

200 microliter into the bottle labeled 1

100 microliter into the container labeled 0.5

50 microliter into the container labeled 0.25

``

Annex 3 Vendors view on practice of customers' checking of expiry date of cosmetic products before purchase, Jimma town, 2016/17

Respondents source	**Customers practices of reading expiry date of cosmetic products before purchase**			
	Most	All	Some	**Total**
Pharmacies	24(55.81)	4 (9.30)	0(0)	28(65.12)
Boutique	7(16.28)	1(2.32)	3(6.98)	11(25.58)
Cosmetics shop	0(0)	1(2.33)	1(2.33)	2(4.65)
Supermarket	2(4.65)	0(0)	0(0)	2(4.65)
Total	34(76.74)	6(13.95)	4(9.30)	43(100)

Annex 4 Colony morphology and biochemical characteristics of isolated bacteria species

isolated bacteria genus	Colony morphology on selected media	KOH	Catalase	Morphology	Indole	Citrate	Coagulase	urease	Fermentation	Gas production
Staph	Golden yellow	-	+	Blue cocci	-	+	-	+	+	+
Bacillus	Golden yellow	-	+	Blue rod	-	+	X	_	+	-
Coliform	Pink	+	+	Red rod	+	+	X	+	+	+

``

Annex 5 Association between brands of baby lotion and coagulase negative *Staphylococcus* and *coliform* group

	Value	**Df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	**.264[a]**	**3**	**0.967**
Likelihood Ratio	**.263**	**3**	**0.967**
N of Valid Cases	**118**		

Annex 7 variation of aerobic mesophilic bacterial count among selected brands of baby lotion purchased from pharmacies.

Source	DF	64	MS	F	P
Type	3	1.85051E+15	6.16837E+14	13.89	0.000
Error	61	2.70855E+15	4.44024E+13		
Total	64	4.55906E+15			