

**PHYTOCHEMICAL INVESTIGATION OF THE ANTIOXIDANT ACTIVITY AND SUN
PROTECTION FACTOR OF THE ESSENTIAL OIL OF *CITRUS LIMON***

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UNIVERSITY OF BENIN,

BENIN-CITY,

EDO STATE.

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AWARD OF DOCTOR OF PHARMACY (PHARM.D) DEGREE OF THE UNIVERSITY
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CERTIFICATION

This is to certify that this project work was carried out by **EWEKA OSAMUDIAMEN ISABELLE** in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin-City, Edo State.

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DEDICATION

This project work is dedicated to God Almighty and my beloved parents, Mr and Mrs H.U.

Eweka.

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ABSTRACT

Over time there has been a need to go back to “our roots” in the areas of medicine, considering the endless report of adverse drug reaction to synthetic has created a need for proper utilization of medicinal plant hence the rise in natural product. The use of these natural product also finds useful application in skin care and in this study essential oils are the product of choice. Proper investigation on the phytochemical constituents which shows positive for alkaloids, terpenoids, tannins, and phenolics, the antioxidant activity and sun protection factor of the essential oil of Citrus limon were carried out. The experimental study shows the process of essential oil extraction via hydro-distillation as well as the comparison of its antioxidant activity with a known standard –ascorbic acid where it showed moderate antioxidant activity. The sun protection factor was determined by ultra violet-visible light spectrophotometric analysis to be 0.553 at 0.01% and 3.45 at 0.1%. The mild sun protection activity and moderate antioxidant property makes it a viable ingredient for organic skin care product.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Throughout mankind history there have been obvious improvements in medical and health science with regards medications used to treat infections and disorders. The skin being the largest organ protecting all others has to be properly cared for, over time there has been research into skin care including the use of natural and synthesized chemical active ingredients. This review however will focus on the naturally sourced chemical actives used in the treatment and management of skin infections, anti-aging and UV protection agents and the medicinal plants containing them.

The human skin is the largest organ of the body, and acts as the first line of defense. It is made up of three main layers: the epidermis, dermis, and hypodermis, each with its own specific functions. The epidermis is the outermost layer, which can vary in thickness depending on its location on the body, with the thinnest being on the eyelids and the thickest on the palms and soles. The dermis is located beneath the epidermis and can also vary in thickness, with the thinnest being on the eyelids and the thickest on the back. The hypodermis is a layer of fat and connective tissue beneath the dermis that helps to regulate the temperature of the skin and the body. The size of this layer can vary between individuals and on different parts of the body. The skin also contains various specialized cells and structures, including hair follicles, sweat glands, and sebaceous glands ⁽¹⁾

Around the world, there is ongoing research into finding natural remedies for skin care. Some researchers reviewed the importance of seed oils from six species used in cosmetics, as well as their traditional and medicinal uses.⁽²⁾ A researcher Mabona focused on the dermatological

applications of 47 southern African medicinal plants, highlighting their antimicrobial effects against skin pathogen.⁽³⁾

1.0 Medicinal plants

According to the World Health Organization (WHO) Medicinal plant is any plant which, in one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs, Plants which have been found useful pharmacologically on the human or animal body. With full regards to skin care and the treatment and management of some skin infections, there have been discoveries of medicinal plants having such properties. These include Aloe vera, Carica papaya, Cassia fistula, etc. Skin infections and concerns such as rashes, ringworm, eczema, acne, hyperpigmentation, rosacea, melasma, sunburn, hives, alopecia, etcetera have found to be curable or managed using these medicinal plants that will be discussed.

The use of plant-based medicines is becoming more popular because of their various benefits. These benefits include fewer side-effects, better patient tolerance, lower cost, and a long history of use. In addition, herbal remedies can offer a sensible way to treat certain diseases that are difficult or impossible to cure with other forms of medicine. Due to these advantages, many plants have been studied for their potential in treating a range of skin ailments, from minor itching to serious conditions like skin cancer. To date, 31 plants have been identified as effective treatments for various skin diseases.⁽⁴⁾

1.1.Skin infections

Skin infections are conditions affecting the skin which are caused by bacteria, fungi or viral parasites living in or on the skin. The continuous growth or thriving of these microbes presents with what is termed an infection. Some of these presentations include; symptoms of rash, patches,

Redness, swelling, pus formation. Drugs such as antibiotics are formulated into topical dosage forms such as creams and ointments to locally treat these infections. There are some skin conditions however that are not caused by microbial infections but disorder due to hormonal imbalance as seen in puberty, pregnancy, or pigmentation caused by acne scars, sunburn, trauma to the skin, post inflammatory hyperpigmentation, photo-aging. Skin problems are prevalent and affect individuals of all ages, from newborns to the elderly and can cause harm in various ways. While there are over a thousand conditions that can impact the skin, they can generally be categorized into nine common types, these include the following;

- i. Rashes
- ii. Viral infections
- iii. Bacterial infections
- iv. Fungal infections.
- v. Parasitic infestations
- vi. Pigmentation
- vii. Tumors
- viii. Trauma.

There are various skin conditions that do not fit into clear categories, including wrinkles, rosacea, spider veins, and varicose veins. Wrinkles occur when the skin's collagen and elastin fibers break down, resulting in loose skin. Rosacea is a long-lasting condition characterized by redness and pimples on the face, and sometimes nose enlargement, with an unknown cause. Spider and varicose veins occur when blood vessels enlarge and become visible through the skin.⁽²⁾

Table 1.0 some medicinal plants and uses ⁽⁵⁾

S/N	NAMES OF PLANTS	PARTS OF PLANTS USED	USES
1	<i>Achyranthesaspera</i>	Roots	Ring worm
2	<i>Aloe vera</i>	Leaves	Acne, inflammation
3	<i>Catheranthusroseus</i>	Leaves	dermatitis
4	<i>Cyperusrotundus</i>	rhizome	Ant-inflammation
5	<i>Curcoma longa</i>	rhizome	Rosacea, melasma
6	<i>Cassia fistula</i>	Flowers, leaves	Wound healing
7	<i>Carica papaya</i>	Fruit	hyperpigmentation
8	<i>Daucuscarota</i>	Root	hyperpigmentation
9	<i>Annona muricata</i>	Seed, fruit	Psoriasis, dry skin
10	<i>Trifoliumrepens</i>	Flower, leaves	Eczema, psoriasis
11	<i>Riccinuscommunis</i>	Seeds	Ringworm, acne
12	<i>Perseagratisima</i>	Fruits	Acne,
13	<i>Polypodiumleucotomos</i>	Leaves	Psoriasis,eczema
14	<i>Calendula officinalis</i>	Flower	Acne, anti-aging
15	<i>Silybummarianum</i>	Seeds,flower, roots	Sagging skin, wrinkles

1.2.The phytochemistry of medicinal plants used to treat skin diseases and disorders

Phytochemistry which is derived from the Greek term "phyto" meaning plant, are naturally occurring chemical compounds that are biologically active and offer health benefits to humans beyond those provided by macronutrients and micronutrients. These compounds are found in plants. The historical use of plants and their derivatives for medicinal purposes dates back to the

beginning of human civilization. According to the World Health Organization (WHO), approximately 80% of people in developing countries rely on traditional medicine for primary healthcare needs, with plant-based drugs being the most commonly used⁽⁶⁾.

Organic compounds found in plants are responsible for their medicinal properties, with alkaloids, saponins, flavonoids, tannins, glycosides, anthraquinones, steroids, and terpenoids being among the most important bioactive constituents.⁽⁷⁾ These compounds are synthesized by the primary or secondary metabolism of living organisms. Secondary metabolites, in particular, are highly diverse and taxonomically complex compounds with unclear functions. They are widely utilized in human therapy, veterinary medicine, agriculture, scientific research, and numerous other fields.⁽⁸⁾.

1.3. Classifications of phytochemicals in medicinal plants

1.3.1. Phenols and phenolic acids

Due to the vast range of phytochemicals, an exact classification of them has not yet been possible. However, in recent years, phytochemicals have been categorized as either primary or secondary constituents based on their role in plant metabolism. Primary constituents consist of commonly found sugars, amino acids, proteins, nucleic acid components such as purines and pyrimidines, as well as chlorophyll. On the other hand, secondary constituents include a diverse group of plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids, and glucosides.⁽⁹⁾ Phenolic phytochemicals represent the largest and most widely distributed category of phytochemicals in the plant kingdom. The three key groups of dietary phenolics are flavonoids, phenolic acids, and polyphenols. Phenolics are characterized by the presence of hydroxyl groups (-OH), are a class of chemical compounds in which the (-OH) group is directly bonded to an aromatic hydrocarbon

group. Phenolic compounds found in plants exhibit distinct molecular structures and are identified by the presence of “hydroxylated aromatic rings”⁽¹⁰⁾

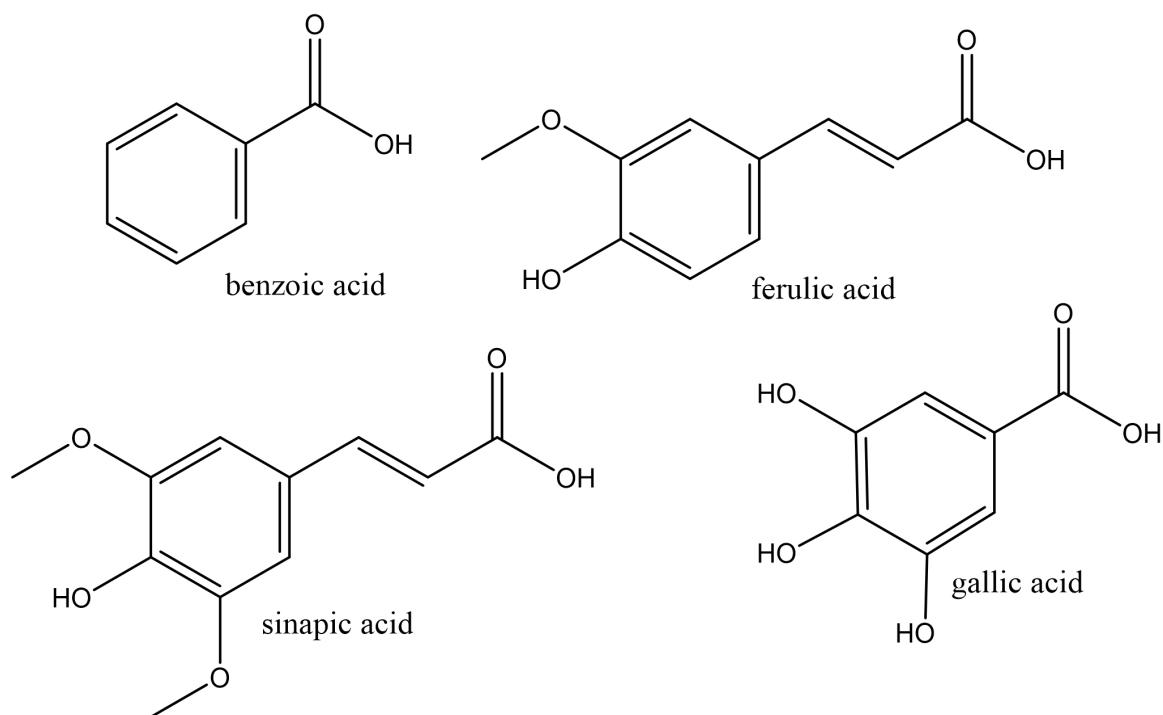


Fig 1.0 Structure of phenols

1.3.2. Tannins

Defining tannins from a chemical standpoint presents a challenge as the term encompasses a wide range of oligomers and polymers with diverse properties.⁽¹¹⁾ Tannins are a heterogeneous group of polyphenolic compounds with high molecular weight. They have the ability to form reversible and irreversible complexes with various biomolecules such as proteins, polysaccharides (cellulose, hemicellulose, pectin), alkaloids, nucleic acids, and minerals.⁽¹²⁾ Based on their structural properties, tannins can be classified into four major categories; Gallotannins, ellagitannins, complex tannins, and condensed tannins.

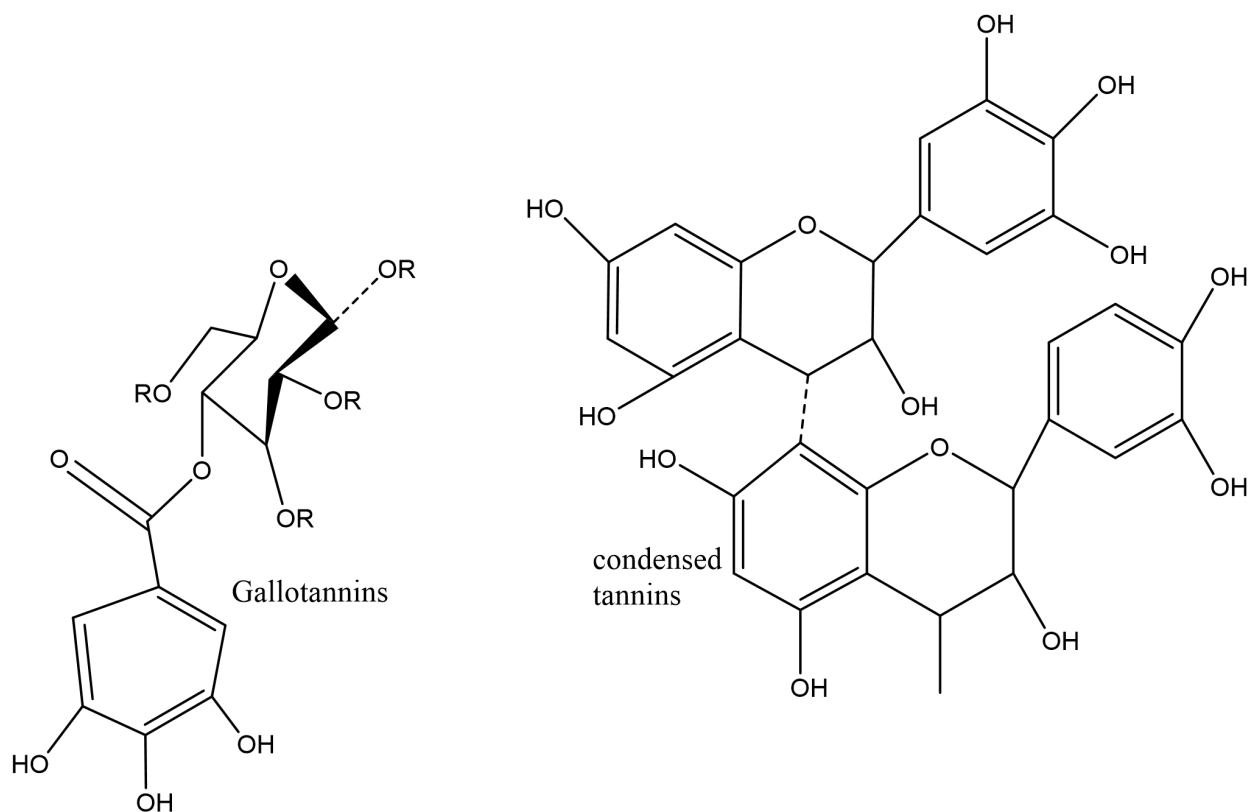


Fig 1.1 Structures of tannins

1.3.3. Flavonoids

Flavonoids are a class of polyphenolic compounds that are widely distributed in nature. A lot of different flavonoids have been identified, with many occurring naturally in vegetables, fruits, and beverages such as tea, coffee, and fruit juices⁽¹³⁾To date, over 4,000 flavonoids have been identified within plant parts that are commonly consumed by humans. Among these, approximately 650 flavones and 1,030 flavanols have been characterized.⁽²⁶⁾Due to their wide-ranging biological and pharmacological activities, flavonoids have garnered significant attention in recent years. These compounds have been shown to possess diverse properties such as antimicrobial, cytotoxic, anti-inflammatory, and antitumor effects. However, one of the most extensively studied properties of flavonoids across various groups is their potent antioxidant activity, which helps protect the human body against free radicals and reactive oxygen

species⁽¹⁵⁾ some flavonoids are said to possess better antioxidant activity than some well known nutrient antioxidants such as vitamin C, vitamin E and beta-carotene. Flavonoids are crucial in safeguarding biological systems from the destructive impacts of oxidative processes on macromolecules such as carbohydrates, proteins, lipids, and DNA ⁽¹⁶⁾.

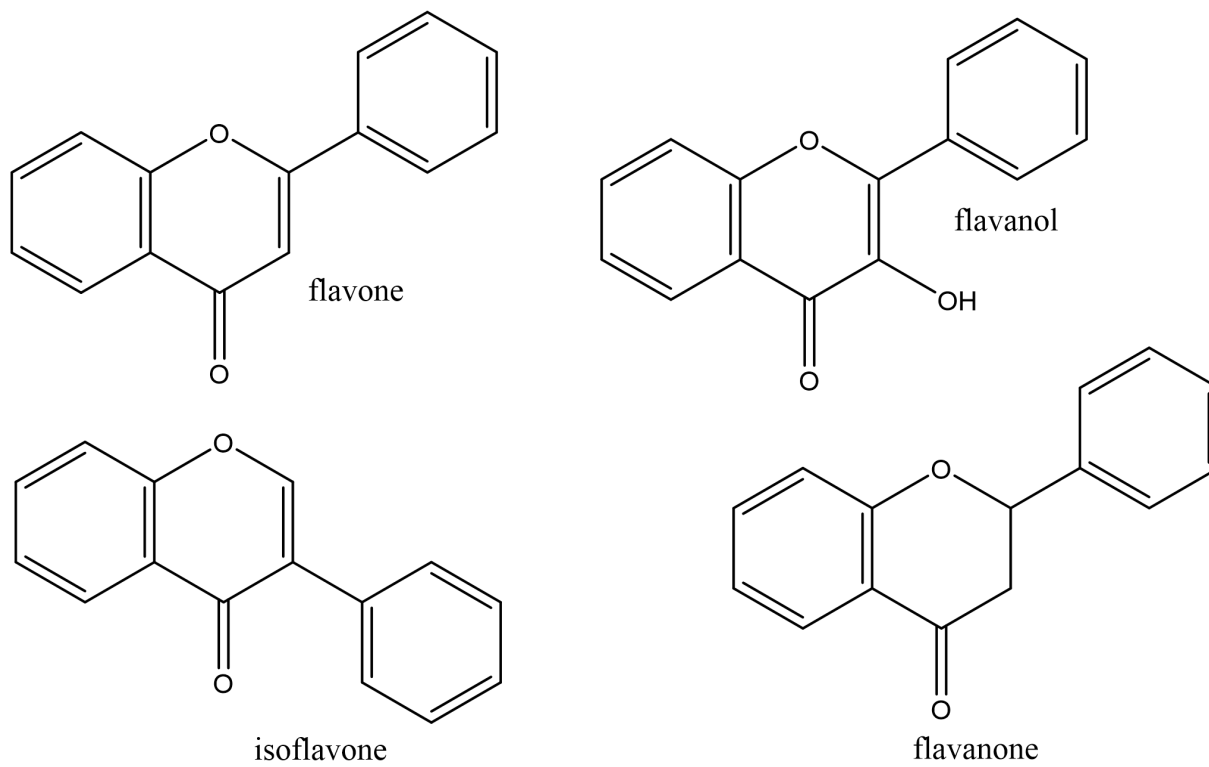


Fig 1.2 Structures of flavonoids

1.3.4 Alkaloids

Alkaloids are a class of natural products that are characterized by the presence of heterocyclic nitrogen atoms and exhibit basic properties. The term "alkaloids" originates from "alkaline" and was historically used to refer to any nitrogen-containing base⁽¹⁷⁾. Alkaloids are categorized into several families based on the heterocyclic ring system they contain. Pyrrolidine alkaloids have a pyrrolidine ring system, while pyridine alkaloids have a piperidine ring system. Pyrrolidine-

pyridine alkaloids have both pyrrolidine and pyridine rings, and pyridine-piperidine alkaloids have a pyridine ring joined to a piperidine ring. Quinoline alkaloids have a quinoline ring system, while isoquinoline alkaloids have an isoquinoline ring system. Examples of alkaloids in each family include Hygrine, Coniine, Myosmine, Quinine, and Opium alkaloids such as morphine and codeine ⁽¹⁵⁾.

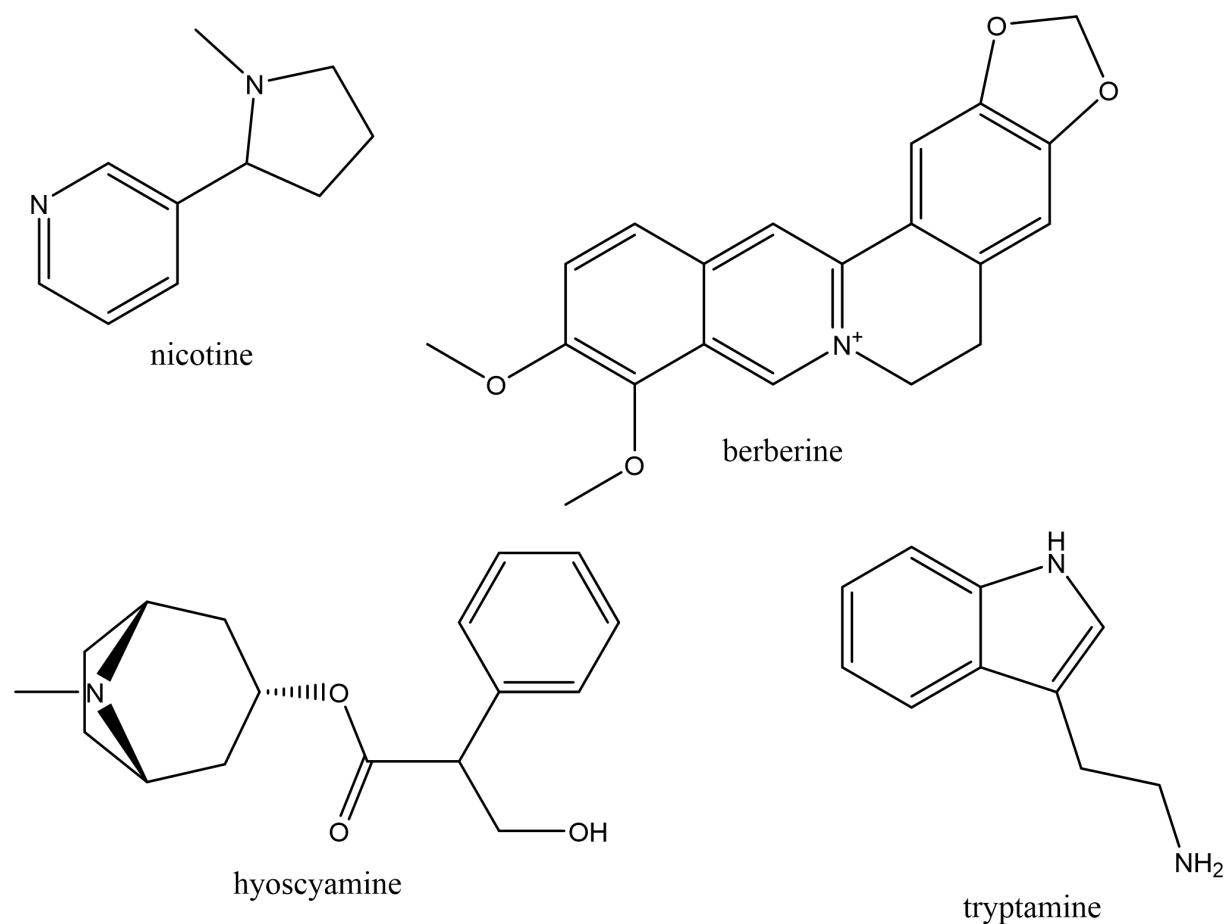


Fig 1.3 Structure of alkaloids

1.3.5 Terpenoids

Terpenoids are a group of natural products that originate from isoprene units consisting of five carbons. They have complex structures that vary in their basic carbon skeletons and functional groups. Terpenoids are found in all living organisms and are the largest group of natural products. They have many commercial applications ⁽¹⁸⁾. They are wide spread in nature and are classified according to their isoprene units;

Terpenoids are a class of natural products derived from isoprene units. Hemiterpenoids consist of a single isoprene unit while monoterpenoids have two units and can be linear or contain rings. Sesquiterpenes have three isoprene units and diterpenes are composed of four units. Triterpenes consist of six isoprene units and examples include lanosterol and squalene found in wheat germ and olives.

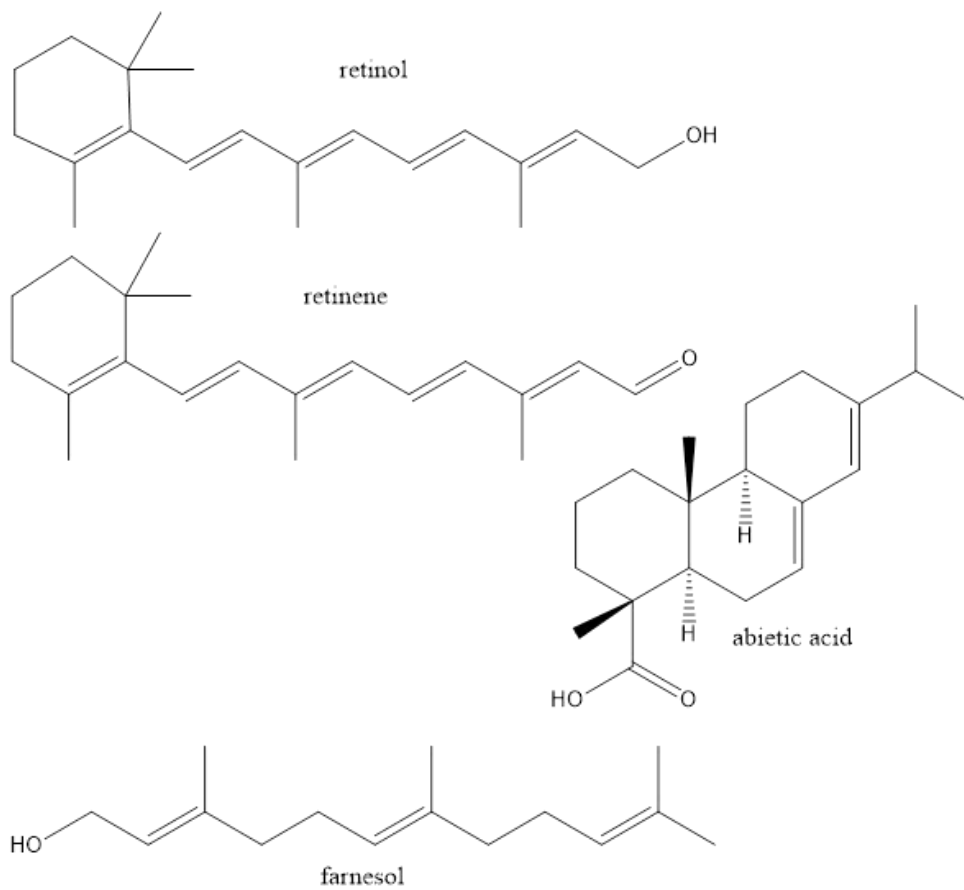


Fig 1.4 Structure of terpenoids

1.4. Phytochemicals of medicinal plants useful for combating skin photo-aging

1.4.1. Phytochemicals with Antioxidant activity

According to research, oxidative stress is a significant factor in skin aging and dermatological issues ⁽¹⁹⁾. The sun's ultraviolet radiation is the primary external factor that causes harm to the skin. Repeated exposure to environmental factors can cause changes in the connective tissue by producing reactive oxygen species (ROS) and lipid peroxides, as well as through enzyme activity, leading to various skin disorders ⁽²⁰⁾. Free radical formation is naturally controlled by various beneficial compounds known as antioxidants. These are radical scavengers providing protection to the human body by inhibition of various oxidizing chain reactions ⁽²¹⁾.

Plant phenolics, which are widely distributed in plants and important components of the human diet, include a diverse array of compounds such as flavonoids (anthocyanins, flavonols, flavones, etc.) and non-flavonoids (phenolic acids, lignins, stilbenes).⁽²²⁾ These bioactive substances have antioxidant properties and can prevent the formation of free radicals by scavenging or promoting their decomposition, and suppressing disorders. Certain compounds can inhibit the initiation or propagation of oxidative chain reactions, thereby preventing or repairing oxidative damage caused by the body's cells through oxygen⁽²³⁾.

The antioxidant activity of phenolic compounds can vary depending on their molecular structures⁽²⁴⁾. The relationship between structure and activity has shown that the number of hydroxyl groups is the most crucial factor determining the antioxidant activity of phenolic compounds⁽²⁵⁾. Therefore, compounds with a higher number of hydroxyl groups possess stronger antioxidant activity. Phenolic-rich plants can also be used to prevent the harmful effects of UV radiation on the skin ⁽²⁶⁾. The photo protective properties of phenolic compounds are attributed to their ability to neutralize the reactive oxygen species generated by UV radiation, thereby

reducing the risk of skin damage and skin cancer. In conclusion, phenolic compounds have emerged as promising natural antioxidants with multiple health benefits.

1.4.2. Anti- aging properties

The skin serves as a protective barrier against the external environment, regulating temperature, fluid balance, and protecting the body from harmful microbes and UV radiation from sunlight

⁽²⁷⁾The skin is composed of three layers: the epidermis, dermis, and subcutaneous layers, with the epidermis being particularly vulnerable to extrinsic factors. As we age, the effects become increasingly visible on the skin, which starts to show signs of aging from the moment we are born. Many people desire to live longer and look younger for an extended period of time

⁽²⁸⁾Current trends in anti-aging skincare products are focused on developing new plant extracts and botanical ingredients based on their traditional medicinal uses ^(29,30). Skin aging process is usually propagated by two factors: intrinsic factors (this refers to physiological factors caused by natural aging of cells marked by decline in cell functions and cell degradation) and the extrinsic factors (non physiological factors such as effect of chemicals on the skin, free radical damage, UV radiation which causes photo aging, environmental pollution etc). Almost 90% of the skin changes occur due to extrinsic factors Understanding the mechanisms of skin aging is important for developing effective strategies to prevent or slow down the aging process. These strategies may include the use of antioxidants, anti-inflammatory agents, and agents that promote extra cellular matrix synthesis and repair.

Table 1.1 Classes of phytochemicals with anti aging properties

FREE RADICAL SCAVENGERS	SKIN MATRIX PROTECTANTS	UV-RADIATION PROTECTANTS
Apigenin	Phenolics	Catechin
Curcumin	Arctin	Phenolics
Licorice	Terpenoids	Flavonoids
Pycnogenol		Silymarin

1.4.3. Skin matrix protectants

The skin is the largest organ in the human body, and it serves as a protective barrier against environmental factors such as UV radiation, microorganisms, and mechanical stress. The skin is composed of three main layers: the epidermis, dermis, and hypodermis (also known as the subcutaneous tissue). The dermis is the middle layer of the skin and is responsible for providing the skin with its strength and elasticity. The dermis is made up of various components, including collagen, elastin, and glycosaminoglycan (GAGs), which form the extracellular matrix (ECM). The ECM provides structural support to the cells of the skin and helps to maintain its shape and function. Collagen is the most abundant protein in the ECM and is responsible for the skin's tensile strength. As we age, the amount and quality of collagen in the skin decreases, leading to the formation of wrinkles and sagging skin. Factors such as UV radiation, smoking, and poor nutrition can also contribute to the breakdown of collagen in the skin. To maintain healthy skin, it is important to protect it from environmental factors and to consume a diet rich in nutrients that support collagen synthesis.⁽³¹⁾

UV radiation is a well-known source of ROS, and excessive exposure to UV radiation can lead to the formation of ROS in the skin. The accumulation of ROS in the skin can lead to oxidative stress, which can trigger inflammation and damage to the extracellular matrix (ECM)

1.4.4. Ultraviolet radiation protectants

Excessive exposure to UV radiation can lead to photo aging and skin cancer. UV radiation can be divided into three bands: UVA (320-400 nm), UVB (290-320 nm), and UVC (200-290 nm). UVC has the highest energy and is capable of causing the most biological damage, but it is typically filtered out by the ozone layer and is therefore not considered a significant factor. UVB is mainly associated with erythema (skin redness) and sunburn. UVB radiation can cause a variety of damaging effects to the skin, including sunburn, premature aging, and an increased risk of skin cancer. One way that UVB radiation can cause damage to the skin is by stimulating the expression of matrix metalloproteinase (MMPs). MMPs are enzymes that can break down the extracellular matrix (ECM) of the skin, including collagen and elastin, which are crucial for maintaining skin structure and elasticity. Over time, excessive MMP activity can lead to photo aging and wrinkles.⁽³²⁾

1.4.5. Free radical damage protection

Free radicals are molecules that contain one or more unpaired electrons, which make them highly reactive and capable of damaging other molecules in the body, including those in the skin. These free radicals are produced as a byproduct of normal cellular metabolism, and their production can also be triggered by exposure to environmental stressors, such as pollution and UV radiation. When the skin is exposed to these stressors, free radicals are produced, which can damage the skin's cell membrane and trigger enzymes such as matrix metallo-proteases (MMPs), which are

known to break down collagen and elastin fibers in the skin⁽³³⁾. This damage can lead to premature skin aging, wrinkles, and other skin problems.

Fortunately, there are many natural compounds found in plants that can act as free radical scavengers and neutralize these harmful molecules. Phytochemicals such as flavonoids and phenolic compounds are known for their antioxidant properties and act as free radical scavengers by donating electrons or hydrogen ions to the unstable free radicals, thereby stabilizing them and preventing them from causing further damage to the skin.

The hydroxyl functionality attached to the aromatic ring of these phytochemicals is particularly effective at donating electrons or hydrogen ions to free radicals. By neutralizing free radicals, these compounds can help to protect the skin from oxidative damage and maintain its overall health and appearance.

Flavonoids and phenolic compounds, mostly act as free radical scavengers. The hydroxyl functionality attached to the aromatic ring has a strong ability to donate a single electron or hydrogen and trap the free radicals.

1.5 Essential oils –Bioactivity in skin care

These are also known as volatile oils; they are called essential oils because they carry the essence of the plant (the true characteristics ; odor, chemical composition, extracts) , according to the NCI Dictionary of cancer terms it is “the scented liquid taken from certain plants using steam or pressure”. This definition clears the confusion between fixed and essential oils where fixed oils are gotten via expression by subjecting the plant material under pressure or heat.

There are a plethora of medicinal plants which have proved useful in the treatment of certain skin infections when applied topically or administered otherwise. Some of these plants however have also been known to produce essential oils which in some cases are more active than the

plants themselves, some of these oils are extracted from the leaves, seed, bark, stem, seeds or from the flesh of the plants itself, some of these essential oils would be itemized.

1.5.1 Essential oils which have proved useful in skin care

1. Peppermint oil
2. Frankincense oil
3. Myrrh oil
4. Clarry sage oil
5. Ylang-ylang oil
6. patchouli oil
7. Rose Neem oil
8. Lemon essential oil
9. Sandalwood oil
10. Helichrysum oil

It is pertinent to know essential oils cannot be applied directly to the skin as they can cause irritation and inflammation so they are usually diluted with carrier oils such as coconut oil, argan oil, avocado oil, jojoba oil, grapeseed oil and the likes. These essential oils are formulated into topical dosage forms as well as ingredients in the cosmetic industry. The uses of these oils would be briefly discussed.

Lavender oil: Lavender essential oil is extracted from the flowers of the lavender plant through steam distillation. It has been used for centuries for its therapeutic benefits and is widely recognized for its calming and soothing properties. Apart from its relaxing effects on the mind, lavender oil is also known for its potential benefits for the skin. One of the most notable benefits of lavender oil for the skin is its strong anti-inflammatory properties. ⁽³⁴⁾Lavender oil can help to

reduce inflammation and redness in the skin, making it an excellent natural remedy for people with sensitive or irritated skin. In addition to its anti-inflammatory properties, lavender oil can also boost the wound healing process and promote the production of collagen in the skin. ⁽⁷¹⁾Collagen is a protein that is essential for maintaining skin elasticity and preventing the appearance of fine lines and wrinkles. By enhancing collagen production, lavender oil can help to keep skin looking youthful and radiant.

Turmeric oil: Like every other essential oil turmeric oil has to be diluted with a carrier oil, *Curcuma longa*, commonly referred to as turmeric, possesses antimicrobial and antioxidant properties that aid in the treatment of various skin conditions such as psoriasis, eczema, and hyperpigmentation. Additionally, it exhibits anti-inflammatory and anti-allergic properties and aids in wound healing. Turmeric essential oil is acknowledged as an excellent skin rejuvenator that revitalizes the skin and delays the emergence of signs of aging, including wrinkles ⁽³⁵⁾. Curcumin is the primary active component present in turmeric essential oil, and it contains anti-inflammatory enzymes that can suppress cell proliferation and alleviate skin irritations. Moreover, this oil is effective in reducing dark spots on the skin, thereby enhancing skin tone. A study has demonstrated that using turmeric oil in lotion form can improve skin brightness within three weeks, and the results can be sustained for an equivalent duration ⁽³⁶⁾.

Rosehip oil: Extracted from the seeds of rosehip plants, Rosehip essential oil (also known as rosehip seed oil) is a potent source of essential fatty acids such as oleic, palmitic, linoleic, and gamma-linolenic acid that improve skin texture and hydration levels. This oil is also rich in vitamin C, an essential ingredient for anti-aging, and retinoids that can help combat acne and brighten the skin. The combination of vitamin C and fatty acids in the oil boosts collagen production while preventing early signs of aging ⁽³⁷⁾.

Carrot seed oil: Carrots have been recognized for their health benefits for centuries, and the seeds of this root vegetable have recently come to the forefront as a rich source of essential oils with numerous health benefits. Among these, the carrot seed oil is highly valued for its anti-oxidative properties that can be extremely beneficial for the skin. Antioxidants found in the carrot seed oil can neutralize these free radicals, preventing the skin from aging prematurely ⁽³⁸⁾.

Ylang-ylang oil: Ylangylang essential oil is derived from the flowers of the *Canangaodorata* tree, which is native to tropical regions of Asia, including Indonesia, Malaysia, and the Philippines. The oil is extracted through steam distillation of the freshly picked flowers, resulting in a sweet, floral fragrance that is commonly used in perfumes, soaps, and other cosmetic products. Ylangylang essential oil has natural astringent properties that can help to regulate sebum production, which can prevent breakouts and acne. Moreover, ylangylang essential oil contains antioxidants that can protect the skin from damage caused by free radicals and other environmental stressors. These antioxidants also facilitate skin cell regeneration, which can improve skin elasticity and reduce the appearance of fine lines and wrinkles ⁽³⁹⁾.

Patchouli oil: Patchouli (*Pogostemoncablin*) essential oil is derived from the leaves of the patchouli plant, which is native to tropical regions of Asia. This essential oil is well-known for its distinctive, musky scent and is commonly used in aromatherapy, perfumes, and other scented products. In addition to its anti-inflammatory effects, patchouli essential oil has been found to have other health benefits. It has been shown to have antimicrobial and antioxidant properties, which can help protect the body against infection and oxidative stress. Ultraviolet radiation is extremely responsible for skin damage and ageing. Patchouli essential oil seems to have protective effects towards this harmful radiation.

1.7. The process of extraction of essential oils

Essential oils come from different parts of plants, such as flowers (e.g. rose, jasmine, carnation, clove, mimosa, rosemary, and lavender), leaves (e.g. mint, *Ocimum* spp., lemongrass, and jamrosa), leaves and stems (e.g. geranium, patchouli, petitgrain, verbena, and cinnamon), bark (e.g. cinnamon, cassia, and canella), wood (e.g. cedar, sandal, and pine), roots (e.g. angelica, saffra, vetiver, saussurea, and valerian), seeds (e.g. fennel, coriander, caraway, dill, and nutmeg), fruits (e.g. bergamot, orange, lemon, and juniper), rhizomes (e.g. ginger, calamus, curcuma, and orris), and gums or oleoresin exudations (e.g. balsam of Peru, Myroxylonbalsamum, storax, myrrh, and benzoin). There are four different extraction methods

- i. Azeotropic distillation (hydro distillation)
- ii. Solvent extraction
- iii. Maceration
- iv. Cold press extraction

Details of oil extraction would be discussed in later chapters.

1.8. Sun protection factor -why do we need it, how can we improve it with natural products.

SPF stands for Sun protection factor, according to Kate Lohnes in the encyclopedia Britannica it is the “relative measurement for the amount of time the sunscreen will protect you from ultraviolet (UV) rays”. It goes on to explain how the value of SPF tells you how long you’re protected for? Well, let’s say you typically burn after being outside for 30 minutes and have an SPF 15 sunscreen you plan to use. You’d multiply 30 by the SPF, in this case 15. That means that you are technically protected for 450 minutes, or 7.5 hours.

Chronic exposure of human skin to solar ultraviolet (UV) radiation can have detrimental effects on the skin. Sunburn, skin cancer, oxidative stress, and photo aging are some of the common skin damages that can occur due to prolonged exposure to UV radiation. The extent of the damage caused by UV radiation depends on various factors such as the intensity and duration of exposure, the type of UV radiation (UVA or UVB), and the individual's skin type and susceptibility ⁽⁴⁰⁾. Oxidative stress occurs when the skin's natural defense mechanisms are overwhelmed by excessive exposure to UV radiation, resulting in the production of harmful reactive oxygen species (ROS). These ROS can damage the skin's DNA and proteins, leading to premature aging and increased risk of skin cancer. It has become common knowledge that besides the effect of tanning on our skin the effects of long exposure to UV radiation has health risk, an easy way out would be to avoid UV radiation but it is the sun, we must go out and even sometimes at peak UV radiation hours of the day hence the need for products containing Sun protection factor can not be over emphasized. Ingredients with the ability to protect the skin from the sun (oxybenzone, titanium dioxide, zinc oxide, avobenzonetc) have been formulated in creams, lotions, roll on ,liquid and aerosol sprays etc, these products are called “sun screen” or “sun block”.

1.8.1. Why do we need it

Protection from the sun is not for just cosmetic reasons but also for our health, ultraviolet radiation from the sun has the potential to be carcinogenic. It is divided into two three types ; ultraviolet A, ultraviolet B, ultraviolet C, this would be discussed properly later on.The most prevalent form of cancer in light-skinned populations worldwide is skin cancer. There are two primary types of skin cancer: melanoma and non-melanoma skin cancers (NMSCs), which include basal and squamous cell carcinomas (BCC and SCC, respectively). Melanoma is

responsible for the majority of cancer-related deaths, while NMSCs are generally considered to have a less severe course with locally aggressive characteristics⁽⁴¹⁾

It is important to know that there are different types of ultra violet radiation that reaches the earth and in essence our skin, they are;

- i. **Ultraviolet A ray:** Around 90-99% of this radiation actually reaches the surface of the Earth, as it is not filtered by the ozone layer in the stratosphere. These rays have a long wavelength and low energy, enabling them to penetrate deeper into the skin. Although once thought to be harmless, excessive and prolonged exposure to these rays is now considered harmful. They can lead to skin aging, immediate and long-lasting pigmentation (tanning), and can even penetrate glass.⁽⁴⁵⁾
- ii. **Ultraviolet B ray:** Roughly 1-10% of this radiation manages to reach the Earth's surface, as it is filtered by the ozone layer in the stratosphere. These rays have a short wavelength and high energy, allowing them to penetrate the upper layers of the skin, including the epidermis. They are responsible for causing sunburns, tanning, wrinkles, photoaging, and skin cancer. Furthermore, they are **carcinogenic** and can lead to thousands of cases of cancer each year. It does not penetrate glass.⁽⁴⁵⁾
- iii. **Ultraviolet C ray:** Before reaching the Earth's surface, these rays are filtered by the ozone layer in the stratosphere. Germicidal lamps are the primary artificial sources of this radiation. Exposure to this type of radiation can cause skin burns and contribute to the development of skin cancer.⁽⁴⁵⁾

1.9.1.2. Skin color and ultraviolet ray protection

The reason for the low incidence of skin cancer in darker-skinned groups is primarily due to the photoprotection provided by increased epidermal melanin. This melanin provides an inherent sun

protection factor (SPF) of up to 13.4 in people with black skin. Compared to people with Caucasian skin, the epidermal melanin in people with black skin filters twice as much UVB radiation. Additionally, the epidermis of dark skin transmits 7.4% of UVB and 17.5% of ultraviolet A rays, while the epidermis of Caucasian skin transmits 24% and 55% of UVB and ultraviolet A rays, respectively.⁽⁴⁶⁾ The larger, more melanized melanosomes in the epidermis of dark skin absorb and scatter more light energy than the smaller, less melanized melanosomes in white skin, resulting in this difference. The dose of UVR required to produce a minimally perceptible erythema is estimated to be 6–33 times greater in people with black skin than in people with white skin. This is one of the primary reasons why the use of sunscreen is not popular with people of color, but nonetheless ultraviolet ray protection is necessary for all races.

1.9.1.3. Ultraviolet radiation and aging

In the absence of inactivation, reactive oxygen species (ROS) can cause damage to macromolecules such as lipids, proteins, and DNA. UV radiation, especially longer-wavelength UVA, is a well-known inducer of ROS, and UV-induced oxidative stress may be a significant contributing factor to melanoma.⁽⁴⁷⁾ ROS can activate signaling pathways inappropriately, interfere with genome maintenance, and affect apoptosis. Several studies have investigated the effects of solar radiation and oxidative stress on the skin, and oxidative stress has been linked to the age-related loss of skin elasticity, defective cellular signaling, and photo aging.⁽⁴⁸⁾ Since oxidative stress triggers cellular damage pathways, it activates cellular senescence, which is believed to directly lead to photo aging. The use of sunscreen or sunblock is to shield the skin from the aging effect as well as the tanning effect of the ultraviolet radiation.

1.8.2. How can we improve it

The use of SPF has become more popular over the years, its use has been emphasized by dermatologist and aestheticians, makeup products and skin care products have been infused with SPF (usually from SPF 15 and above). According to dermatologist the formulation to be used on the face and body to properly protect the skin should be at least SPF 30 and above which brings us to ask; How is this factor calculated and how do we improve this using natural products.

1.8.2.1. Calculating Sun protection Factor

The efficacy of a sunscreen is usually expressed by its Sun Protection Factor (SPF). SPF is a measure of how well a sunscreen can protect the skin from UVB radiation, which is the primary cause of sunburn and skin cancer. The SPF of a sunscreen is calculated using the Equation below which compares the amount of UV energy required to produce a minimal erythema dose (MED) in protected and unprotected skin^(49,50)

$$\text{SPF} = \frac{\text{MED in sunscreen unprotected skin}}{\text{MED in non sunscreen protected skin}}$$

Here, the MED is the smallest amount of UV radiation that causes redness or inflammation in the skin. It is a measure of the skin's sensitivity to UV radiation. For example, suppose a person's skin normally starts to turn red after being exposed to 10 minutes of direct sunlight. Using a sunscreen with an SPF of 15, it would take 150 minutes of exposure to the same amount of UV radiation to produce the same level of redness.

SPF values typically range from 15 to 50, with higher values indicating greater protection. However, it's essential to note that no sunscreen can provide 100% protection from UV radiation. Even with a high SPF sunscreen, some UV radiation will still reach the skin, so it's essential to reapply sunscreen frequently, especially after sweating or swimming.

The use of in vitro screening methods can be an efficient and practical way to reduce the need for in vivo experiments and the associated risks of UV exposure to human subjects, as long as the technical test parameters are appropriately set and improved.⁽⁵¹⁾ Two main types of in vitro methods exist, which involve either measuring the absorption or transmission of UV radiation through sunscreen product films in quartz plates or biomembranes, or determining the absorption properties of the sunscreen agents by analyzing dilute solutions with spectrophotometry.⁽⁵²⁾

Using the spectrophotometric formula we have;

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

where CF correction factor (10), EE (l) erythrogenic effect of radiation with wavelength l, Abs (l) spectrophotometric absorbance values at wavelength l. The values of EE & I are constants.⁽¹⁾

With this formula, using spectrophotometric analysis one can calculate the SPF of any chemical substance which in the case is an essential oil of a known plant, to further improve the activity, efficacy and safety profile of sunscreen using natural products, analytical studies would be done on the essential oil of *Citrus limon*.

1.9. Use of natural products for anti-aging and ultraviolet radiation protection

In this study, the use of medicinal plants for treatment of skin infections and disorders have been discussed and well as the application of essential oil in skin care, properly the analytical study of the plant *Citrus limon* would be thoroughly discussed, research on its phytochemistry, bio activity, efficacy, safety profile and effectiveness. The process of essential oil extraction as well as the method of analytical studies would also be discussed.

1.10. *Citrus limon*(lemon oil) bio activity, and phytochemical studies

1.10.1. Plant taxonomy

Kingdom: Plantae

Subkingdom: Angiosperms

Phylum: Eudicots

Class: Rosids

Order: Sapindales

Family: Rutaceae

Genus: *Citrus*

Species: *C. Limon*

1.10.2. Common names:lemon, zitrone, citroen, citron, citron, sitron, sítróna

1.10.3. Botanical description: Citrus trees yield fruits of various shapes and sizes (from round to oblong), are evergreen, and are rich in flavor, scent, and juice ⁽⁵³⁾. Lemon leaves are dark green in colour, 6.5 to 10mm in size, and alternately placed on the stalk. There are five lemon petals with a white aromatic color. Lemon pulp is quite acidic, and its blossom is bisexual and male. Lemon cultivar 'Pink Lemonade' is the source of the lemon flower. This cultivar's fruit is striped, and the leaves are variegated. Lemons are commonly oval in shape and have a smooth, spongy surface. ⁽⁵⁴⁾ Lemons come in a variety of forms and sizes, with some being similar to grapefruit and others being slightly larger. Lemons are generally greenish yellow in hue, but once they reach their full size, they turn bright yellow instead of greenish yellow. Small seeds are contained within the fruit known as floppies. ⁽⁵⁵⁾.

1.10.4. Geographical description:It is native to northern India, but it is also grown in Turkey, Mexico, the United States, Argentina, Iran, Brazil, Africa, Italy, Spain, and the People's Republic of China. Lemon fruit can be utilized for both non-culinary and culinary reasons all around the world.

Lemon is mostly used for its juice, although the pulp and flavor are also often utilized in baking and cooking ⁽⁵⁶⁾

1.10.5. Ethnomedical uses: Lemon was used to heal the plague, and a syrup known as acridinecitri is made from its juice, which is used to alleviate someone's thirst while also being fought over. Honey and lemon are boiled together and used as digestives. It is also used to treat pimples and dark spots on the face ⁽⁵⁷⁾. Lemon juice was utilized to eliminate intestinal worms and remove kidney stones and gravel ⁽⁵⁷⁾. According to various reports, drinking large amounts of a citrus juice mixture reduces oxidative stress, prevents atherogenic modifications of LDL cholesterol, improves blood lipid profile, platelet aggregation, and increases HDL-cholesterol concentrations. It has been discovered that its extract has anti-thyroid activity suggesting its potential use to manage hyperthyroidism.

Citrus is the primary source of vitamin C1 for primates. Ascorbic acid, which is required for connective metabolism, notably scar tissue, teeth, and bones, improves iron absorption from the small intestine in the body. It is essential as an anti-stress agent and a protector against chills, dampness, and cold. It quenches and scavenges free radicals produced by UV radiation stabilization, and it also acts as an antioxidant in the skin ⁽⁵³⁾. Vitamin C is also required for collagen synthesis. Collagens help in skin advancement and renewal, a possible anti-aging property. It smoothes out fine creases. Its extracts, which are responsible for the development of protein serum, such as d-glucosamine, increase the total amount of protein in the body and its

energetic constituents. Citrus fruits and peels have been shown to aid in the treatment of a variety of metabolic and inflammatory problems due to the number of bioactive substances contained in them.⁽⁵⁸⁾

Some definite pharmacological properties includes anti-cancer , anti-oxidant, anti-diabetic and anti-microbial properties.

1.10.6. Phytochemical properties: Lemon is one of the citrus fruits high in phytoconstituents.

Citrus fruit contains a high concentration of flavonoid glycosides, coumarins, and volatile oils ⁽⁶⁰⁾. Citrus fruit contains a lot of flavonoids, glycosides, coumarins, and sitosterol. Citrus fruits account for 0.9% of total daily calories and 1.7% of total daily carbs. Polymethoxylated flavones are extremely important and are extremely rare in other plants.

The oil extracted from the peel has high amount of terpenes which exist as limonene, beta-pinene, terpinene, it also contains neral, terpinen-4-ol, geranial, citronella , and thymol. Limonene was found in highest amount and is responsible for the characteristic scent of the oil and its well known antioxidant activity.⁽⁶¹⁾

1.11 JUSTIFICATION OF THE STUDY

In the world of skin care, synthesized chemicals are the leading actives and are most prevalent in products. Overtime though unwanted reactions have been noticed in the use of these chemicals leading to the rise in the use of natural ingredient gotten from plants, such as the leaves, oil extract, petals of flowers, the roots, the juices from the fruits etc. Amongst all other skin concerns, fine lines, wrinkles and signs of aging happens to be what most people want to solve, aging often times is caused by extrinsic factors of which the most is owed to chronic exposure to ultraviolet radiation produced by the sun. The use of creams and formulation containing sun

protection factor(SPF) has become popular nowadays and the need to produce these formulation from natural sources has also grown.

This project work delves into the skin care application of phytochemicals found in *Annona muricata*, exploring its bio activity on the skin and the possible anti aging properties as well as its ability to act as sun protection, it would involve the collection, oil extraction and UV spectroscopic analysis of Graviola oil. This will be of good information as other activity of the plant has been tested time before, better exploration would be done in the course of this project towards the efficacy, effectiveness and safety of Graviola oil to be used against possible disorder or disorder caused by Ultraviolet radiation.

1.12 AIM

To isolate, identify, characterize and determine the phytochemicals for the antiaging property of lemon essential oil, its antioxidant activity and its sun protection factor.

1.13 OBJECTIVES

Extraction of the essential oil from *citrus limon*

To determine phytochemicals, antioxidant activity and sun protection factor.

CHAPTER TWO

METHODOLOGY

2.1 Area of study

The entire study was carried out in the natural product research laboratory, department of pharmaceutical chemistry, University of Benin, Benin city, Edo state.

2.2 Study design

The study is an experimental study.

2.3 Laboratory analysis

The analysis involved phytochemical assay of sample extract, antioxidant screening(DPPH Assay) , screening for physiochemical properties, Ultraviolet/Visible light spectrophotometric analysis to determine SPF.

2.4 Materials and reagents

2.4.1 Glassware

Measuring cylinder (25ml, 50ml), conical flasks, test tubes, pipette (5ml, 10ml), volumetric flasks, burette (50ml), round bottom flask (500ml), sample bottle, glass stirrer, beakers.

2.4.2 Apparatus

Water bath, heating bath, julabo cooling bath, Clevenger-like apparatus, Gas chromatography-Mass spectrometer, Ultra violet-Visible light spectrophotometer, micro pipette.

2.4.3 Reagents

Ethanol (99.8%), benzophenone, Dragendorff's, Mayer's, Hager's and Wagner's reagent, 1% naphthol, concentrated acid(sulphuric acid, nitric acid, hydrochloric acid), ferric chloride, lead acetate, 20% sodium hydroxide, 1% gelatin, chloroform.

2.5 Sample collection, identification, preparation, extraction and storage.

2.5.1 Sample collection and identification.

The fresh fruits of *Citrus limon* were purchased from a local sales person not too far from the university campus (GPS Coordinates). There after it was identified by a known plant biologist and biotechnologist Dr. H.A. Akinnibosun, there a voucher number was given to the sample – UBH-C525.

2.5.2 Sample preparation and extraction

The fresh lemons were washed to remove extraneous materials and peeled, the peels were weighed and there after subjected to hydro-distillation using the Clevenger type apparatus (the Clevenger apparatus is associated with a julabo cooling bath, a suitable round bottom flask, and a heating mantle). Each round of extraction takes about 45 minutes to 1 hour and an average of 350-400g of lemon peel is used to extract an average of 1.8-2.5 ml of essential oil.

2.5.3 Sample storage

After a successful extraction process, the oil is decanted from the water and dried using anhydrous sodium sulphate salt (a hygroscopic salt which absorbs moisture and becomes molten), there after the dry oil is decanted from the molten salt and transferred to a glass sample bottle and stored at 4 °C until needed for analysis.

2.6 Determination of physiochemical properties

2.6.1 Organoleptic properties

The organoleptic properties of the oil such as appearance, odour, taste and texture were determined with 0.1 ml of lemon essential oil and results recorded immediately.

2.6.2 Determination of specific gravity

Specific gravity is the relative heaviness or lightness of a material. 1ml of the sample was weighed into a sample bottle for 3 consecutive times using an analytical weighing balance and the values gotten were 0.828 g, 0.828 g and 0.828 g. The average of the values was 0.828 g
Specific gravity = weight of oil per 1 ml of oil The specific gravity of scent leaf is 0.828 g/ml.

2.6.3 Determination of refractive index

The refractive index of the oil extract was determined using an Abbe refractometer. Firstly the instrument was turned on for 5 minutes in order to warm up, the lenses were cleaned with cotton wool and acetone and left to dry. A clean stirring rod was used to transfer a drop of lemon essential oil to the bottom lens of the instrument and the top lens was covered with it, the sodium lamp was brought close to the lens in order to have a view, using the control knob the refractometer was adjusted till the view was clear and the coarse adjustment knob was used to align the boundary line with the 'X'. The read button was pressed at a temperature of 27 C, the refractive index was measured and displayed on the screen, triplicate readings were taken and recorded.

2.7 Phytochemical assay screening

2.7.1 Sample preparation for phytochemical assay

The part of the plant where the essential oil was collected from was tested for the presence of phytochemicals . The lemon peel was zested and dried indoors and 5 g was weighed out for analysis. The dried zest was boiled with 70 ml of water for 30 minutes and filtered using a filter paper after cooling. The filtrate was then subjected to phytochemical test.

2.7.2 Test for alkaloids

2ml of filtrate was shaken with 1% HCL for 2mins. The mixture filtered and few drops of dragendorff's reagent were added. Formation of a orange precipitate indicated the presence of alkaloids.

2.7.3 Test for saponins

1ml of filtrate was shaken vigorously with 10ml of distilled water for 10mins. Frothing was thereafter observed for 15mins.

2.7.4 Test for tannins

2 ml of filtrate was swirled with 2 ml of 1 % gelatin solution in 10 % sodium chloride.

2.7.5 Test for phenols

2 ml of filtrate was added to 5 ml of distilled water. To the solution was added 2 drops of 5 % in ferric chloride solution, the mixture turned a dark color compared to the blank which was clear.

2.7.6 Test for flavonoids

2.7.6.1 Alkaline reagent test

To 2 ml of the filtrate 20% sodium hydroxide was added followed by few drops of dilute hydrochloric acid solution, colour of the mixture intensified, no precipitate observed.

2.7.6.2 Lead acetate test

To 2ml of the filtrate was added few drops lead acetate solution, formation of light brown precipitate was observed.

2.7.7 Test for terpenoids (Salkowski test)

To 5 ml of the filtrate 2 ml of chloroform was mixed and there after concentrated sulphuric acid was added, formation of a second layer and evolution of bubbles was observed.

2.7.8 Test for carbohydrate

To 2ml of filtrate was added 2 drops of 1% alcoholic naphthol. 2ml of concentrated sulphuric acid was thereafter added at a slanted position (about 45°), formation of a purple ring at the interface of both mixtures was observed.

2.7.9 Test for anthraquinones

To 2 ml of the filtrate 2 ml of petroleum was added and shaken, formation of a layer of ether was observed, thereafter washed with dilute ammonia solution and formation of bubbles was observed.

2.8 Determination of antioxidant activity (DPPH assay)

2.8.1 Reagent preparation

0.1mM DPPH solution was prepared by dissolving 0.0020g of DPPH in 50ml of methanol.

2.8.2 Working procedure

Stock solution (1mg/ml) of Ascorbic acid and lemon essential oil were prepared in two different beakers by weighing 0.010 g of each sample into a beaker and 10 ml of distilled water and 10 ml of acetone respectively were used to dissolve the powder and oil. Serial dilutions were done and eight concentrations of 1 µg/ml, 2 µg/ml, 5 µg/ml, 10 µg/ml, 20 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml were prepared from the stock solution (1mg/ml) and distilled water and acetone were used to make up to 3ml total volume. 1µg/ml was prepared by placing 3 µl of the essential oil in a dried test-tube and adding 2997 µl of acetone to make up to 3ml total volume, triplicate determinations were made for each concentration. A blank solution was prepared containing 3ml of methanol and 1ml of DPPH; this was done in triplicate. 1ml of 0.1mM of DPPH in methanol was then added to all test tubes and test tubes were covered with foil paper. The reaction was shaken thoroughly and left in the dark at room temperature for 30 minutes. The absorbance was

thereafter determined using the UV-Visible Spectrophotometer at 517 nm. Ascorbic acid was used as standard.

2.9 GC-MS analysis

Determination of quantitative and qualitative data were done by GC-MS analysis. Lemon essential oil was injected into a Thermo-Scientific Trace GC ULTRA system coupled to DSQ II mass spectrometer, equipped with an AS 3000 auto sampler and a split/split-less injector. The column used was a TR-5MS, coated with 5% diphenyl-95% polydimethylsiloxane. Carrier gas was helium at 30cm/s linear velocity, inlet pressure 99.8KPa; detection temperature, 280°C. The components of the sample were identified based on their retention factors. Confirmation of the identity of the active compounds was done by comparing their mass spectra with published spectra and reference compounds from Library of National Institute of Standard and Technology database.

2.10 Determination of Sun Protection Factor (SPF)

The determination of SPF values was made through the UV-Vis spectrophotometric method and the Mansur equation was applied. 1 % concentration of the lemon *essential* oil was prepared by measuring 100mcL(0.1ml) of the oil and adding 9.9ml of ethanol. From the 1% ethanolic extract of the essential oil, 0.01 % concentration was prepared using a dilution factor of 1:100(0.01ml of the oil in 10ml of ethanol). The absorption of the ethanolic essential oil extract was measured using a Ultra Violet-Visible light spectrophotometer at a wavelength of 290-320nm with an interval of 5nm. The SPF value was then calculated using the Mansur equation to determine the category of the sunscreen protection.

CHAPTER THREE

RESULTS

3.1 Results from determination of physiochemical properties

Physiochemical property	Results
Appearance (colour)	Pale yellow
Odour	Aromatic, Fruity
Taste	Slightly bitter
Texture	Non greasy
Specific gravity	0.828 g/ml
Refractive index	1.468

Table 3.1 Results from phytochemical screening

Phytochemicals constituents	Results
Alkaloids	+
Tannins	-
Flavonoids	+
Saponins	+
Phenolics	+
Terpenoids	+
Carbohydrates	+

Table 3.2 Results from Antioxidant oxidant screening (DPPH Assay)

Concentration (mcg/ml)	Average Absorbance (standard)	Average Absorbance (lemon oil)	Radical scavenging activity [standard (%)]	Radical scavenging activity [lemon oil(%)]
1	0.248	0.282	44.30	51.50
2	0.175	0.376	53.99	35.30
5	0.149	0.317	64.01	45.50
10	0.149	0.279	64.52	51.90
20	0.159	0.223	64.53	61.60
50	0.142	0.222	66.76	61.70
100	0.163	0.321	63.50	44.60
200	0.155	0.206	65.20	64.50

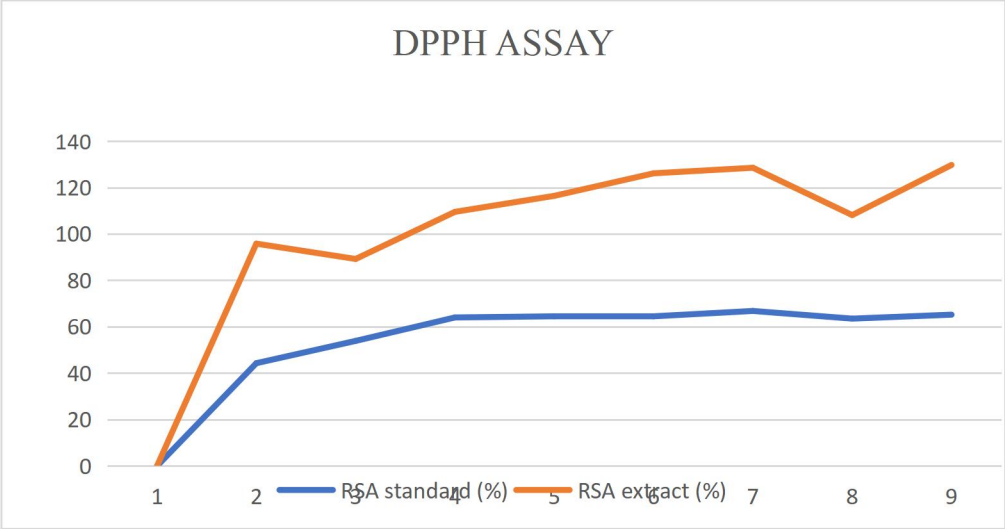


Fig 3.1 Graph of antioxidant activity of lemon oil and ascorbic acid.

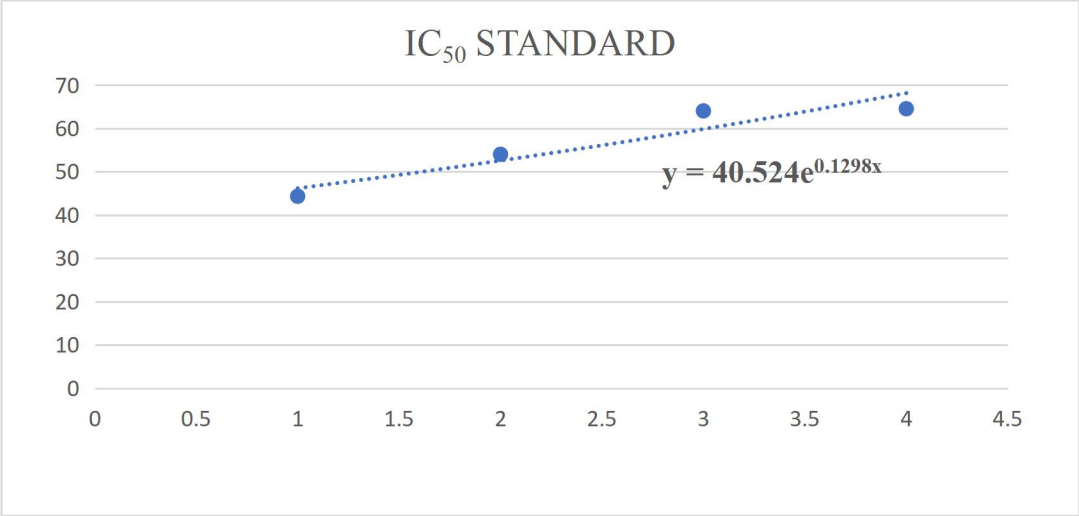


Fig 3.2 Graph of IC₅₀ of standard (ascorbic acid)

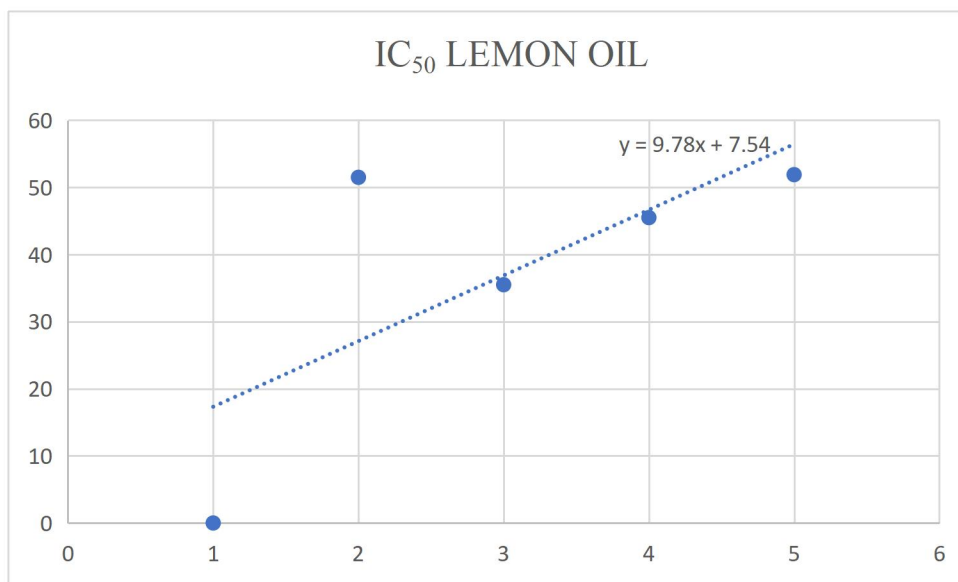


Fig.3.3 Graph of IC₅₀ of lemon oil extract

Table 3.3 Ultra violet-Visible light spectrophotometric results for Sun Protection Factor determination (standard –Benzophenone 0.01%)

Wave length(nm)	Absorbance
295*	0.425
300	-9.999
305	-0.013
310	-0.075
315	-0.021
320	0.061

Table 3.4 Ultra violet-Visible light spectrophotometric results for Sun Protection Factor (SPF) determination (lemon essential oil 0.01%)

Wave length(nm)	Absorbance(A)	Absorbance(B)	Absorbance(C)
295*	0.748	0.754	0.926
300	0.006	0.019	0.014
305	0.005	-0.029	-0.016
310	0.030	0.024	0.017
315	-0.148	-0.122	-0.700
320	0.010	0.004	0.008

Table 3.5 Ultra violet-Visible light spectrophotometric results for Sun Protection Factor (SPF) determination (lemon essential oil 0.1%)

Wave length (nm)	Absorbance (A)	Absorbance (B)	Absorbance (C)
295	2.576	2.584	2.584
300	0.43	0.50	0.54
305	-0.004	0.001	-0.002
310	-0.019	-0.010	-0.024
315	-0.181	0.015	0.013
320	-0.005	-0.004	-0.00035

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where Where: EE – erythemal effect spectrum;

I – solar intensity spectrum

(EE and I are constant at different wavelength)

Abs - absorbance of the analyte

CF – correction factor (= 10).

Table 3.6 The values of EE X I at different wavelength

Wavelength (nm)	EE X I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

The SPF of the lemon essential oil at 0.01 = 0.553

The SPF of lemon essential oil at 0.1 = 3.45

CHAPTER FOUR

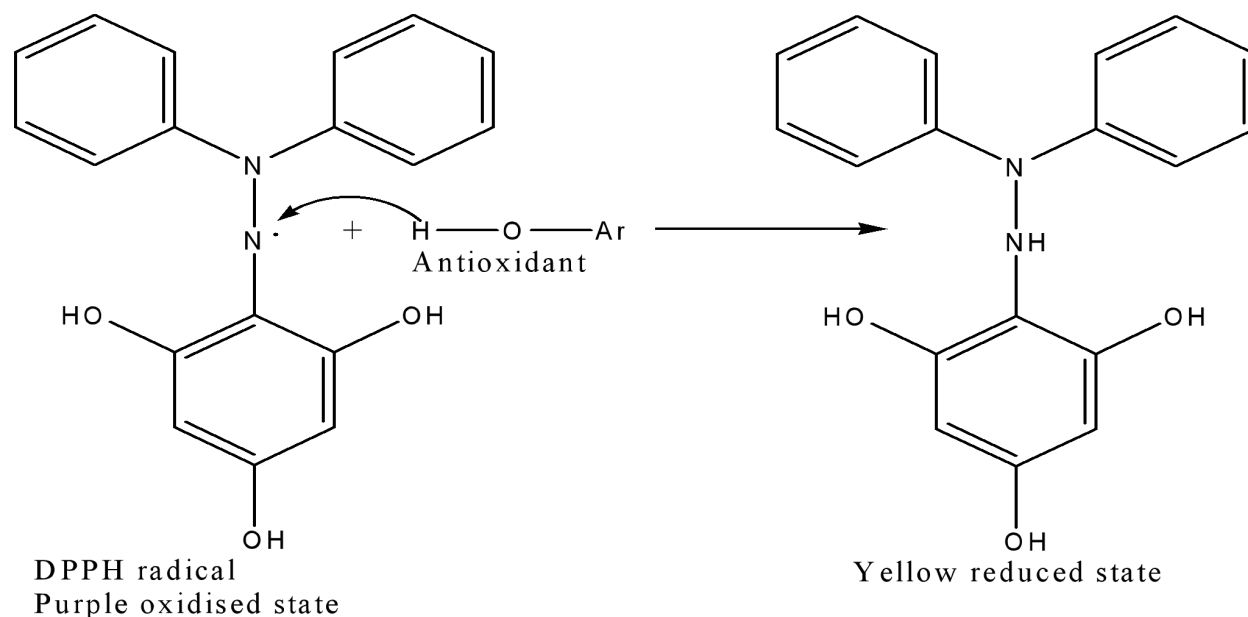
DISCUSSION

The results obtained shows the qualitative and quantitative analysis of the essential oil of lemon (*Citrus limon*) which aids the investigation of the phytochemical, antioxidant and Sun Protection Factor of the analyte. From the physiochemical results we can see that the essential oil according to literature truly carries the characteristics of the plant especially in its odour –a fruity scent which is distinctive to citruses and the lemon plant itself. According to literatures this is owed to the terpenes present in the composition of the oil, from the phytochemical screening the presence of terpenoids⁽¹⁾ was confirmed and its composition of determined via the GC-MS analysis. The physiochemical screening also tells us about the color and texture of the oil extract which can be use to check for purity and also compare with commercially available oils and reduce chance of adulteration. The texture of the oil is non greasy which is a sharp contrast with regular fixed oil extract. The specific gravity of the oil was gotten as 0.828 g/ml which is less than water (1 g/ml) which explains the process by which it is extracted as seen floating on top water in the apparatus. The refractive index of the oil was determined to be 1.468 (less than 1.5)⁽²⁾ which shows it would have a long shelf life and not easily go rancid (as is characteristic with most essential oil). From the phytochemical screening the oil tested positive for the presence of alkaloids, saponins, terpenoids, phenolics, anthraquinones, flavonoids and carbohydrates but tested negative for tannins, which is different from the results of literature⁽³⁾ and research, this could be owed to possibly the nature of lemon samples, geographical location and weather were it was grown and possibly quality of specific reagent used for the analysis. The various phytochemical tested present can be confirmed from the GC-MS analysis result and their relative compound

composition as well. The presence of flavonoid from first analysis shows a good antioxidant potential of the oil, as studies have shown that flavonoids have anti-oxidative properties⁽⁴⁾.

The antioxidant property of the essential oil was carried out using DPPH assay, where the absorbance at different concentration of the oil in a suitable solvent was related with a blank determination and further compared with a standard in this case is ascorbic acid. The minimum inhibitory concentration (IC50) was determined to be **1.083** for the standard and **4.34** for lemon oil extract. According to research the higher the IC50 value the lower the antioxidant activity⁽⁵⁾

The oil extract clearly shows a weaker antioxidant activity than the standard but not so far of so its antioxidant property could be described as moderate from the graph illustrated in the results the activity can be seen side by side. The principle of this that the reagent DPPH -2,2-Diphenyl-1-picrylhydrazyl contains free radicals which allows it to cause oxidative damage the action of an antioxidant is to donate hydrogen to DPPH which then scavenges or 'eats up' the free radicals turning DPPH into a stable diamagnetic molecule. The reaction is observed physically by a change in color of the reagent from the characteristic purple to yellow showing a reduction reaction.⁽⁶⁾ During the processes of the antioxidant assay of the standard and the extract, it is noticed that the process is carried out in darkness as well as the incubation period this is because DPPH is photo sensitive and would be further oxidized in the presence of light which would hinder accuracy of the results.



From later results as seen in fig the various absorbance of the standard and oil extract using the spectrophotometric formulae , it is seen that the SPF of lemon oil was calculated to be 3.45 at 0.1% and 0.553% at 0.01%, although low in combination with its antioxidant activity can be very useful additive in cosmetic and dermatological product. More studies can be done to observe the sun protection factor in higher concentrations. When compared to past literatures the value determined are not so different, possible reasons for slight discrepancies might be due to difference in specie of lemons used to carry out the experiment, effect of weather on the general composition of the essential oil extracted.

The distinct scent of *Citrus limon* oil which is owed to the large amount of limonene ⁽⁷⁾ can be very useful as fragrances in dermatological products as its aroma is none irritant and appealing. Its level of antioxidant activity allows it to be useful as food preservation to reduce damage and deterioration by reactive oxygen species.

CHAPTER FIVE

CONCLUSION, RECOMMENDATIONS AND LIMITATIONS

5.1 CONCLUSION

The phytochemical screening, antioxidant assay and sun protection factor determination of the essential oil of *Citrus limon* agree with past literature and shows it is a good material for additive into dermatological product, its strong antioxidant activity makes allows it to be useful in combating the damaging effect of ultraviolet radiation by scavenging free radicals.

5.2 RECOMMENDATIONS

From the results determined it is highly recommended that more studies be done to further potentiate the usefulness of this essential oil, finding other natural products which could work synergistically with the essential oil of *Citrus limon*.

5.3 LIMITATIONS OF THE STUDY

This study due to factors beyond human control did not include assay of toxicity profile of the oil extract nor in situ studies on biological systems which could have made more positive impact on the results obtained. This was due to reduced time allotted for the study and also time wasted because the extraction process was elongated due to lack for electric power supply.

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APPENDIX



Figure 1: *Citrus Limon*



Figure 2: Peel of *Citrus Limon*



Figure 3: Essential oil of *Citrus Limon*