

**Deanship of Graduate Studies
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**Extraction of oleuropein from Palestinian olive leaf by
using simple extraction methods and apply the extract in
cosmetic products**

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Extraction of oleuropein from Palestinian olive leaf by using simple extraction methods and apply the extract in cosmetic products

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Al-Quds University
Deanship of Graduate Studies
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Thesis Approval

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Declaration

I certify that this thesis submitted for the degree of master, is the result of my own research, except where otherwise acknowledged, and this thesis has not been submitted for the higher degree to any other university or institution.

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Acknowledgments

At the end of my thesis, I would like to thank people at Al-Quds University for giving me the opportunity to achieve the M.Sc. degree.

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Special thanks for the family of Raed Cosmetics, and also for the staff in Beit Jala Pharmaceutical Company (BJP) for their help during my experimental work.

Abstract:

The main goals of the present study were to study the extraction of oleuropein from Palestinian olive leaves; the effect of the extraction solvent (type, composition), the effect of pH and temperature of the extraction methods (Maceration and Soxhlet) and to develop a stable topical formulation containing oleuropein.

Oleuropein contents in green olive leaves dried at ambient temperature, and at elevated temperature (50° C) from Palestinian olive trees located in Beit Sahour area and collected in the middle of November were determined by high-performance liquid chromatography HPLC and compared to dry olive leaves which are collected dry from the tree. The results showed that the highest concentration of oleuropein was obtained from olive leaves dried at room temperature (10.0 mg/g), meanwhile for the olive leaves dried at elevated temperature at (50°C) it was 1.7 mg/ g, and for the dry olive leaves collected dry from the tree a concentration of 2.5 mg/g was extracted. Oleuropein content of dried olive leaves was compared with olive leaves that were collected and immediately chopped and extracted, results show that these fresh leaves showed the lowest oleuropein content (< 0.1 mg/g) proving that drying of leaves is necessary for increasing the amount of oleuropein being extracted.

Mixture of the solvents gave higher oleuropein content. The solvent with 80% ethanol give the highest oleuropein content followed by the solvent with 20% acetonitrile, meanwhile using the pure water, methanol and ethanol solvents are not good for oleuropein extraction. Temperature of extraction was found to have a significant effect on the oleuropein content where higher temperature gave higher oleuropein content. It was found also that acidic pH of pure water gave higher oleuropein content compared to basic pH. Soxhlet extraction was found to give higher oleuropein content compared to maceration method.

Oleuropein hasn't been widely incorporated into cosmetics and dermatological formulation, however, oleuropein has several pharmacological properties, including antioxidant, anti inflammatory, skin protectant and anti-aging. The prepared cream was found to be natural, stable and safe. All physical and rheological properties of all the prepared samples were nearly the same as commercial product. Stability studies showed a stable homogeneous appearance and effective during three months storage period at room temperature.

The oleuropein contents decreased significantly with increasing the temperature and storage period, therefore by giving the product an expiry date of one year the consumer will still have a sufficient active concentration for product efficacy even with low concentration (0.1g/100 g of cream).

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Chapter One: Introduction

“A woman without paint is like food without salt” Roman philosopher, Plautus.

1.1 Cosmetics:

“A cosmetics product means any substance or preparation intended to be placed in contact with the various external parts of human body (epidermis, hair system, nails, lips and external genital organs) or with teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, changing their appearance and/or correcting body odors and/or protecting them or keeping them in good condition.” This definition is according to Directive 93/35/EEC, the Sixth Amendment to the original Cosmetics Directive of 1976, which is also build by Palestinian National Authority Ministry of Health on December 2007 and displayed as a Guidelines for Cosmetics Products Registration in Palestine part 3.

1.2 Cosmetics in the ancient world:

Men and women in Egypt used oils and ointments to clean and soften their skin and mask body odor (Gunther *et al.*, 2005). Oils and creams are used for protection against the hot Egyptian sun and dry winds. Myrrh, Thyme, Chamomile, Lavender, Lily, Peppermint, Rosemary, Roses, aloe Vera, Olive Oil, Sesame Oil and Almond Oil provide the basic ingredients of most perfumes that Egyptians use in religious ritual. Then they applied galena mesdemet (made of copper and lead Ore) and malachite (bright green paste of copper minerals) to their faces for color and definition. They employ a combination of burnt almonds, oxidized copper, different colored coppers Ores, lead ash and ochre- Together called Kohl to adorn the eyes in an almond shape (Cosmetics and your health, 2004). Chinese people began to stain their fingernails with gum Arabic, gelatin, bees wax and egg (Huo, 2011). Grecian woman paint their face with white lead and apply crushed Mulberries as Rouge (Lesley, 1998). Japanese used rice powder to make their faces white. Eye brows are shaved off, teeth painted gold or black and henna dyes applied to stain hair and faces (Naomi, 2001).

1.3 Cosmetics in the modern world (100 AD – 21st century):

In Rome people put barely flower and butter on their pimples and sheep fat and blood on their fingernails for polish. Henna is used in India as a hair dye, and as an art form painted on to the hands and feet, especially before a wedding. Henna is also used in some North African cultures. In England, dyed red hair comes into fashion. Society women wear egg whites over their faces to create the appearance of a paler complexion. Yet, some thought cosmetics blocked proper circulation and therefore posed a health threat. Queen Elizabeth of England was one well-known user of white lead, with which she created a look known as “the mask of Youth”. With a blond hair; Mixtures of black Sulphur, alum, and honey were painted onto the hair and lift it to work in the sun (History of Cosmetics, 2010). Italy and France were the main centers of cosmetics manufacturing. Arsenic is used in face powder instead of lead. European women were lighten their skin using a variety of products, including white lead paint. Zinc oxide become widely used as a facial powder, replacing the used deadly mixtures of lead and copper after discovered the toxicity of it and physical problems including facial tremors, muscle paralysis and even a death (Agata, 2013). Beauty salons increased in the beginning of the 20th century because women needed

assistance to look younger. In 21th century the market of cosmetics has a different dynamic compared to the 20th century, some countries are driving this economy.

According to Euromonitor international 2008 the worldwide market top ten are accordingly as follow: USA, Japan, Brazil, China, Germany, France, United Kingdom, Russia, Italy and Spain (Euromonitor, 2008).

According to the national bureau of statistics of china (NBS) 2012, china today is the largest emerging cosmetics market in the world by reaching 134 billion Yuan. It is developing very fast, with an annual growth of 15%. The skin care and make up are the two main sectors of the market.

An Overview of the broad cosmetics industry (also referred to the cosmetics and toiletries (C&T) industry) in the EU, Japan, China, and the U.S. reveal that Europe's market size is almost large as the US and Japan combined, due to its large population (Global, 2007). In 2009, the US. Cosmetics market was €38.2 billion, while Japan's was €23.7 billion and China's €8.2 billion. The total EU 27 cosmetics market was valued at €63.5 billion in 2006. Among the EU countries, Germany has the largest cosmetics market, valued at €11.7 billion followed by France (€10.4 billion), the U.K. €10.0 billion, Italy €8.8 billion and Spain €7.4 billion (Global, 2007).

In the last few years, new forms of treatments brought alternatives to traditional cosmetics products. Skin care and Sun care have clearly been the most dynamic product segments in the cosmetics industry and are being targeted both the medical devices and pharmaceuticals industries.

1.4 Cosmetics industry in Palestine:

Cosmetics sector in Palestine is not an independent one. It is found under a chemicals industry and/or pharmaceuticals industry.

1.4.1. General information of chemical industries:

The sector is represented by an industrial association that needs to be strengthened and institutionalized. The estimated number of regulated companies working in the industry is 60, in which only 5 of them are producing cosmetics, and the other in the production of paints, inks, and detergents. The actual number of producers (unregulated) is much more than that. Some factories produce both detergents and cosmetics.

1.4.2. Cosmetics Industry problems and needs:

It is clear that cosmetics sector in Palestine suffers from unfair mechanism of the local market. And hence regulating the local market is a priority. This will force the incompatible illegal products to leave that market. The availability of raw material is a real threat to the industry combined with proper technologies.

Encouraging small companies to merge with others or from a strategic relation with them will enhance their competitiveness in the market. Besides, this will minimize the negative effects of family business administration and practices in the industry. Lack of training and technical assistance is seen to be an important matter to develop the industry.

1.4.3. Regulated cosmetics companies in Palestine:

1. Raed Cosmetics Company (Beauty Code) Beit- Sahour ((Products for hair care, products for body care and for facial care).

It is a national company operating with the hands of qualified, scientific and practical Palestinian team. It also employs world best raw materials with an emphasis on what is available in Palestine. Such as Olive oil and Olive leaf extract. Hence Raed Cosmetics took the decision to incorporate Olive tree, oil, and extracts among the ingredients of its products. And put 3 olive tree leaves in its logo to indicate its origin from the Holy Land.

Research and Development department of Raed Cosmetics started to study the possibility of extracting the active ingredient Oleuropein from the Palestinian olive leaves. Encouraged by the abundance of the stuff, and most people consider it as discarded byproduct.

2. Palkarm Cosmetics Ltd, Nablus. Perfumes, cosmetics, hair products and creams.
3. Holy Land Industry, Nablus. Specialized in producing shampoo, soap and creams
4. Arab Industrial Company (Star) Ramallah. Powder and liquid detergents, personal hygiene products, Soap and Dead Sea products.
5. Swan Cosmetics laboratories. Gaza. Perfumes, cosmetics, hair color, skin and hair care.

1.5 Plant Extracts:

The use of plant extracts in cosmetics formulation is increasing, mostly because of the poor image that animal –derived extracts have acquired during the past few years, when the common woman in the street finally discovered the source of collagen and elastin, those ingredients may hold less appeal than they currently enjoy. So to increase the consumer appeal the GREEN conscience has become a widely supported.

Applications of plants and plants extracts in cosmetics are widely spread and where used for purposes such as moisturizing, whitening, tanning, color cosmetics, sunscreen, radical scavenging, anti-aging, anti oxidant, immune stimulant, washing, preservatives, thickeners (Blum *et al.*,2007).

1.5.1. *Olea europaea* L:

The olive tree, botanically-classified as *Olea europaea* L. is one of the most important fruit trees in Mediterranean countries. The characteristic green to blue-black fruit of this shrub yields useful edible oil. Both the oil and the dried green-grayish colored leaves are used medicinally (Wern *et al.*, 1985).The olive tree has been held in high esteem throughout history. The oil is symbolic of purity and goodness, while the olive branch represents peace and prosperity. Historically, the knowledge of the medicinal properties of the olive tree date back to the early 1800s, when it was used in liquid form for malaria treatment (Wern *et al.*, 1985). In the early 1900s a bitter compound was found in the leaves of certain olive trees called "oleuropein," which was thought to be part of olive tree's potent disease-resistant structure (Walker *et al.*, 1997).The olive tree has been the source of natural healing agents down through the ages including the olive oil produced from its fruit. For centuries, teas and other preparations made from olive leaves have been used successfully to treat fevers and gastrointestinal complaints, including parasites in human patients. Olive leaf extract is effective against a broad spectrum of microbial agents, including viruses, bacteria and even parasites, and can considered one of the most useful and safe natural anti- microbial herbal extracts yet discovered (Pooley *et al.*, 1997). It can inhibit and kill over 100 microorganisms which can cause disease and death on abroad scale, thus it can be considered nature antibiotic remedy to be used to prevent and treat numerous animal infectious

conditions and health problems related to: viruses, bacteria, parasites, allergy conditions, skin problems (psoriasis), inflammation (arthritis, sinusitis, bursitis, etc.), gastrointestinal problems, ulcers, free radical overload, over burdened immune system, and wound healing.

Olive leaf extract was derived from the leaves of the olive tree. It is a source of many phytochemicals. It was found to be part of a compound produced by olive trees that make them particularly vigorous and resistant to insect and bacterial damage (Pooley *et al.*, 1997) The *Olea europaea* L. leaves represent a typical herbal drug of the Mediterranean area, commonly used in traditional medicine as vasodilatory, hypotensive, anti-inflammatory, antirheumatic, diuretic, antipyretic, and hypoglycemic agents (Somova *et al.*, 2000). The active constituents of olive leaf have a wide number of ingredients, including the chief constituent oleuropein (60-90 mg/g) and several types of polyphenolic compounds. The following polyphenols were detected in olive leaf tissue: hydroxytyrosol (figure (1.1)), hydroxytyrosol-glucoside, tyrosol (figure (1.2)), elenolic acid derivatives, caffeic acid, oleuropein, verbascoside, rutin, luteolin 7-O-glucoside, luteolin 4-O-glucoside, apigenin-7-O-rutinoside and apigenin 7-O-glucoside (Benavat *et al.*, 2000). There are at least six active substances (oleuropein, hydroxytyrosol, caffeic acid, vanillin, luteolin-7-glucoside, and verbascoside) in the extract. These six substances work together synergistically to prevent resistance by pathogen microorganisms. While oleuropein is the ingredient most studied, there are in fact 95 different chemicals in the leaf and a balance of ingredients seems to work the best. Oleuropein content varies from 17% to 23% depending upon the time of year the leaves are harvested (Le toutour *et al.*, 1992).

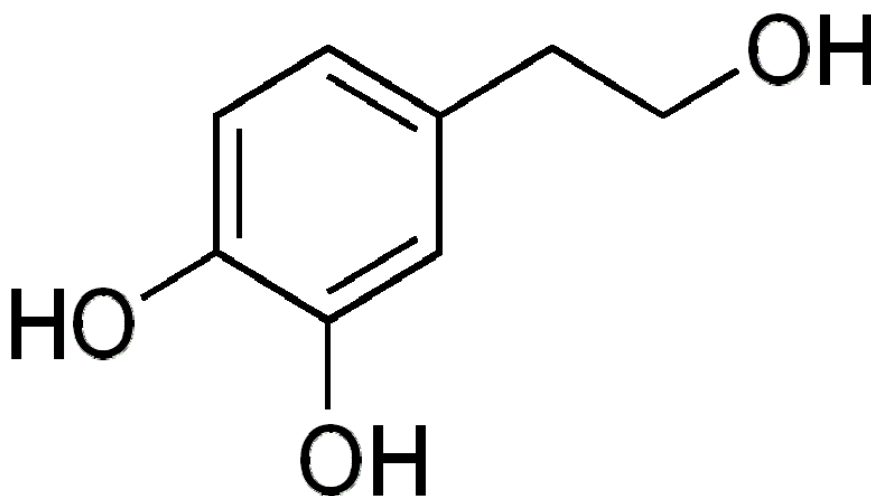


Figure 1.1: chemical structure of Hydroxytyrosol.

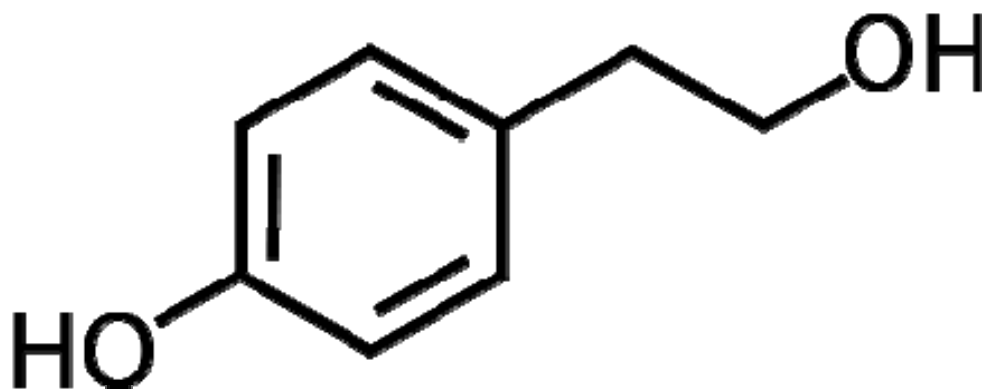


Figure 1.2: Chemical Structure of Tyrosol.

1.5.1.1. Terpenic compounds in olive leave:

In 1969, the study showed that there is a presence of terpenic acids in Olive Leaf. The important one is Oleanolic acid (3-beta-hydroxy-28-carboxyolean) (figure (1.3)). This compound found extensively throughout the plant kingdom, and in olive leaf it occurs in free acid form in considerable proportions accounting for 3% of dry leaf weight (Albi *et al.*, 2001).

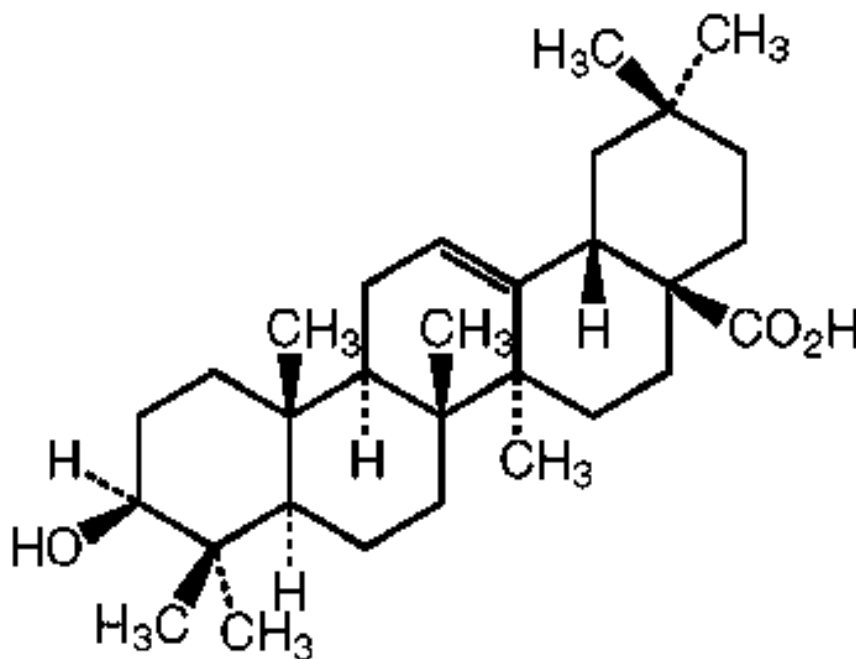


Figure 1.3: Oleanolic Acid chemical structure

Oleanolic acid is biologically active with antiabortive, anti-cariogenic, anti-fertility, anti-hepatotoxic, anti-inflammatory, cancer prevention, cardiotoxic, diuretic, hepatoprotective properties (Saady *et al.*, 1994).

1.5.1.2. Liposoluble compounds in olive leaf:

In 1973, liposoluble compounds present in olive leaves (hexane extract) were isolated by thin layer chromatography and analyzed the fatty acids of the triacylglycerols from leaves. The main liposoluble compounds present in olive leaves are:

Saturated hydrocarbons, Squalene (figure (1.4)), Ester waxes, Alpha-tocopherol (figure (1.5)), Triglyceride, Beta carotene, linear alcohols, Alpha-and beta- amyryne, and Beta-sytosterol (figure (1.6)). These compounds have multiple applications in the pharmaceutical, cosmetics, and food additives industrials.



Figure 1.4: Chemical structure of Squalene

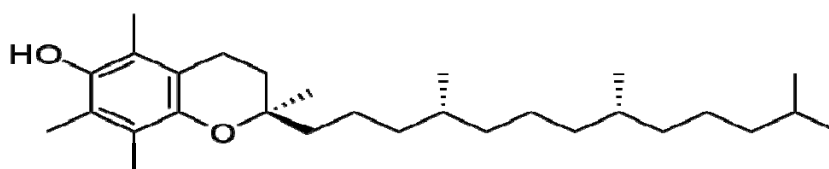


Figure 1.5: Chemical structure of Alpha-Tocopherol

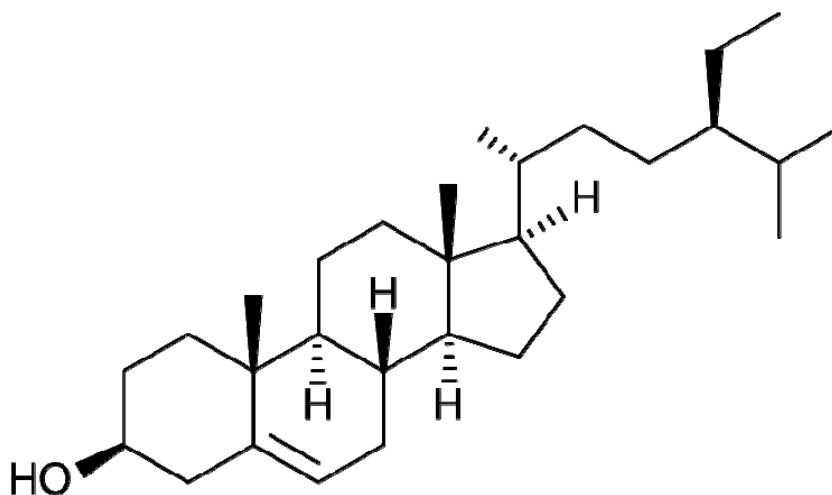


Figure 1.6: Chemical structure of Beta-Sytosterol

1.5.1.3. Other Compounds:

The olive leaves contain Mannitol (figure (1.7)), a hexitol derivatives of mannose. It offers a series of properties with advantageous applications in the food and pharmaceutical industries. Its sweetening potency is equivalent to 70% that of sucrose; it has a low caloric value (2kcal/g), it does not cause caries and its metabolism in humans is not dependent on insulin, which makes it suitable for consumption by diabetics. Additionally, it has healthful ef-

fects as an antioxidant (Wisselink *et al.*, 2002) and is of therapeutic use in severe head injuries, as it is effective in the reduction of intracranial pressure (Unterberg *et al.*, 1997).

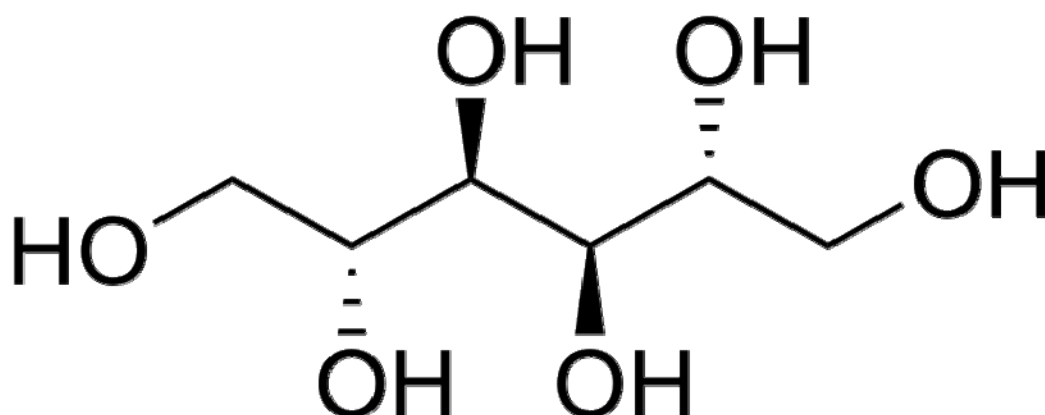


Figure 1.7: Chemical Structure of Mannitol

1.5.2. Oleuropein:

Oleuropein (figure (1.8)) is a natural product of secoiriodoid group; hetrosidic ester of elenolic diteracid and 3,4-dihydroxyphenel ethanol, containing a molecule of glucose, the hydrolysis of which yields elenolic acid glucoside and hydroxytyrosol . Many molecules isolated from *Olea europea* fruits or leaves are thought to have been originated from Oleuropein via aglycon, by opening of olenolic acid ring with a final rearrangement into the secoiriodoid compound, such as hydroxytyrosol (Syed *et al.*, 2010).

The amount of oleuropein in olive leaves depends on several factors, including olea europea variety, time collection, possible infestation by olive fly *Dacus Olea*, climate, conditions of storage, and the methodology of extraction. Oleuropein content varies from 17%-23% depending the time of year the leaves are harvest.

When oleuropein and β -glucoside make contact, oleuropein is broken down and converted into other compounds (see figure (1.9)), that have high protein – denaturing, protein crosslinking and lysine –alkylating activities.

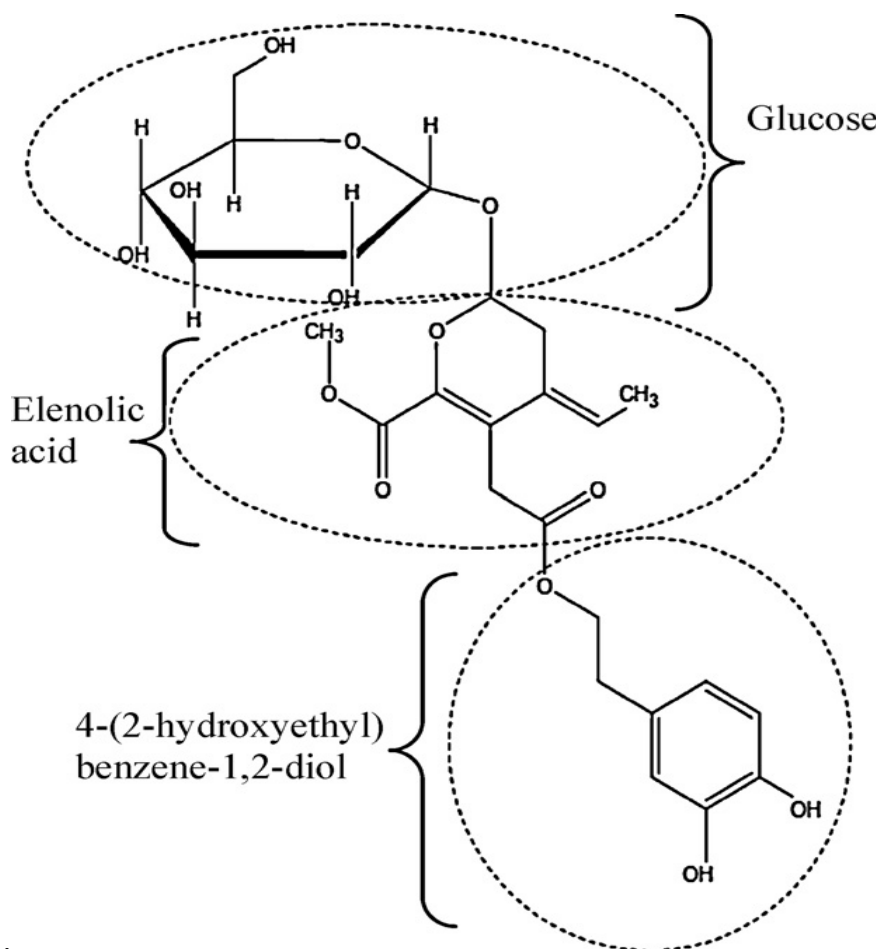


Figure 1.8: chemical structure of Oleuropein

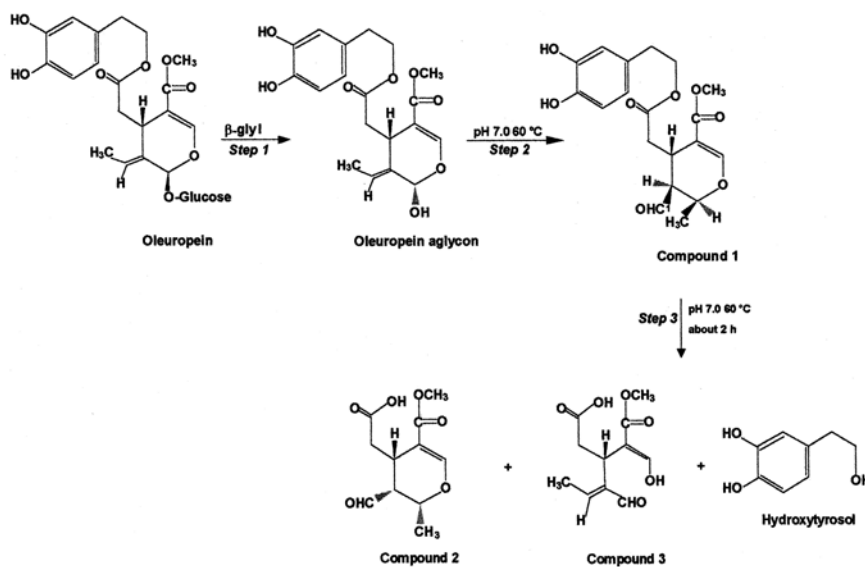


Figure 1.9: The main reaction products obtainable from Oleuropein hydrolysis by hyperthermophilic β -glycosidase at pH 7.0 at 60°C. Rearrangement product of oleuropein aglycon (compound 1), two forms of elenolic acid (compounds 2 and 3) (Syed *et al.*, 2010).

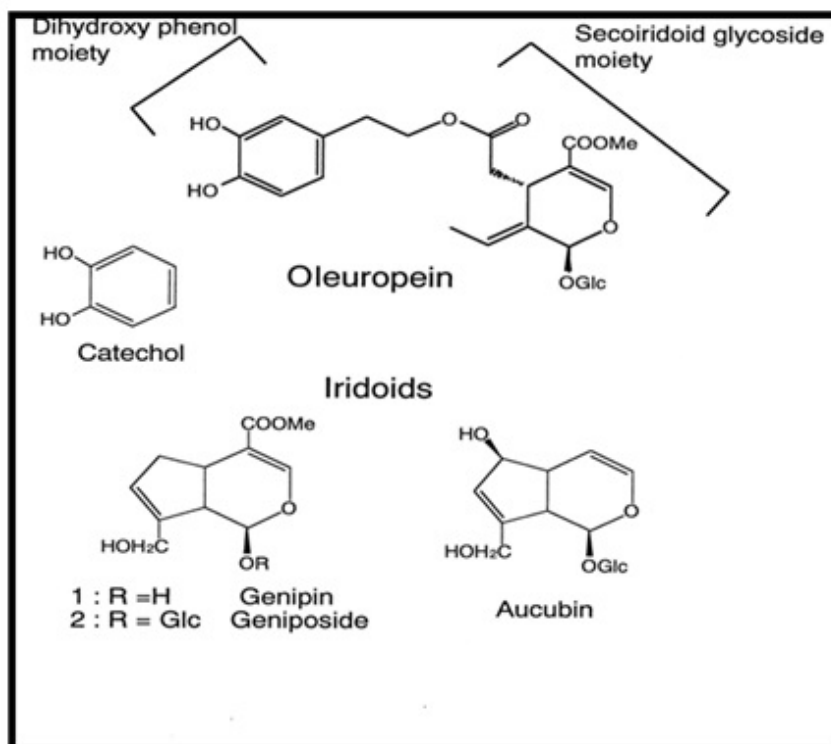


Figure 1.10: Structures of oleuropein and its related compounds. (Kotaro *et al.*, 1999)

1.5.2.1. Pharmacological properties of oleuropein:

Oleuropein has several pharmacological properties, including antioxidant, anti-inflammatory, anti-atherogenic, anti-cancer, antimicrobial, and antiviral, and for these reasons, it is commercially available as food supplement in Mediterranean countries. In addition, Oleuropein has been shown to be cardioprotective against acute adriamycin cardiotoxicity and has been shown to exhibit anti-ischemic and hypolipidemic activities (Syed *et al.*, 2010).

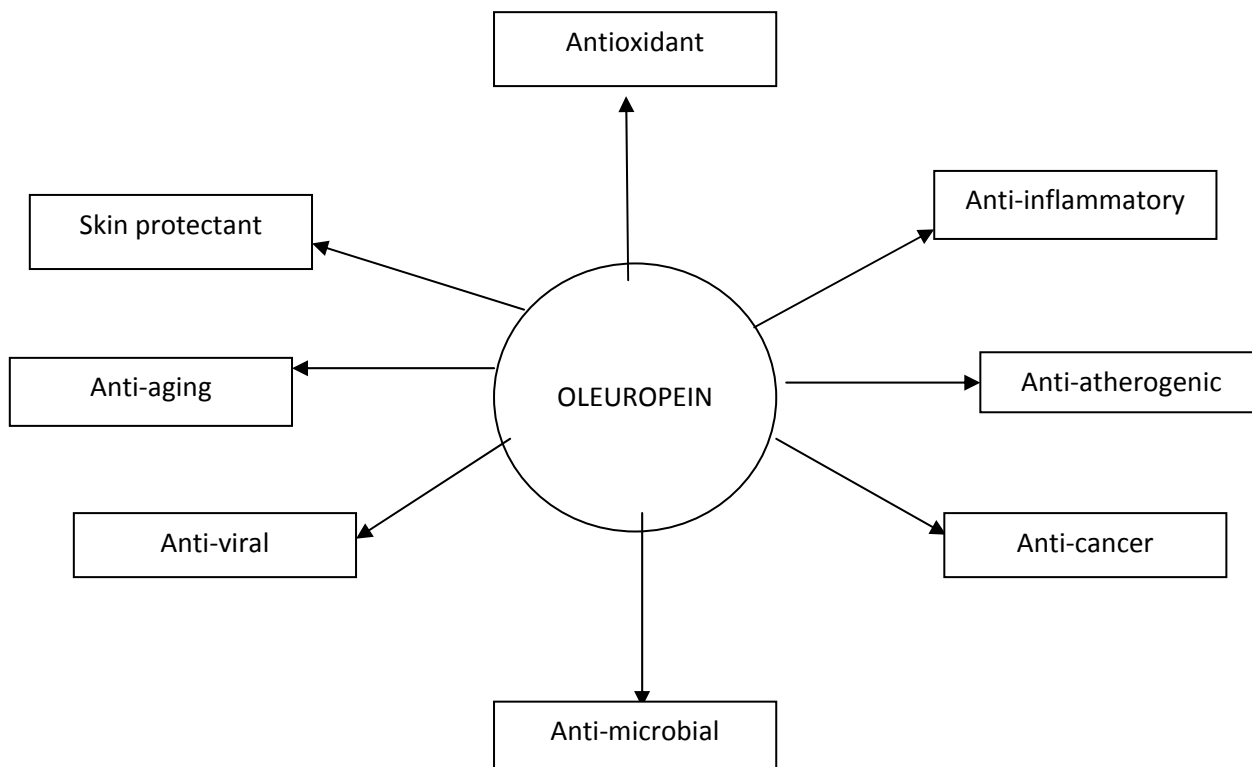


Figure 1.11: Pharmacological effects of oleuropein

1.5.2.1.1. Antioxidant activity:

Oleuropein has high antioxidant activity in vitro, comparable to a hydrosoluble analog of Tocopherol. Oleuropein scavenges superoxide anions and hydroxyl radicals, and inhibits the respiratory burst of neutrophils and hydrochlorous acid-derived radicals (Visioli *et al.*, 2002).

1.5.2.1.2. Anti-inflammatory effect:

Recent studies showed that Oleuropein increases nitric oxide (NO) production in macrophages challenged with lipopolysaccharide through induction of the inducible form of the enzyme nitric oxide synthase, thus increasing the functional activity of these immunocompetent cells. It is well known that Oleuropein elicits anti-inflammatory effects by inhibiting lipoxygenase activity and the production of leukotriene B4 (Visioli *et al.*, 1998).

1.5.2.1.3. Anti-atherogenic effect:

Oleuropein reduces monocytoid cell adhesion to stimulated endothelium as well as vascular cell adhesion mRNA and protein. Reflow in ischemic hearts was accompanied by a prompt release of oxidized glutathione; in ischemic hearts pretreated with Oleuropein, this release was significantly reduced and was accompanied by prevention of membrane lipid peroxidation, which is considered a key factor in the pathogenesis of atherosclerosis (Carlucci *et al.*, 2003).

1.5.2.1.4. Antimicrobial effect:

Oleuropein has been shown to have strong antimicrobial activity against both Gram-negative and Gram-positive bacteria as well as mycoplasma. Phenolic structures similar to Oleuropein seem to produce its antibacterial effect by damaging the bacterial membrane and/or disrupting cell peptidoglycans. The exact mechanism of the antimicrobial activity of

Oleuropein is still not completely established, although some authors have proposed that it is due to the presence of the ortho-diphenolic system (catechol) (Owen *et al.*, 2000).

1.5.2.1.5. Skin protectant:

Recent studies have shown that the phenol components of olive leave have a direct anti-oxidant action on skin, especially Oleuropein, which acts as a free radical scavenger at the skin level (Ancora *et al.*, 2004).

1.5.2.1.6. Anti-aging:

Normal human fibroblasts undergo replicative senescence due to both genetic and environmental factors. The proteasome, a multicatalytic nonlysosomal protease, has impaired function during aging, while its increased expression delays senescence in human fibroblasts. Oleuropein enhances proteasome activities in vitro more effectively than other known chemical activators, possibly through conformational changes of the proteasome. Moreover, continuous treatment of early passage human embryonic fibroblasts with Oleuropein decreases the intracellular levels of reactive oxygen species (ROS), reduces the amount of oxidized proteins through increased proteasome-mediated degradation rates and retains proteasome function during replicative senescence. Importantly, Oleuropein-treated cultures exhibit a delay in the appearance of senescence morphology, and their life span is extended by approximately 15 % (Katsik *iet al.*, 2007).

1.5.2.1.7. Other activities:

Further pharmacological activities of Oleuropein includes diverse healing properties due to its vasodilatory, anti-platelet aggregation , hypertensive, anti-rheumatic, diuretic and anti-pyretic effects. Prevention of free radical formation by Oleuropein occurs through its ability to chelate metal ions, such as Cu and Fe, which catalyze free radical generation reactions, and through its inhibitory effect on several inflammatory enzymes like lipoxygenases. Previously, Oleuropein was reported to have an anti-hyperglycemic effect in diabetic rats. Oleuropein inhibits hyperglycemia and oxidative stress induced by diabetes, which suggests that administration of Oleuropein is helpful in the prevention of diabetic complications associated with oxidative stress (Syed *et al.*, 2010).



Figure 1.12: Olive leaves extract (oleuropein)

1.5.2.2. Olive leaf extracts (oleuropein) and cosmetics application:

Table 1.1: Oleuropein in personal care and cosmetics application:

Trade name	INCI.name	Company	Function	Recommended use
Inoveol Oleu.	Aqua and Oleurophenyl Glucoside	Induchem AG.	Hydro boosted version of Oleuropein	
Olive Extract H.GL.	Glycerine, water, OleaEuropea (olive) leaf extract	Provital group.	Antiseptic, antioxidant, anti-irretant	Up to 10% of creams and lotions
Olive leaf extract 20-40% Oleuropein	olea europaea leaf extract	Phyto Nutra-ceutical Inc.	Promote Skin regeneration, also from UV ultra-violet rays, keeping skin soft flexible and effective.	0.1-1.0 %
Herbalia®Olive 50% oleuropein	Olea europaea leaf extract, <i>Maltodextrin</i> , <i>Silca</i> .	Cognis part of Basf	Anti-inflammatory, anti-microbial, antioxidants.	0.1-1.0%

1.6 Uses of the olive leave extracts:

The enormous interest in these substances in recent years as additives or natural food or natural ingredients has gone beyond food preparation and conservation, into the new area, known as nutraceuticals. This science studies the therapeutic effects of the components of foods and cosmetics (Hassel *et al.*, 1998). At present, several natural extracts among them that of the olive leaf, are used in the manufacture of functional foods and cosmetics (Micol *et al.*, 2003). Until now, the use of the olive leaf has been made principally through formulations which include an extract of the same, without any previous separation of the active compounds. There are numerous preparations of this type on the market; they are used as complements to the treatment of certain diseases or as nutritional supplements. New patents appear daily for functional foods or cosmetics based on olive leaf extracts. The patents are quite varied in their formulation; for example, there are liquid preparations with alcohol and with glycerin (Oliveda Network SL, 2005). Equally diverse are the applications to which these formulations are destined: manufacture of dietetic bread for diabetics (Scheneder *et al.*, 1985); preparations which contain olive leaf extract for use in food or cosmetics (Coll *et al.*, 1999); use of olive leaf extracts in dietetic cookies (Shtukatur, 2003); the manufacture of a nutritional supplement with physiological effects containing olive leaf extract (Stueckler, 1998); an extract of olive leaves to combat free radicals, and their use in dietetic foods and in cosmetics (Amari, 1998).

1.7 Objectives of the study:

The main objective of this work is to extract olive leaf rich with Oleuropein from Palestinian olive leaf (*Olea Europaea*. L), and to use the extract in different cosmetic preparations (moisturizing day cream, shampoo, and anti-aging cream).

This goal will be achieved by the following objectives:

- 1- Extraction of oleuropein from Palestinian olive leaves obtained from Palestinian.
- 2- Comparing the efficiency of simple green extraction with Soxhlet extraction for extraction of oleuropein.
- 3- Studying the factors affecting Oleuropein during extraction process (types of solvents, the concentrations of the solvents, PH of the solvents, temperature and drying of the collected sample).
- 4- Apply the crude olive leaf extract into different cosmetic preparations (moisturizing day cream, anti aging cream and shampoo)

1.8 Hypotheses and research questions:

1. What is the best extraction method of oleuropein from Palestinian Olive leaf, simple green extraction or Soxhlet extraction?
2. Is it possible to obtain a large amount of Oleuropein from Palestinian Olive leaves by using simple extraction methods?
3. What is the effect of the pH of water on the extraction Of Oleuropein from Palestinian olive leaves?
4. What are the effects of the type of the solvents on the extraction of Oleuropein from the olive leaves?
5. What are the effects of drying on the extraction of Oleuropein from the olive leaves?

Is Oleuropein stable in cosmetic preparations which are applied with olive leaf extracts?

Chapter Two: Literature Review

Scientific literature does not contain any reports that deal with Olive leaf extracts, oleuropein and the application of cosmetics from Palestinian olive tree. Therefore, a detailed study of the Palestinian olive leaf constituents will be a valuable addition to the available literature. In fact abundant literature with olive leaf extract came out of the Europe and Asia region.

This part reviews literature on the following subjects: sampling, olive leaf extract, oleuropein, extraction methods of oleuropein from olive leaf, instrumental method of analysis and factors affecting oleuropein.

2.1 Introduction:

The olive tree is amongst the oldest known cultivated trees in the world. It is uncertain the exact origin of the olive tree, the genetic and archaeological studies indicated that the original centers of olive cultivation were Palestine, Lebanon, Syria, Cyprus and Crete. The olive tree was firstly wide spread on the Greek islands and the mainland of Greece, Italy, and then probably introduced into Spain by the Greeks, Romans and Arabs (Kiritsakis, 1998). The Romans invented the press used to take the oil from olive fruits. The olive tree was cultivated in Southern Europe and this is why it is called *Olea europea*. The expansion of olive tree has continued from Mediterranean countries to California, South Africa, Australia, Japan, China, Indian and other countries. The earliest record of olive oil production in California was in 1803 and the first trees were planted in Sydney, Australia around 1805. Today, the olive tree is cultivated all over the world (Kiritsakis, 1998).

The olive (*Olea europea* L.) is the most important crop in Palestine agriculture in terms of area covered as well as economic returns. It covers about (100000) hectares distributed all over the West Bank and Gaza strip and it is distinguished as the major tree in the rain fed area, covering about 45% of the total cultivated area in the West Bank and contributes to about 40% of total fruit production (PCBS, 2005).

2.2 Extraction of oleuropein from olive leaves.

2.2.1. Sampling preparation:

Fresh olive leaf generally needs drying and milling before extraction. As a preservation method, drying is carried out to remove the water from the leaves to protect the leaves against spoilage and degradation of oleuropein by enzyme action. Milling the dried leaves can reduce particle size and facilitate solvents entering into the cells of the leaves. It also improves extraction efficiency or extractability. Many different drying approaches have been explored by researchers, but air drying, microwave drying and freezing drying have been mostly reported in the literatures. Air drying can be carried out at room temperature or elevated temperatures for different time periods (Sayadi, 2005; Malik *et al.*, 2008). Generally longer times are required for lower temperatures to achieve the same extent of dryness of leaves. Carefully temperature control is needed during the drying since it may cause degradation of Oleuropein. Malek (2008) reported that air drying at 25°C or elevated temperature such as 30 °C and 40 °C would result in substantial losses of polyphenols possibly due to degradation of oleuropein and other polyphenols. Galyb *et al.*, (2012) dried the fresh leaves in ventilated oven for 72 hours at 40°C while Ansari *et al.*, (2010) dried the fresh olive leaves under ambient temperature. Freezing drying is an alternative way to ef-

fectively avoid thermal degradation while removing the water from leaves. The leaves are immediately frozen in liquid nitrogen, and lyophilized before extraction (Briante *et al.*, 2002). The elimination of water through lophilization generally does not affect the phenolic compounds excessively, and allows samples to be kept for longer periods (Galyb *et al.*, 2012). The temperature of -80 °C is commonly used to store biological samples; it is also used for storage of plant tissues for the purpose of keeping bioactive compounds in the plant. However it was not recommended as a sample preparation method for olive leaves extraction, thawing frozen olive leaf samples caused a sharp reduction in oleuropein levels. That is possible due to breakage of cell membrane during thawing and consequently release of active oleuropein-degrading enzymes. Furthermore, small discrepancies in sample handling would easily cause inconsistent results when frozen and stored at -80 °C. (Malik *et al.*, 2008).

Microwave drying was reported to avoid ester hydrolysis of saponins which occurs during air drying, and higher concentrations of oleuropein have been reported after using this drying method (Sayadi *et al.*, 2005). Infrared drying was recently suggested as a good method for preserving olive leaves because it allows the retention of the green colour of fresh leaves. Infrared drying resulted in considerable moisture removal from the fresh leaves (more than 85% by weight) during a short drying period (varying from about 162 min at 42° C to 5 min at 70° C). The total phenolic content of infrared dried olive leaves was greater compared to fresh ones (Boudhrioua *et al.*, 2009).

There is little literature reporting extraction of phenolic from fresh olive leaves, and it was reported that increased levels of oleuropein were found in dried leaves than in fresh leaves probably due to the conversion of oleuropein glucoside into oleuropein by β -glucosidase present in fresh leaves (Silva *et al.*, 2006).

2.2.2. Methods of extraction:

Traditionally, there are a number of methods to make herbal remedies such as infusions, tinctures and decoctions, and many substances have been used as a base for extracts. Infusion is the simplest way to prepare leaves and flowers for use as a medicine. By simply pouring boiling water into a handful of dried herbs and infuse for a certain length of time, water based extracts are obtained. Tinctures are made by soaking dried or fresh herbs in alcohol for 10-14 days, and then removing the herbs using a wine. (Chevallier, 2000).

Solvent extraction was the main method adapted by most researchers to extract phenolics from olive leaves. This is a process designed to separate soluble compounds by diffusion from a solid matrix using a liquid matrix. This process takes place by two steps, which are adsorption of solvent into the solid phase by osmotic forces, by capillary and by salvation of the ions in the cells, then followed by diffusion from the solid phase (Escribano *et al.*, 2003). The aim of extraction is to concentrate antioxidant components of raw materials; the extraction process involves a more or less vigorous agitation of the ground raw materials with extraction solvent at ambient or elevated temperatures and subsequent separation of the residue by filtration. Repeated extraction steps may be accomplished to increase the extract yield. Alternatively, a packed bed of the ground material can be used which is leached by the extraction solvent under refluxing conditions (Oreopoulou, 2003).

Besides conventional solvent extraction, other methods such as ultrasound-assisted, microwaves-assisted and supercritical fluid extraction can offer a good yield and preserve the properties of antioxidants. These methods can be used for the extraction of polyphenol

from plant tissue. Ultrasound assisted extraction is faster and more complete in comparison with traditional methods (Escribano *et al.*, 2003). The use of ultrasound can increase extraction yield and shorten extraction time, therefore it was suggested by Albi *et al.*, (2004).

Microwave assisted extraction is a new technique that combines microwave with traditional solvent extraction. Some studies shows that it has many advantages over conventional extraction methods include higher extraction rate, simple and cheap. It has been used for extraction phenolic compounds from tea leaves and grape seeds (Escribano *et al.*, 2003).

In terms of olive leaf extraction, microwave and ultrasound assistance during extraction were proposed by Japon *et al.*, (2006) to accelerate the extraction and reduce the extraction time. Complete extraction of targeted analyses can be achieved in 8 minutes and 25 minutes when using microwave and ultrasound assistance, respectively. The high recovery and low maintenance costs make it potential for industrial implementation. Le Floch *et al.*, (1998) used carbon dioxide modified with 10% methanol as a supercritical fluid to isolate phenolics from olive leaves, however, extraction did not achieve satisfactory yield. Too many variables such as modifier, temperature, and pressure and collection system resulted difficulty in controlling extraction (Le Floch *et al.*, 1998). A static-dynamic superheated extraction approach for olive leaves was developed by Japon *et al.*, (2006) in order to obtain more concentration extracts. This methods involved high temperature and pressure, which may cause thermal degradation of phenolic compounds (Japon *et al.*, 2006). A recent study provided a relatively simple and rapid method, the olive leaf powder was refluxed with 80% ethanol at 80°C for 3 hours, and the extraction was repeated three times (Lee *et al.*, 2009). Another recent research used fresh olive leaves and ethanol and the extraction took place at room temperature for two weeks (Poudyal *et al.*, 2010). Simple green and inexpensive water based procedure was developed by Ansari *et al.*, (2010) to extract oleuropein from olive leaves. Dried olive leaves were macerated in different solvents (water, ethanol, and methanol) with different PHs at various times of extraction (Ansari *et al.*, 2010). Another recent study used dried powdered of olive leaves and methanol and the extraction took place at room temperature for two hours (Galyb *et al.*, 2012). After extraction, suspension was filtered using filter paper or glass wool or centrifuged. The solvent was removed by various methods such as rotary evaporators, vacuum evaporators and freezer drier, and then a dry or a concentrated extract was obtained (Sayadi *et al.*, 2005), (Lee *et al.*, 2009), (Ansari *et al.*, 2010), (Galyb *et al.*, 2012). There are number of important parameters which affect extraction yield of polyphenols. They are the type of solvent, concentration of solvent, extraction temperature and the time, the ratio of liquid –to-solid and pH of the solvent.

2.2.3. The effect of the extraction solvent:

The extraction yield is strongly influenced by the solvent, due to the different polarity of compounds extracted. Therefore, organic solvent of higher polarity are more effective in quantitative recovery of phenolic compounds than non-polar solvent and methanol was reported in many studies as a good solvent for extraction of phenolics from the plants including olive leaves. However, it may lead to unacceptable levels of toxic residues in the final extracts; ethanol and water are the most widely employed solvents for safety and abundances reasons (Moure *et al.*, 2001; Oreopoulou, 2003). Altiok *et al.*, 2008 revealed that ethanol alone was not effective as a solvent for extraction of phenolic compounds from olive leaves, and water has important role in extraction process by increasing the diffusion

of extractable polyphenols through the plant tissues (Altiok *et al.*, 2008). Changes in ethanol concentration modify the physical properties of the solvent such as density, dynamic viscosity, and dielectric constant. Solubility of compounds would also be modified by changes in the ethanol concentration, and this may influence the extraction of phenolics (Cacac *et al.*, 2003). Mylonaki *et al.* (2008) used 40%, 50% and 60% ethanol (v/v) in the study to investigate the effect of ethanol concentration on total phenolics yields. They discovered that ethanol concentration in the medium had seemingly biphasic effect, as an intermediate ethanol level (50%) appeared to provide the lowest yield. In the contrast, the trends observed when decreasing or increasing the ethanol concentration indicated that polyphenol extraction was favored in both cases. Since many of olive leaf phenolics are glycosides, it can be assumed that their solubility may be higher at 40% ethanol (v/v) than that at 50% ethanol (v/v). On the other hand, oleuropein, which is significantly less polar, would be more soluble in 60% ethanol (v/v) than in 50% ethanol (v/v). This may be the reason for such biphasic tendency in response to ethanol changes (Mylonaki *et al.*, 2008).

Ethanol, methanol, ethyl acetate, boiling water, hexane, diethyl ether, chloroform and butanol were the main solvents used by researchers for olive leaf. Of these solvents, aqueous methanol or ethanol was most commonly used and concentration of solvent varied between 40% and 80% (v/v). Extraction with 80% methanol (v/v) was reported as the most effective method for olive leaf polyphenols (Malik *et al.*, 2008). Boiling of dried leaves was also a very efficient method for extraction oleuropein resulting in 96% recovery of the compound (Malik *et al.*, 2008). Japon *et al.*, 2006 suggested 80% ethanol (v/v) was the optimum solvent for extraction of oleuropein from olive leaf and it can be used as replacement for toxic solvents (methanol, diethyl ether, chloroform) to obtain bioactive phenols for human use (Japon *et al.*, 2006). Lee *et al.*, (2009) also reported total flavonoid and phenolic contents were significantly higher in the 80% ethanol (v/v), butanol, and ethyl acetate extracts than hexane, chloroform and water extracts (Lee *et al.*, 2009). Anssari *et al.*, (2010) reported that higher oleuropein levels was observed with the solvent containing deionized water at 60°C with PH 3 this because oleuropein is water soluble which solubility can be increased by elevating the temperature.

2.2.4. The effect of extraction temperature, time and solvent to solid ratio:

The extraction is a function of how fast the compound is dissolved and the equilibrium is achieved by liquid solvents (Pinelo *et al.*, 2006). The temperature has impact on solubility, diffusion coefficient (mass transfer rate) and stability of oleuropein. An increase in temperature and a decrease of viscosity significantly increase the diffusion rate. However, high temperature may degrade the oleuropein. The driving force for the extraction is the concentration gradient within the particles, which is related to solvent:solid ratio. The rate of extraction increases with a larger concentration gradient (Cacace *et al.*, 2003).

A range of extraction temperatures and time were employed by researchers in the extraction of phenolics compounds from olive leaves. Generally less extraction time is required with the increasing temperature. When ethanol was used as a solvent, the extraction process took place either at room temperature or elevated temperatures of 40°C for 24 -48 hours under agitation (Malik *et al.*, 2005; Japon *et al.*, 2006). A water bath was used to achieve required temperatures. With boiling water, 10 to 30 minutes extraction time was used by researchers (Malik *et al.*, 2008). This kind of extraction is purely a static process and easy to operate. The solvent to solid ratio is normally expressed as the ratio of the volume of solvent (milliliter) to the weight of extraction sample (gram). The solvent to solid ratio employed by the researchers for olive leaf extraction varied hugely from 4 to 100, but

a ratio between 10 and 50 was mostly reported in the literatures (Briante *et al.*, 2002; Sayadi *et al.*, 2005; Malik *et al.*, 2008; Ansari *et al.*, 2010; Galyb *et al.*, 2012).

2.2.5. The effect of the extraction pH:

The pH of extraction medium determines the degree of solubility for soluble compounds and also influences the possible solubilization of the hydrolysable fractions. The influence of the pH of medium on the olive leaf extracts was examined by Japon *et al.*, 2006, and diluted aqueous solutions of hydrochloric acid and sodium hydroxide were used to achieved extraction pH 2 and pH 12, respectively. By comparison of the extracts obtained at pH 2 and pH 12 with pH 8 showed that the amount of olive bioactive phenolics extracted at pH 12 decreased by 35% for oleuropein and this due to the ester bond hydrolysis. However, the concentration of the target analytes at pH 2 was similar to those in the extract at pH 8 (Japon *et al.*, 2006). Another study also indicated increased pH values were unfavorable for extraction of phenolic compounds from olive leaves (Mylonaki *et al.*, 2008). Ansari *et al.*, (2010) reported that higher yields of oleuropein were observed at an optimum pH of 3 that may be related to lower degradation of oleuropein at this pH, while the use of higher or lower pHs, caused a significant decrease in the yields of oleuropein (Ansari *et al.*, 2010).

2.3 High-Performance Liquid Chromatography analysis (HPLC) of quantitative determination of oleuropein in olive leaf:

High performance liquid chromatography (HPLC) is one of the most powerful separation techniques, and it has been extensively used in the food and drug industry for the analysis of components of both raw and processed products. The separation sample into individual components takes place when samples pass through a column carried by a flow of mobile phase. The separated compounds are eluted and detected by a detector, and presented as a set of chromatographic peaks in a chromatogram (Lunn, 2002).

A representative HPLC instrument consists of a mobile phase reservoir, high pressure, pump, an injection device, a separation column, a detector, and a data system. The separation column and detector are the heart of the HPLC system. The properties of the stationary phase and mobile phase as polarity and size of particle determine the mechanism of separation (Lunn, 2002). The sample is separated on the basis of solubility and polarity of the sample components. For normal phase HPLC the solvent (mobile phase) is non-polar and the column packing (stationary phase) is polar. Sample molecules are more or less attracted to the particles in the column as opposed to the solvent; the less polar molecules eluted first. Revised – phase HPLC (RP HPLC) uses polar solvent and non-polar packing. In RP HPLC, the elution order is reversed (Nollet, 2000).

Isocratic elution and gradient elution are two common methods used HPLC, in isocratic elution, mobile phase composition is held constant during the whole run, whereas, a gradient elution, mobile phase composition is held varies with test time. The most commonly used detector is a UV detector, which is based on the UV absorbance of eluting compounds. In diode array detectors (DAD) also known as photodiode array detectors, a series of given wavelengths can be simultaneously used (Lunn, 2002).

Before HPLC analysis, sample preparation normally extraction is often necessary to isolate the components of interest from a sample matrix. A pre-treatment may also be required,

and which includes purification or fractionation step for removal of interfering compounds or an enrichment procedure in case of trace components. The sample pre-treatment can be achieved by liquid-liquid extraction and solid phase extraction on cartridges as mentioned previously (Nollet, 2000).

2.3.1. Methods for separation of phenolic compounds in olive leaf extract:

For separation of phenolic compounds in olive leaf extract, the reversed-phase chromatography on silica-based C18 bonded-phase columns is the most reported. It was suggested by Bouaziz *et al.*, 2004 that C-18 column is more suitable for the resolution of the range of phenols and the C-8 column provided adequate separation of flavonoids. (Malik *et al.*, 2004).

Aqueous acetic, formic, trifluoroacetic acid (TFA). Or phosphoric acids with methanol or acetonitrile as an organic modifier are the solvents commonly used for the mobile phase. TFA (0.05%), 0.01% of phosphoric and formic acid, 0.2-5% of acetic acid were the acids adopted by most researchers. The pH and ionic strength of the mobile phase plays a crucial role in determining retention of phenolics on the column. The pH range most often used for RP-HPLC for phenolics is low, between 2 and 4. Small amounts of acetic acid or phosphoric acid are included in the solvent system and can improve the resolution and reproducibility of analysis (Benavente *et al.*, 2000); Savournin *et al.*, 2001); Hayes *et al.*, 2011).

The detectors used for HPLC analysis of olive leaf extract in previous studies were mainly photodiode array detector (PDA), absorbance at 280 nm, 340 nm, 350 nm were monitored for a variety of phenolic compounds (Japon *et al.*, 2006). UV-VIS detector and mass spectrometer were also used by many researchers (Laguerre *et al.*, 2009). Gradient elution was adopted by most researchers for separation of phenolics compounds and external standard methods were mostly used for quantification of those compounds in the olive leaf extracts (Benavente *et al.*,2000); Savournin *et al.*,2001); Hayes *et al.*, 2011). The internal standard method was also used by some researchers to determine phenolics compounds in olive leaf extract. Savournin *et al* (2002) used coumarin as an internal standard to determine oleuropein concentration. It was also recommended by Savournin *et al.*, (2001) that the quantification of oleuropein using internal standard is of particular importance because commercially available oleuropein standards are not of HPLC grade (Savournin *et al.*, 2001).

Saleh *et al.*, 2008 used an easy and rapid method using capillary electrophoresis coupled with electrospray ionization time of-flight mass spectrometry (CE-ESI-TOF-MS). Ansari *et al.*, 2010 used RP-HPLC system for analysis of Oleuropein. C18 Column, mobile phase: water (adjusted to pH 3):acetonitrile (80:20 V/V), flow rate: 1 ml/min and wavelength: 280 nm. Ghalib *et al.*, 2012 used HPLC-DAD system for analysis of oleuropein. chromatographic separation was achieved with LC system form Agilent (Infinity 1260) coupled with a Diode array (DAD). C18 Column, mobile Phase: water:acetonitrile: formic acid (84.6:15:0.4), flow rate: 1 ml/min, and wavelength: 240 nm.

Chapter Three: Materials & Methods

3.1 Sample collection:

Olive leaves samples were obtained from trees type Nabali Baladi localized in Beit- sahour West Bank /Palestine. The collection was directly from the trees from an old branch exactly closer to the stem in the middle of November 2012. In addition to these samples which were collected fresh (green) from the tree, another sample of olive leaves were obtained from a branch of tree that is dry (brown olive leaves) i.e. this sample is obtained dry from the tree.

3.2 Sample preparation:

Fresh green leaves were dried using two procedures. In the first procedure, fresh leaves were dried in ventilated oven at 50°C. In the second method, fresh leaves were dried at ambient temperature. Then the dried samples were grinded to obtain powder which was stored at room temperature in dark until extraction. In the same way, dry (brown) olive leaves which is already dry were grinded to obtain powder and were stored at room temperature in dark until extraction. Green olive leaves were chopped to obtain small pieces and stored at temperature between (2-4°C) until extraction.

3.3 Chemicals:

Oleuropein (40%) which used as a standard of oleuropein was obtained from Chengdu Biopurity Phytochemicals Ltd China. Chromatographic grade-double distilled water, HPLC grade acetonitrile (Merck), analytical grade acetic acid, ethanol, methanol and ethyl acetate were obtained from Sigma and Aldrich.

3.4 Materials used in moisturizing day cream formula:

Glyceryl mono stearate was purchased from Faci Italy. Stearyl alcohol, propylene glycol, mineral oil, isopropyl myristate, cetiol CC, propyl paraben, methyl paraben, imidazolidinyl urea and perfume were purchased from Basf, Germany.

3.5 Methodology:

3.5.1. Extraction procedures:

3.5.1.1. Simple green (maceration) extraction method:

10 grams of olive leaves powder were macerated in 100 ml solvent for 4 hours. The solvent mixture used for the extraction were: Deionized water at various pHs (3, 5, 7, 9) (adjusted with Hydrochloric acid (0.1N) solution for acidic solution and sodium hydroxide (0.1N) for basic solutions) at 60°C, 40°C and at ambient temperature. The extracts were then filtered through a Whatman No.1 filter (Whatman, UK) to separate coarse particles from the solutions. The filtered extracts were then evaporated in rotary evaporator at room temperature under vacuum. The concentrated extracts were stored in a refrigerator at (2-4°C) until used.

3.5.1.2 Simple extraction (maceration) method with organic solvents:

10 grams of olive leaves powder were macerated in 100 mL of different solvents for 4 hours. The solvents used for the extraction were: methanol (100%), 80% methanol at ambient temperature, ethanol (100%), 80% ethanol, 50% ethanol and 20% acetonitrile at ambient temperature. The extracts were then filtered through a Whatman No.1 filter (Whatman, UK) to separate coarse particles from the solutions. The filtered extracts were then evaporated in rotary evaporator at room temperature under vacuum. The concentrated extracts were stored in a refrigerator at (2-4°C) until used.

3.5.1.3. Soxhlet extraction:

15 grams of olive leaves sample were placed in the thimble in a classical Soxhlet apparatus and extracted with 300 ml of different solvents for 4 hours. The solvents used for the extraction are: 80 % ethanol, 20% acetonitrile at 60 °C. Extract were cooled to room temperature. Then the extracts were filtered through a Whatman No.1 filter (Whatman, UK) to separate coarse particles from the solutions. The filtered extracts were then evaporated in rotary evaporator at room temperature under vacuum. The concentrated extracts were stored in a refrigerator at 2-8°C until used.

3.6 Determination of oleuropein in olive leaf extracts by HPLC:

3.6.1. HPLC conditions:

For determination of oleuropein from olive leaf extract, reversed phase HPLC method was used with silica-based C₁₈ bonded phase column (C₁₈, 250mm × 4.6 ID) with mobile phase consisting of a mixture of water and acetonitrile (80/20 volume ratio) containing 1% acetic acid at a flow rate of 1.0 mL/min. UV detector at 240 nm was used for oleuropein determination. The injection volume used is 20.0 µl for both standard and sample solutions. Identification of oleuropein in olive leaves extracts was based on retention times in comparison with standard of oleuropein. The quantitation was carried out using external standard method. The concentration of oleuropein was calculated using peak area and the calibration curves obtained from oleuropein standard solution. The amount of oleuropein was expressed as milligram per gram of olive leaf powder.

3.6.2. Statistical analysis

Three samples of olive leaves extract of each treatment were independently analyzed in each sampling, and all of the determinations were carried out in triplicate by using HPLC for analyzing the Oleuropein and SPSS for analysis the questionnaires. The results are expressed as means ± standard deviations for oleuropein contents.

3.7 Materials used in formulas:

3.7.1. Moisturizing day cream with Palestinian olive leaf extract rich with oleuropein:

phase	ingredients	Quantity	supplier	functions
A	Glyceryl mono stearate	4.5%	BASF	(O/W) emulsifier.
	Stearyl alcohol	1.5%	BASF	emollient
	Steareth- 12	0.80%	BASF	(o/w)emulsifier
	Mineral Oil	5.0%		Emollient, softening & soothing agent
	Isopropyl myristate	4.0%	BASF	Emollient
	Cetiol CC	1.0%	BASF	Emollient
	Silicon Oil	1.0%		Deforming agent
	Propyl paraben	0.1%	ISP	Preservative
B	Di -water	Add to 100		solvent
	Carbomere	0.10%		thickener
C	Propylene glycol	3.0%	ISP	Moisturizer
	Methyl paraben	0.2%	ISP	Preservative
	Imidazolidinyl urea	0.2%	BASF	Preservative
D	Olive leaf extract	0.1%, 0.4% and 1.0%	Palestine	Antioxidant, protectant
	perfume	Qs		To give odor
E	TEA	Qs	BASF	To adjust the pH

3.7.1.1. Procedure:

1. Dissolve the ingredients in PART A. Heat PART A to 75 °C.
2. In a separate vessel, combine ingredients in PART C and PART B then heat to 70°C.
3. Add PART A to PART (B+C) and mix until homogenous.
4. Cool the mixture to 35 - 40°C.
5. Add ingredients in PART D in the order shown. Mix until homogenous.
6. Add part E to adjust the pH between (6.5-7.0)

3.7.2. Anti- aging cream with Palestinian olive leaf extract rich with oleuropein:

phase	ingredients	Quantity	supplier	functions
A	Emulium Delta (INCI: Cety alcohol (and) Glyceryl stearate (and) PEG-75 stearate (and) ceteth-20 (and) Steareth-20).	6.0%	BASF	(O/W) emulsifier.
	Stearyl alcohol	2.0%	BASf	Lubricants
	Almond oil	1.0%		Emollient, softening & soothing agent
	Tocopheryl acetate	0.1%	BASF	Antioxidant
	Propyl paraben	0.1%	ISP	Preservative
B	Di -water	Add to 100		solvent
C	Propylene glycol	3.0%	ISP	Moisturizer
	Methyl paraben	0.2%	ISP	Preservative
	Imidazolidinyl urea	0.2%	BASF	Preservative
D	Olive leaf extract	0.1%	Palestine	Antioxidant, anti aging
	Vitamin A palmitate	0.5%	BASF	Anti aging
	perfume	Qs		To give odor
E	TEA	Qs	BASF	To adjust the PH

3.7.2.1. Procedure:

1. Dissolve the ingredients in PART A. Heat PART A to 75 °C.
2. In a separate vessel, combine ingredients in PART C and PART B then heat to 70°C.
3. Add PART A to PART (B+C) and mix until homogenous.
4. Cool the mixture to 35 - 40°C.
5. Add ingredients in PART D in the order shown. Mix until homogenous.
6. Add part E to adjust the pH (5.5-6.5).

3.7.3. Shampoo with Palestinian Olive leaf extract rich with Oleuropein:

phase	ingredients	Quantity	Suppliers	functions
A	Cosmedia Guar C261 N O- $\{2\text{-Hydroxy-3(trimethylammino) propyl}\}$ Guar Gum Chloride.	0.8%	Basf	Conditioning agent
	Propylene glycol	1.0%	ISP	Moisturizer
B	Texapon N50 Mixture of special fatty alcohol ether sulphates (Sodium Laureth Sulfate (and) Sodium Laureth 8-Sulfate (and) Magnesium Laureth Sulfate (and) Magnesium Laureth 8-Sulfate (and) Sodium Oleth Sulfate (and) Magnesium Oleth Sulfate	13%	Basf	Surfactant
	Cocamidopropyl betain	5%	Basf	Detergent
	Cocamide DEA	8%	Basf	Foam booster
C	Bronopol	0.05%	Basf	Preservative
	Perfume	Qs		To give odor
	Olive leaf Extract	0.1%	Palestine	Moisturizing Agent/ Penetrates the scalp to rebuild damaged hair and promote healthy hair
D	Citric acid	Qs	Basf	To adjust the pH
E	Di water	Add to 100		Solvent

3.7.3.1 Procedure:

1. Mix phase A until homogenous
2. Mix phase B until homogenous
3. Add B to A
4. Add Phase E with Stirring gently
5. Add phase C with Stirring
6. Add phase D to adjust the pH (5.5-6.5).

3.8 Questionnaires:

The questionnaire consisted of two parts; personal data and product data. Personal data started from question number one to question number three. Product data started from question number four to question number six. All questions in personal data part are about gender, age and type of skin. While all product questions are about frequencies of the applications of the day cream, the sensorial evaluation of the cream (texture, color, odor, consistency, absorbance, after fed, feeding and shininess), improvement in the skin upon use, allergic reaction to the cream, skin nourishment, and satisfaction with the cream.

3.8.1. Questionnaire (1) for the moisturizing Cream:

This questionnaire is used to see how much the consumers are satisfied with our product, and to take their observations to develop our product.

Sex: Male: Female:

Age: 18-30 years
31-45 years
46 + years

1. Type of skin: Normal Oily Dry other

If other please explain.....

2. How often do you reapply the cream?

Once a day twice a day more occasionally

If more explain please:.....

3. Sensorial evaluation:

(Where 1 is the lowest and 10 is the highest).

- a. Texture: (1-10).....
- b. Color: (1-10).....
- c. Odor: (1-10).....
- d. Consistency (1-10).....
- e. Absorbance (1-10).....
- f. After fed (Graceness) (1-10).....
- g. Feeding (1-10).....
- h. Shininess (1-10).....

4. Please answer the following questions? (regards the product use after 3 weeks):

a. Have you noticed a difference on your skin feel?

Yes No

b. Have you seen an improvement upon use:

Yes No

c. Have you experienced an allergic reaction to Moisturizing day cream?

Yes No

d. Have you noticed your skin nourishment:

Yes No

If yes please explain:

Moisturized skin Smooth skin

e. Are you satisfied with our Moisturizing day cream:

Yes No

Please give a percentage (1-100 %).

Notes.....
.....
.....
.....

Thank you

Chapter Four: Results & Discussion

4.1 Effect of drying temperature on oleuropein content of olive leaves:

The results showed that (table 4.1) the highest amount of Oleuropein is obtained from olive leaves dried at ambient temperature (10.0 mg Oleuropein per gram of dried leaf powder). Simple drying of fresh leaves at ambient temperature preserved Oleuropein levels while drying at elevated temperature of 50°C result in the rapid degradation of Oleuropein (oleuropein content is 1.7 mg/g). Oleuropein content in natural dried olive leaves which is dried naturally by the sun and air was 2.5 mg/g which is lower than olive leaves dried at ambient temperature but higher than those dried at 50°C. Browning of leaves occurs when oleuropein is oxidized to highly reactive quinones which then polymerized (browning is an interactions between diphenol oxidase activity and oleuropein content). Oleuropein content of fresh green olive leaves was very low <0.1mg/g, because the surface area was too low to facilitate the penetration of solvents into cells, so that oleuropein stayed protected in leaves cell. Scientific literature does not contain any reports that deal with green olive leaves and its effect on the extraction of oleuropein (Altiok *et al.*, 2008).

These results showed that drying at ambient temperature is the most suitable method of drying olive leaves. This result is in accordance of the results of Malik *et al.*, 2008.

Figures (4.2) and (4.3) show a chromatogram of oleuropein separated by the Current high performance liquid chromatography (HPLC) method. Figure (4.2) shows chromatogram of oleuropein detected in olive leaf dried at ambient temperature, while figure (4.3) shows chromatogram of oleuropein detected in olive leaf dried at 50 °C.

Table 4.1: Oleuropein content of the olive leaf dried at ambient temperature, at 50 °C, natural dried olive leaves and fresh olive leaves.

Conditions	Oleuropein Content (mg/g)	Percentage
Drying at ambient temperature	10.0 ± 0.26	1.0%
Drying at 50°C	1.7 ± 0.13	0.17%
Natural(brown) dried olive leaves (sun and air)	2.5 ± 0.21	0.25%
Fresh green olive leaves	0.0	0.0%

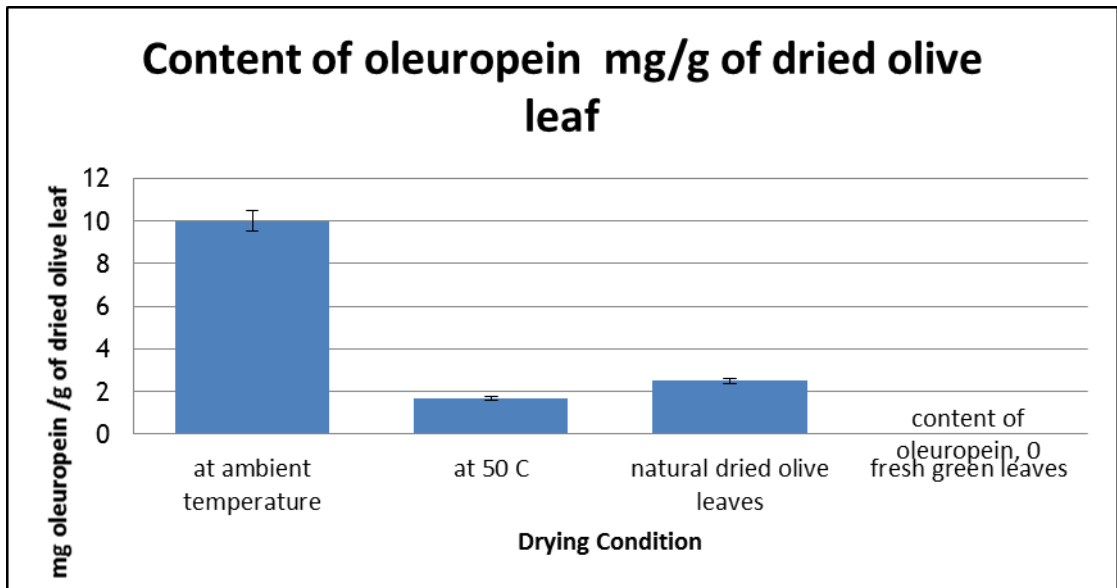


Figure 4.1: Effect of drying temperature on Oleuropein content of olive leaves.

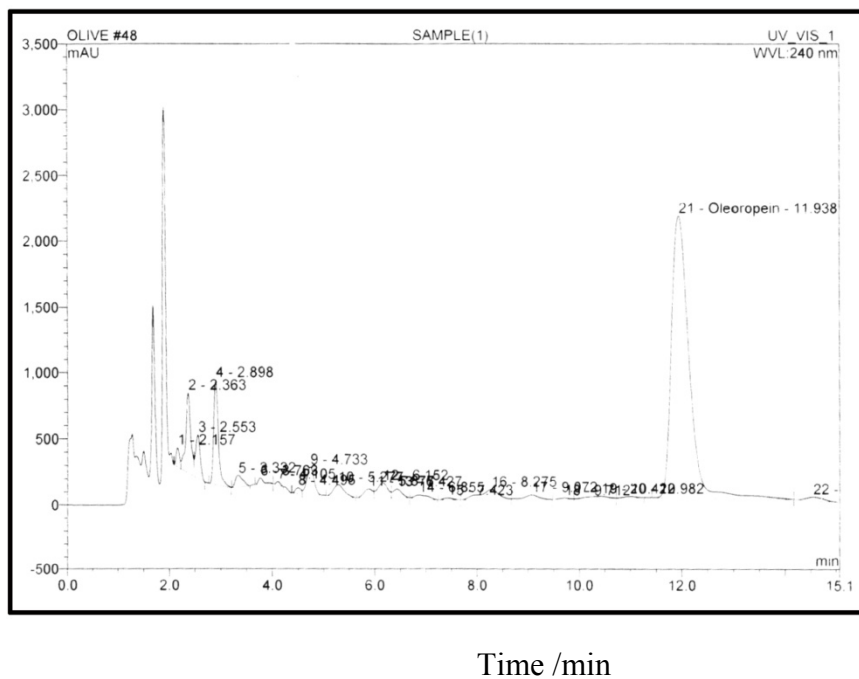


Figure 4.2: Chromatogram of Oleuropein detected in olive leaf dried at ambient temperature.

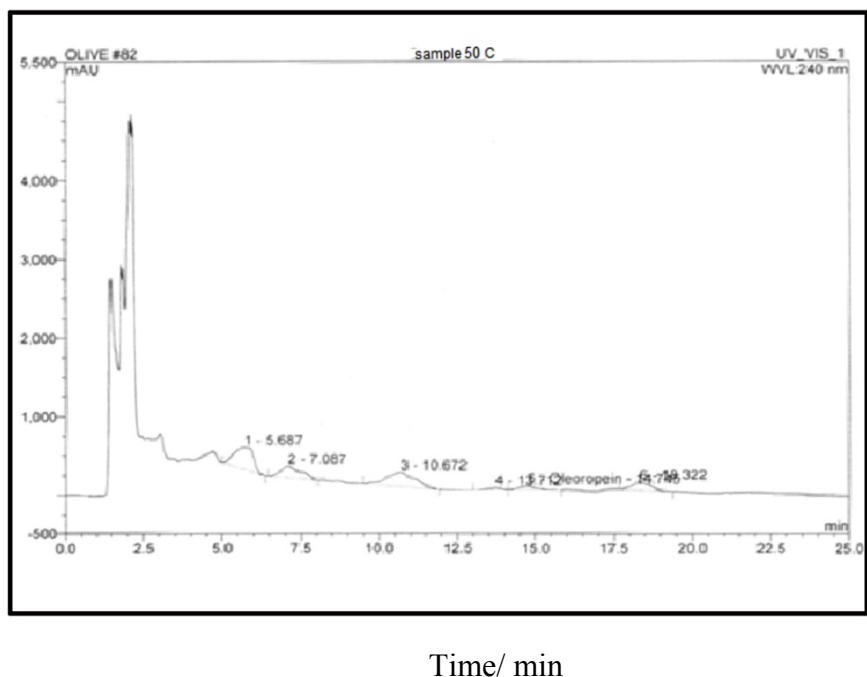


Figure 4.3: Chromatogram of Oleuropein detected in olive leaf dried at 50 °C.

4.2 Effect of extraction solvent on oleuropein content:

Results (table 4.2) showed that the highest oleuropein content was obtained when the olive leaves are extracted with 80% ethanol (13 mg/g) followed by 20% acetonitrile (10.0 mg/g) and the 80% methanol (5.31 mg/g), and the 50% ethanol (2.57 mg/g) and then water (0.16 mg/g). Regards pure methanol and ethanol, the amount of oleuropein extracted using these solvent is low (0.10, and 0.02 mg/g for methanol and ethanol respectively). As we can see from these results, mixture of an organic solvent and water is needed to effecting extract oleuropein from olive leaves as very low amounts of oleuropein was extracted using pure water, methanol, and ethanol, while higher concentration extracted when mix of ethanol/water, methanol/water and acetonitrile/water used as extraction solvents (table 4.2). This can be explained by the effect of solvent which increase the solubility of oleuropein. For example when ethanol increased from 50% to 80%, oleuropein concentration increased from 2.51 to 13 (5 fold increase). Acetonitrile (20%) has a good compact to extract oleuropein from olive leaves (10 mg/g), this can be explained by the fact that Acetonitrile is an organic solvent and has good polarity which increase the solubility of oleuropein. Using water as co-solvent with lipophilic solvents will help to increase the amount of oleuropein in the extraction process. The solvent mixture is the best also because solvent mixture deactivated enzymes during maceration. When oleuropein and β -glucosidase make contact, oleuropein is broken down and converted into other compounds that have high protein-denaturing, protein- cross linking and tysine-alkylating activities (Fransisca *et al.*, 2008)

Table 4.2: Oleuropein content in olive leaves by using different extraction solvents:

Extraction solvents	Oleuropein content (mg/g of olive leaf extract dried powder)	Percentage
Deionized water	0.16 ± 0.01e	0.016%
80% Methanol	5.31 ± 0.16c	0.500%
100% Methanol	0.10 ± 0.01f	0.010%
100% Ethanol	0.02 ± 0.01g	0.020%
80% Ethanol	13.0 ± 0.51a	0.130%
50% Ethanol	2.57 ± 0.24d	0.260%
20% Acetonitrile	10.0 ± 0.69 b	1.000%

Figures (4.5), (4.6), (4.7) and (4.8) show a chromatogram of oleuropein separated by the current high performance liquid chromatography (HPLC) method. Figure (4.5) shows chromatogram of oleuropein detected in olive leaf extracted with 80% Methanol, figure (4.6) shows chromatogram of oleuropein detected in olive leaf extracted with 50% ethanol/Water, Figure (4.7) shows chromatogram of oleuropein detected in olive leaf extracted with 80% ethanol and figure (4.8) shows chromatogram of oleuropein detected in olive leaf extracted with 20% acetonitrile.

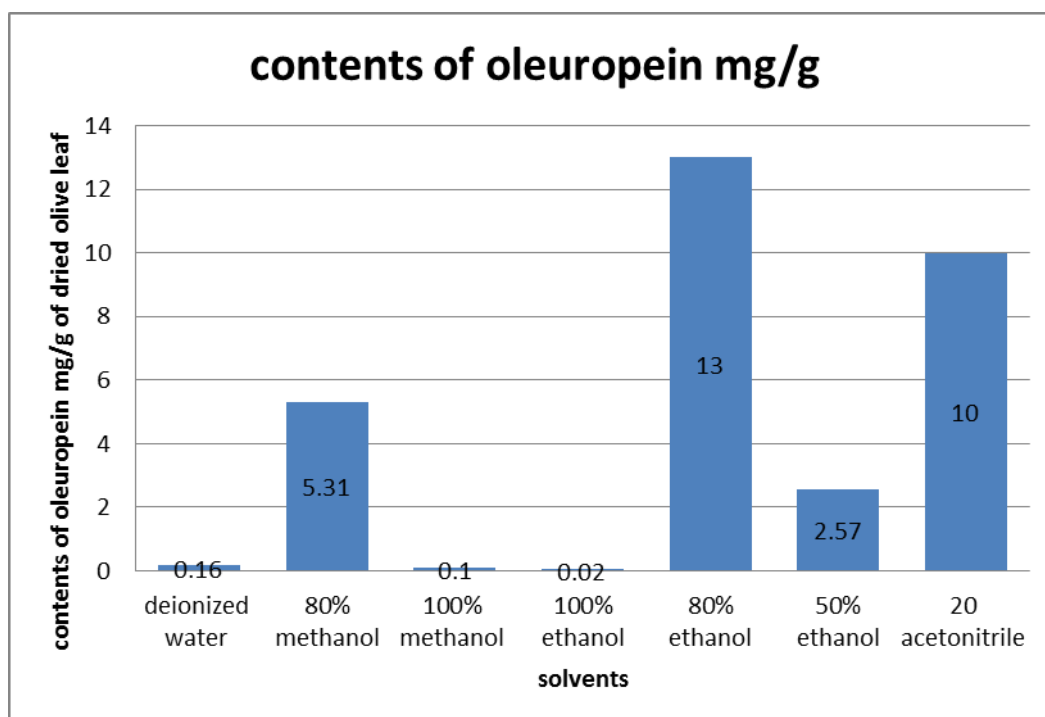
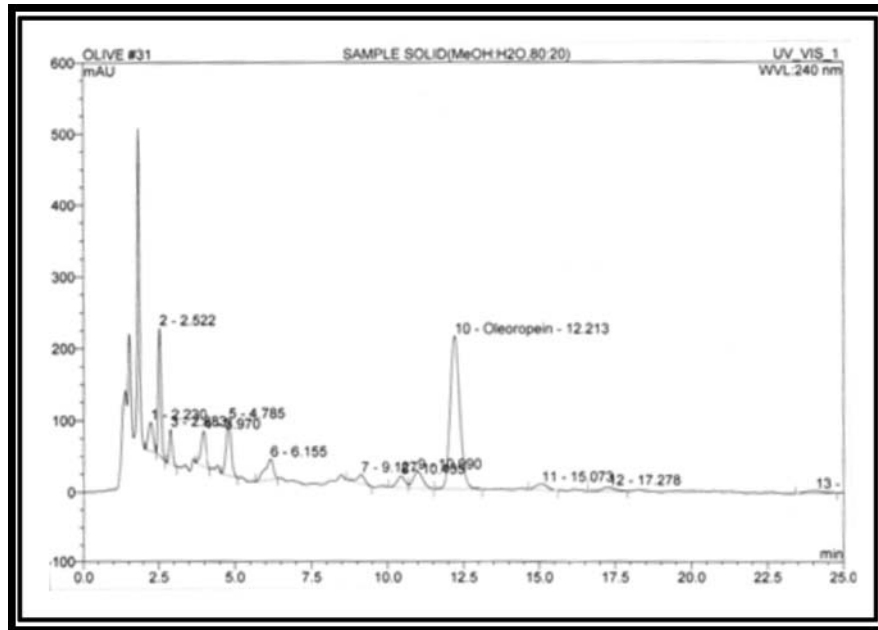
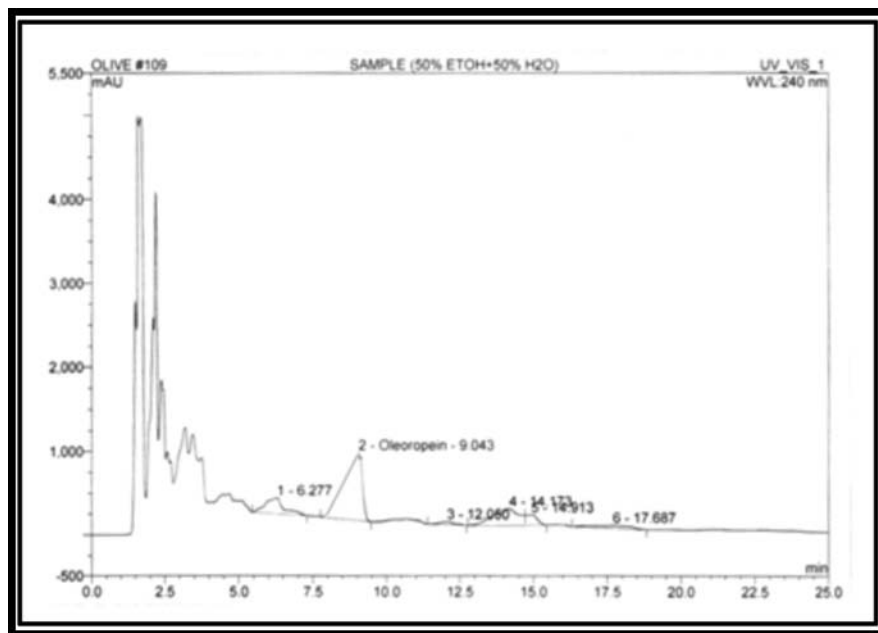


Figure 4.4: Effect of extracting solvent on extraction yield of oleuropein from olive leaves.



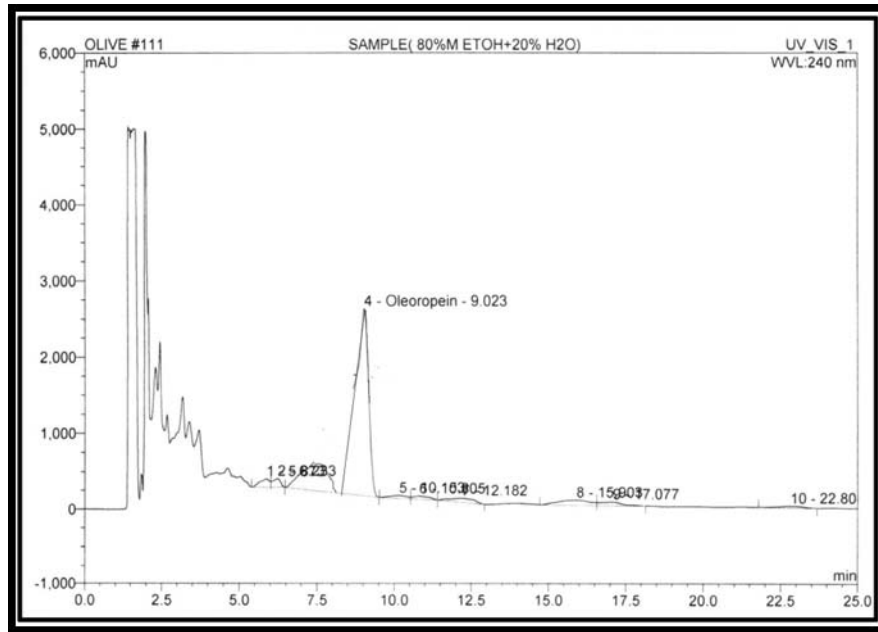
Time/min

Figure 4.5: Chromatogram of oleuropein detected in olive leaf extracted with methanol/water (80:20 v/v).



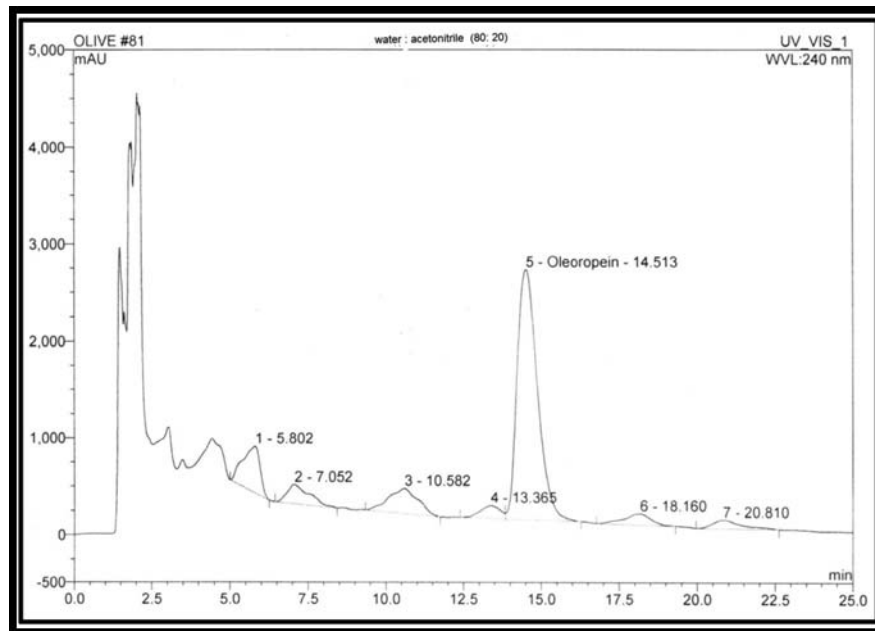
Time/min

Figure 4.6: Chromatogram of oleuropein detected in olive leaf extracted with ethanol/water (50:50 v/v).



Time/min

Figure 4.7: Chromatogram of oleuropein detected in olive leaf extracted with ethanol/ water (80:20v/v).



Time/min

Figure 4.8: Chromatogram of oleuropein detected in olive leaf extracted with water/acetonitrile (80:20 v/v).

4.3 Effect of temperature on oleuropein content:

Table (4.3) show that oleuropein content increase with increasing temperature of extraction solvent where there is 10 fold increase in oleuropein content as temperature increased from 25°C to 40°C and there is 43 fold increase as temperature increased from 25°C to 60°C. This can be attributed to increase in solubility of oleuropein with increasing temperature.

Table 4.3: Oleuropein content in olive leaves extracted at different temperatures:

Temperature (°C)	Oleuropein Content (mg/g)	Percentage
25°C	0.16 ± 0.13c	0.016%
40°C	2.84 ± 0.24b	0.280%
60°C	6.84 ± 0.31a	0.680%

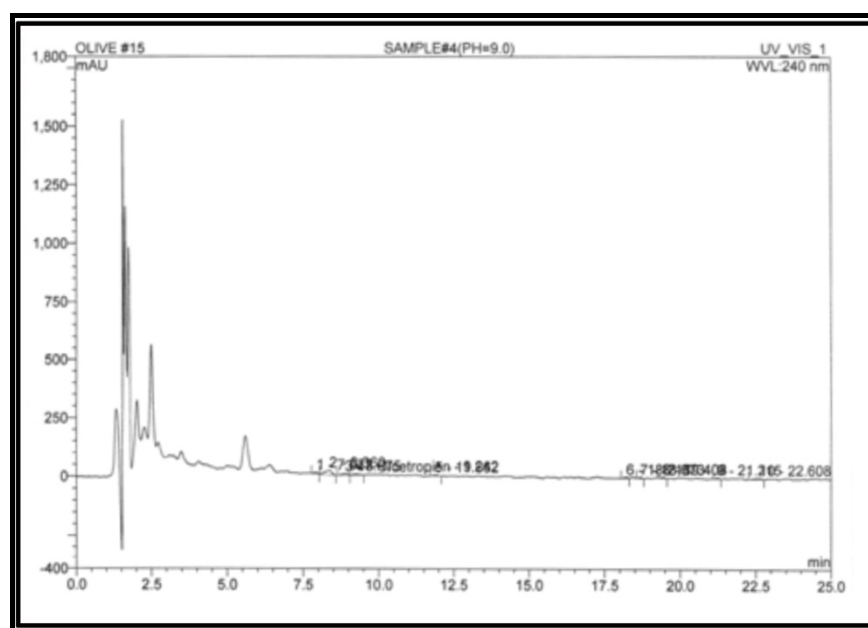
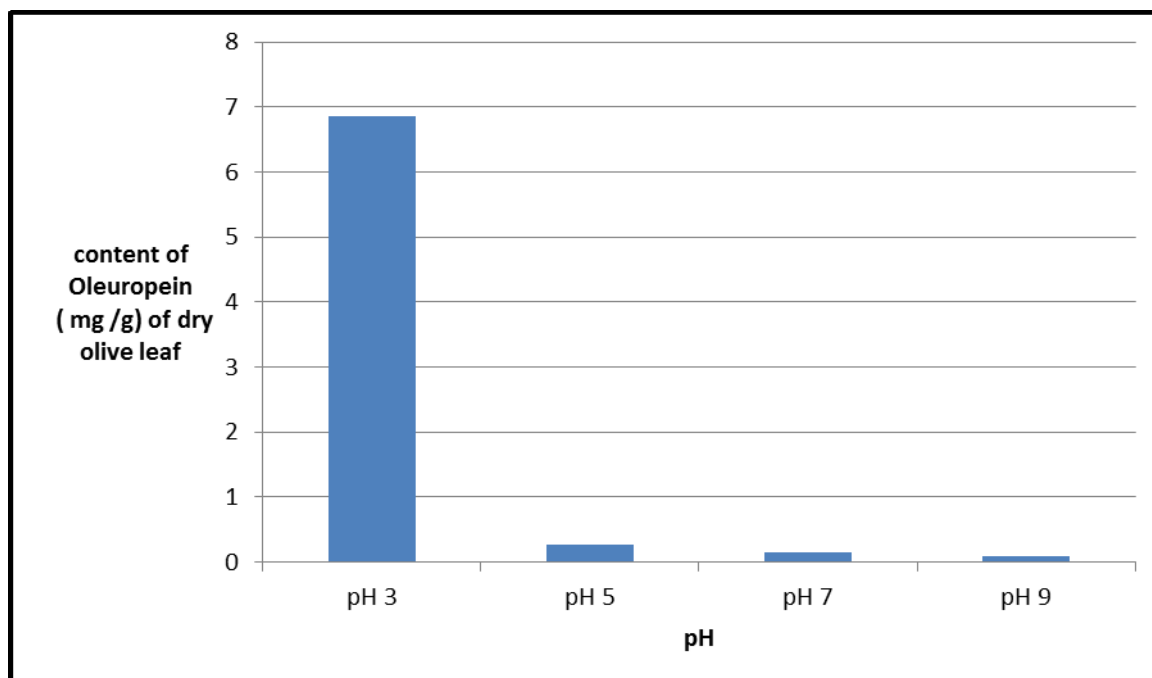
4.4 Effect of pH on the extraction of oleuropein in olive leaf extract:

Results (table 4.4) showed that the highest amount of oleuropein was got when olive leaves are extracted with water at acidic pH (3) (see figure (4.13) peak of oleuropein can be clearly observed) followed by pH (5) (see figure (4.12)), pH (7) (see figure (4.11)) and finally with pH (9) (see figure (4.10)). Then results showed that acidic condition is the best for oleuropein while basic pH is not suitable for oleuropein extraction. It is expected for oleuropein extraction that a basic pH result in degradation of oleuropein while acidic pH is suitable for oleuropein recovery for olive leaves. Statistically there is a significant difference in the amount of oleuropein extraction in water with different pH's (see table 3 when small letter denotes significant changes at confidence of 0.05).

Table 4.4: Oleuropein content in olive leaves by using different pHs:

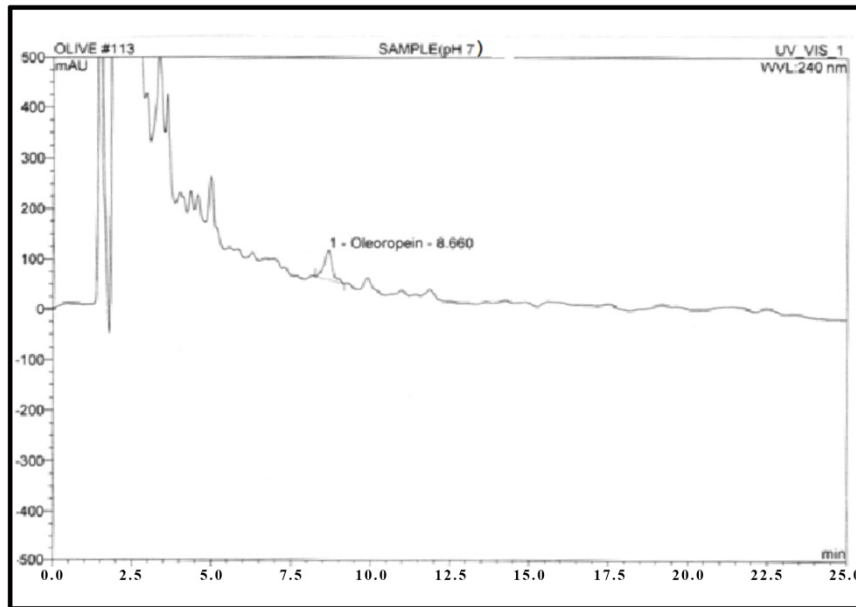
pH of the solvent	Oleuropein content (mg/g)	Percentage
3	6.85 ± 0.17a	0.690%
5	0.26 ± 0.02b	0.026%
7	0.16 ± 0.01c	0.016%
9	0.09 ± 0.01d	0.009%

Figure 4.9: Effect of pH on extraction yield of oleuropein from olive leaves:



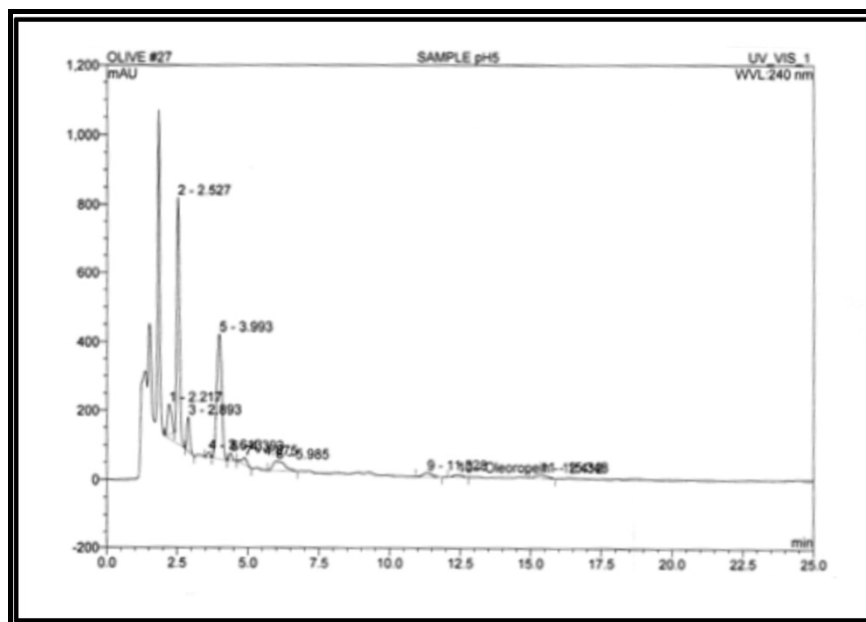
Time/min

Figure 4.10: Chromatogram of oleuropein detected in olive leaf extract with pH 9.



Time/min

Figure 4.11: Chromatogram of oleuropein compound in olive leaf extract with pH 7.



Time/min

Figure 4.12: Chromatogram of oleuropein compound in olive leaf extract with pH 5.

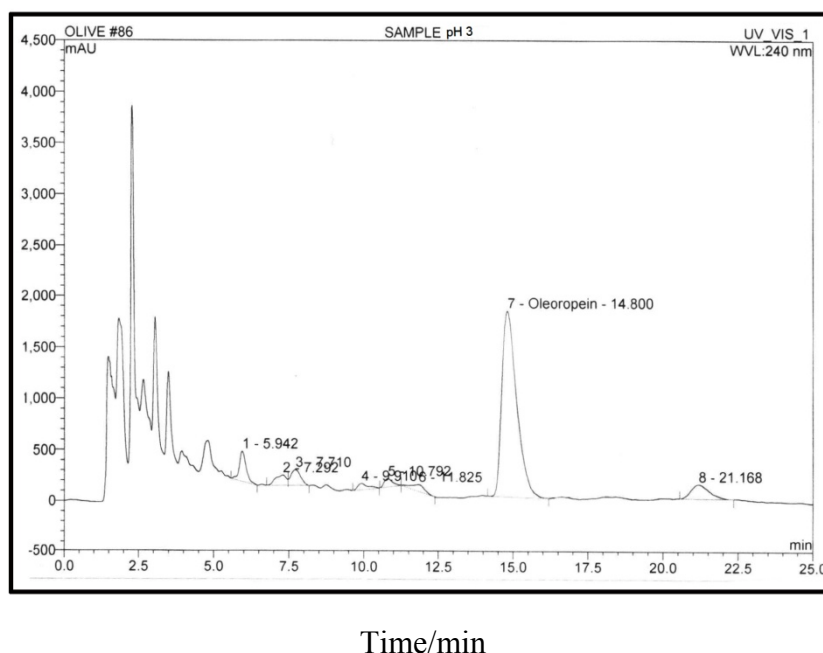


Figure 4.13: Chromatogram of oleuropein compound in olive leaf extract with pH 3.

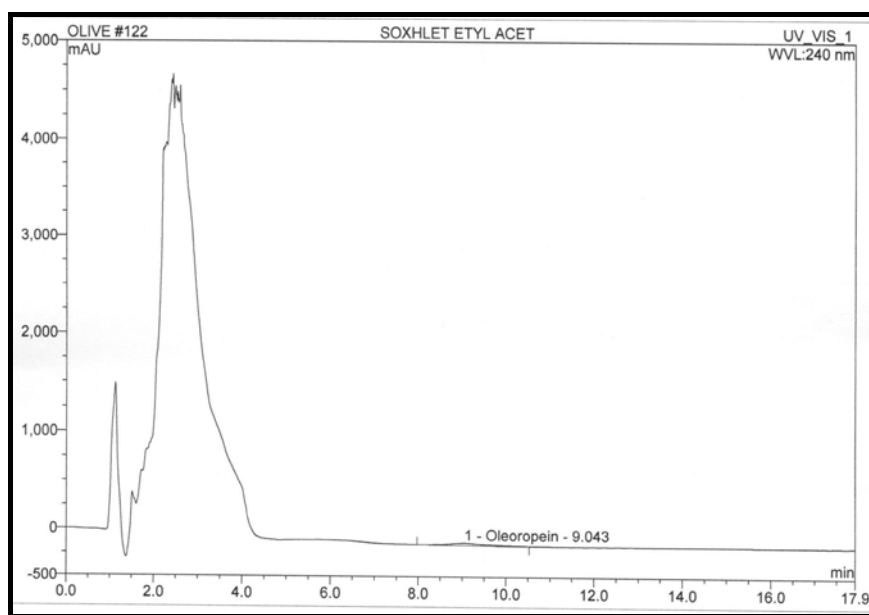
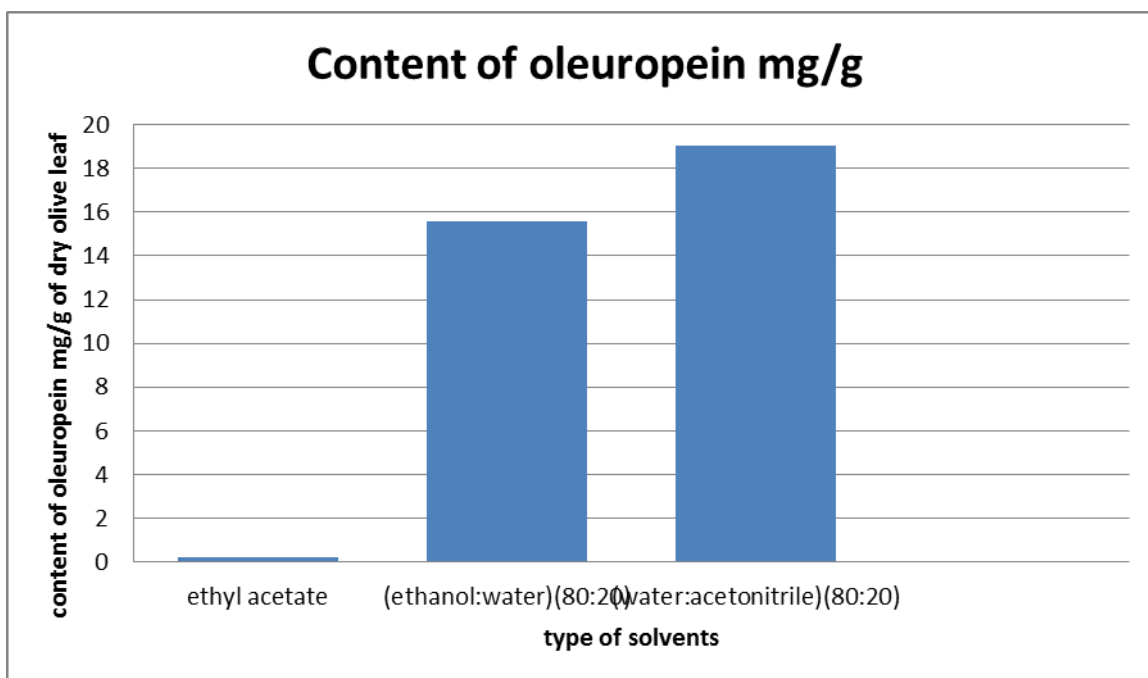
4.5 Effect of type of extraction on the oleuropein content from olive leaves:

Results (table 4.5) showed that maceration methods using two solvents studied. Statistically there is a significant difference between the oleuropein content extracted with the two extraction methods (Soxhlet vs. maceration) i.e. Soxhlet gave a significant higher amounts of oleuropein compared to maceration (see table 4.5). Soxhlet is more effective methods than maceration due to heat energy because it will increase the temperature inside the cell and the wall will rupture thus releasing the oleuropein (Handa *et al.*, 2008). As for maceration methods, 80% ethanol gave higher oleuropein content compared to 20% acetonitrile. Figures (4.15), (4.16) and (4.17) show a chromatograms of oleuropein detected in different extraction solvent by using Soxhlet extraction.

Table 4.5: Oleuropein content in Olive Leaves.

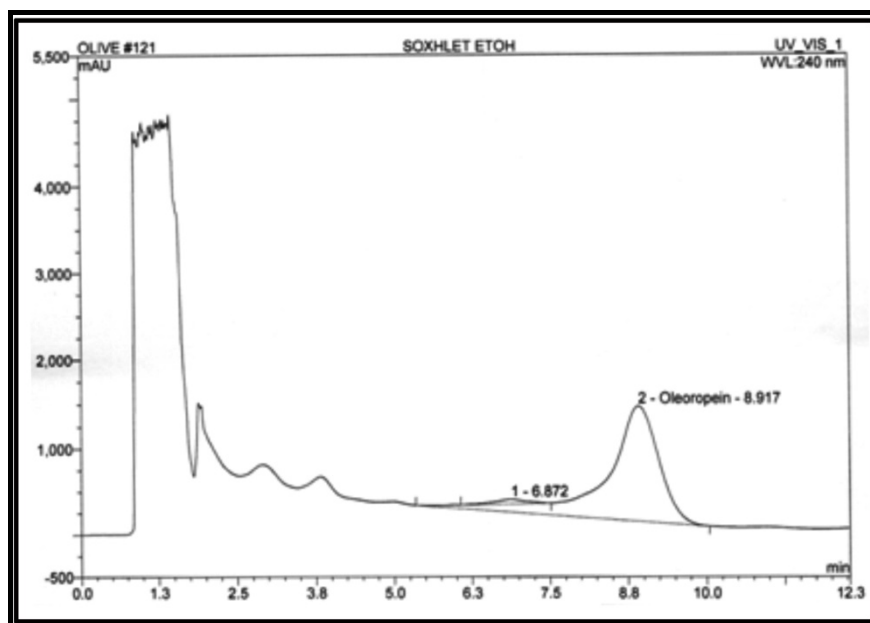
Solvent mixtures	Oleuropein content (mg/g)	
	Soxhlet method	Maceration method
80%ethanol	15.6 ± 0.36a	13.0 ± 0.52b
20%acetonitrile	19.0 ± 0.81a	10.0 ± 0.69b

Figure 4.14: Content of oleuropein mg/g.



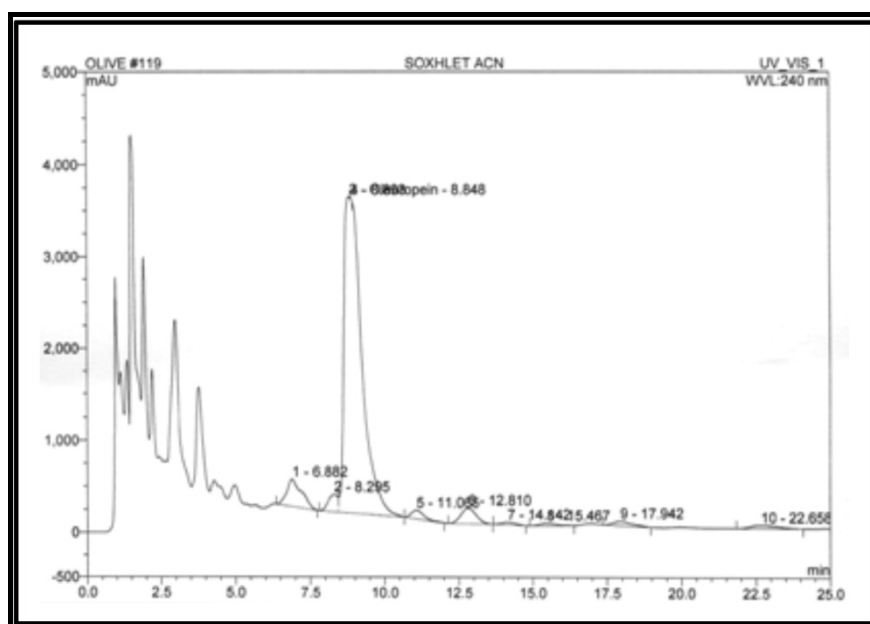
Time/min

Figure 4.15: Chromatogram of oleuropein detected in olive leaf extract by using soxhlet with ethyl acetate.



Time/min

Figure 4.16: Chromatogram of oleuropein detected in olive leaf extract by using soxhlet with (ethanol/Water) (80:20).



Time/min

Figure 4.17: Chromatogram of oleuropein detected in olive leaf extract by using soxhlet with (water: acetonitrile) (80:20).

4.6 Formulation and physical properties evaluation of oleuropein cream:

4.6.1. Formulation of the oleuropein cream:

Base cream containing water and oil phases was prepared. The compositions and the amounts of the formulation ingredients are shown in (3.7.1). For the preparation of the cream, all ingredients were added together according to the formula in table (1), then olive leaf extract was added in different concentrations (0.1%, 0.4%, 1.0% w/w).

4.6.2. Evaluation of physical properties of oleuropein cream formulations:

Physical Characteristics: The prepared formula containing oleuropein extract and the commercial preparations were examined for their physical (pH, color, consistency and homogeneity) as well as rheological properties.

4.6.2.1. Determination of the pH:

Determination of the prepared formula was measured using pH meter (ph meter 211R hanna).

4.6.2.2. Homogeneity:

Homogeneity of various formulas was tested by visual observation and was ranked as follows:

+++ = Excellent, ++ = Very Good, + = Good, and - = Poor.

4.6.2.3. Consistency:

The cone attached to holding rod was dropped from the fix distance to 10 cm such that it should be fall on the center of measuring cylinder travelled by cone was noted down after 10 sec.

4.6.3. Rheological properties:

The prepared formula was evaluated for the following rheological characteristics:

4.6.3.1. Viscosity measurements:

A Brookfield viscometer was used to measure the viscosity (in mPa.s) of oleuropein cream. The spindle was rotated at 2.5 rpm. Samples of cream were allowed to settle over 30 min at the temperature of test ($25 \pm 1^\circ\text{C}$) before the measurement were taken.

4.6.3.2. Spreadability:

Spreadability test was performed by applying the cream on the skin and noticing whether spreading was good or not and was ranked as follows:

+++ = Excellent, ++ = Very Good, + = Good and - = Poor.

4.6.4. Accelerated Stability tests:

Twelve sets of ten gram samples (from formulas 0.1%, 0.4%, 1.0% concentrations) of formulations (0.1%, 0.4% and 1.0% w/w and the commercial one with 0.1% w/w) were stored at different temperatures (ambient temperature, 37°C and 50 °C) for 3 months. After each month their stability was checked regarding oleuropein content, appearance, pH, color, homogeneity and viscosity.

Table 4.6: Composition (amounts of the ingredients) used to make 100 g of oleuropein moisturizing day cream:

phase	Ingredients	Amounts in gram
Oil Phase	Glyceryl mono stearate	4.5
	Stearyl alcohol	1.50
	Stearth- 12	0.80
	Mineral Oil	5.00
	Isopropyl myristate	4.00
	Cetiol CC	1.00
	Silicon Oil	1.00
	Propyl paraben	0.10
Water Phase	Di -water	Add to 100
	Carbomere	0.10
	Propylene glycol	3.00
	Methyl paraben	0.20
	Imidazolidinyl urea	0.20
	Triethanolamine	Qs
	Olive leaf extract	0.10(Cream A), 0.40 (Cream B) and 1.0 (Cream C)
	perfume	Qs

Table 4.7: Physical properties of oleuropein moisturizing day cream and the commercial product:

Parameter	Cream A (0.1%)	Cream B (0.4%)	Cream C (1.0%)	Commercial product (0.1%)
Color	Creamy	Light brown	Brown	Creamy
pH	6.7	6.7	6.7	6.3
Homogeneity	+++	+++	+++	+++
Consistency(60sec)	5mm	5mm	5mm	5mm

Key: Homogeneity: +++ Excellent, ++ Very Good, + Good, - Un-satisfactory

Table 4.8: Rheological Properties:

Parameter	Cream A (0.1%)	Cream B (0.4%)	Cream C (1.0%)	Commercial product (0.4%)
Viscosity (mPa.s)	40.000	39.000	38.000	40.000
Spreadability	+++	+++	+++	+++

Key: Spreadability: +++ Excellent, ++ Very Good, + Good, - Un-satisfactory.

Table 4.9: The effect of the storage time on the physical properties of the oleuropein cream after 3 months at room temperature:

	Commercial product	Freshly prepared one (cream A)	Freshly prepared one (cream B)	Freshly prepared one (cream C)	3months prepared cream A	3 months prepared cream B	3 months prepared cream C
pH	5.9	6.7	6.7	6.7	6.3	6.1	6.2
color	Creamy	Creamy	Light Brown	Brown	Creamy	Light Brown	Brown
Homogeneity	+++	+++	+++	+++	+++	+++	+++
Viscosity mPa.s	40.000	40.000	40.000	40.000	40.000	40.000	40.000

Table 4.10: Oleuropein content determined by HPLC analysis before and after accelerated stability tests:

Parameter	Cream A (0.1%)	Cream B (0.4%)	Cream C (1.0%)	Commercial product (0.4%)
T1 at ambient temperature	92%	94%	95.7%	91%
T1 at 37°C	82.6%	84.7%	86%	80%
T1 at 50 °C	72.5%	74.4%	76%	71.5%
T2 at ambient temperature	80%	82%	84%	80%
T2 at 37°C	23.4%	26.5%	39.2%	30%
T2 at 50 °C	3.1%	16.5%	29.5%	15.7%

T1: first month, T2: second month.

4.7 HPLC analysis of oleuropein in cream samples:

For determination of oleuropein from olive leaf extract, reversed phase HPLC method was used with silica-based C₁₈ bonded phase column (C₁₈, 250mm × 4.6 ID) with mobile phase consisting of a mixture of water and acetonitrile (80/20 volume ratio) containing 1% acetic

acid at a flow rate of 1.0 mL/min. UV detector at 240 nm was used for oleuropein determination. The injection volume used is 20.0 μ l for both standard and sample solutions. Identification of oleuropein in olive leaves extracts was based on retention times in comparison with standard of oleuropein. The quantitation was carried out using external standard method. The concentration of oleuropein was calculated using peak area and the calibration curves obtained from oleuropein standard solution. The amount of oleuropein was expressed as milligram per gram of olive leaf powder.

Creams are semisolid dosage forms intended mainly from external use and commonly consist of two immiscible phases; an oily internal phase and an aqueous external phase. Due to emulsified nature of skin surface, cream dosage form was chosen because cream interacts more effectively with skin and more readily penetrated through biological membranes. Oleuropein was put in the cream dosage form to penetrate through membrane and keep it moist.

As shown in (table (4.7)), physical properties were found to be equivalent to the commercial ones. Consistency test showed no sticky and adhesion to skin and all the products are homogenous when no separation of the phases observed. As shown in (table (4.8)) rheological properties, the Spreadability of the product was excellent and the viscosities were within limit which indicated suitability of creams for applications on the skin. Accelerated stability tests (tables (4.9) and (4.10)) showed that pH of these creams was stable and suitable for skin application (pH cream A 6.3, pH cream B 6.1 and pH cream C 6.2). Viscosity was stable over the study period (40,000 mPa.s for all concentrations). Color was stable where no significant change was observed of the color of creams at different concentrations. Stability studies (table (4.9)) showed a stable homogenous appearance during three months storage period and no separation phases occurred. The Oleuropein contents (table (4.10)) decreased significantly with increasing temperature and period of storage time and this is due to the heat sensitivity of oleuropein. The results in (table (4.10)) show the degradation profile of oleuropein incorporated into cream and provide important data for the establishment of the shelf life of this product. One month stored at temperature 50°C equal 8 months stored at room temperature and three months stored at temperature 37°C equal one year at room temperature. So if we put the expiry date of the cream 1 year, the consumer will still have a sufficiently active concentration for product efficacy even the concentration of the active ingredient is low (0.1 g/100 g of cream).

4.8 Analysis of oleuropein moisturizing cream questionnaires:

A moisturizing day cream formula (o/w) emulsion was designed with different concentrations of crude olive leaf extract (oleuropein). Each formula was given a letter which represented the concentration of the olive leaf extract as follows: Formula A contains 0.1 % crude olive leaf extract; formula B contains 0.4% crude olive leaf extract, formula C contains 1.0% crude olive leaf extract and formula D contains 0.0% (placebo) crude olive leaf extract.

The study is concentrated on Raed cosmetics consumers from Bethlehem region in Palestine only instead of all consumers due to time limitation and points focusing. A, B, C and D samples were given to each volunteers with different period of time; about 3 weeks be-

tween one sample and the other. This is to give the volunteers an enough time to apply the cream sample.

4.8.1. Data analysis:

Principally the data were analyzed by SPSS version 16.0. In order to analyze all answers obtained from the questionnaires, all answers are summarized as descriptive statistic as follows:

Gender: the sample consisted of 100 volunteers, 100% were females.

Age: the samples consisted of 100 volunteers from age between 18 years old to above 46 years old. 57% of the volunteers are between (31-45 years old), while 29% are between (18-30 years old) and only 14% are above (46 years old) (14%). Type of skin: 39% of the volunteers have dry skin, followed by oily skin (33%) and normal skin (28%).

Product data analysis for cream A, B, C and D:

How often do you reapply cream A? : 62% answered once a day, 22% answered twice a day and the rest of them (16%) answered occasionally

How often do you reapply cream B? : 60% answered once a day, 24% answered twice a day and the rest of them (16%) answered occasionally.

How often do you reapply cream C? : 60% answered once a day, 23% answered twice a day and 17% answered occasionally.

How often do you reapply cream D? : 62% answered once a day, 22% answered twice a day and 16% answered occasionally.

Sensorial evaluation for cream A: the volunteers have to give a mark from 1 to 10 to each evaluation where 1 is the lowest and 10 is the highest.

Table (4.11) analysis of sensorial evaluation for cream A (see appendix A):

Marks \ Evaluation	1	2	3	4	5	6	7	8	9	10
Texture						1%	12%	46%	37%	4%
Color			1%			10%	11%	31%	38%	9%
Odor	1%	2%	3%	5%	7%	19%	23%	27%	13%	
Consistency						2%	15%	52%	31%	
Absorbance							5%	50%	42%	3%
After fed(Graceness)	4%					7%	20%	44%	25%	
Feeding						3%	13%	48%	36%	
Shinness		4%	3%		5%	13%	16%	38%	21%	

Form table (4.11) it is seen that most of the consumers were satisfied with cream A. Almost 46% gave a texture mark (8), 38% gave the color mark (9), 52% gave the consistency also mark (8), 50% gave the absorbance mark (8), 44% gave Graceness mark (8) and 48% gave the feeding mark (8) and 38% gave shininess mark (8), where 8 is considered a high mark. For the odor, 27% of them gave mark (8), and 23% gave mark (8) which means that the consumers were satisfied with the odor of the cream.

Sensorial evaluation for cream B: the volunteers have to give a mark from 1 to 10 to each evaluation where 1 is the lowest and 10 is the highest.

Table 4.12: analysis of sensorial evaluation for cream B (see appendix B):

Marks \ Evaluation	1	2	3	4	5	6	7	8	9	10
Texture							12%	48%	37%	3%
Color							15%	50%	35%	
Odor	2%	2%	4%	7%	12%	21%	27%	21%	4%	
Consistency						3%	14%	52%	31%	
Absorbance							6%	50%	41%	3%
After fed(Graceness)						4%	21%	47%	28%	
Feeding						1%	10%	54%	35%	
Shininess	7%	3%	5%	1%	7%	10%	15%	35%	17%	

Form table (4.12) it is seen that most of the consumers were satisfied with cream B. Almost 48% gave a texture mark (8), 50% gave the color mark (8), 52% gave the consistency also mark (8), 50% gave the absorbance mark (8), 47% gave Graceness mark (8) and 54% gave the feeding mark (8) where (8) is considered a high mark. For the odor, 27% of them gave mark (7), and 21% gave mark (8) which means that the consumers were satisfied with the odor of the cream. About 77% gave shininess marks between (6-9) which is high. So most of the consumers have not felt their skin shinning which is considered as an advantage of the cream

Sensorial evaluation for cream C: the volunteers have to give a mark from 1 to 10 to each evaluation where 1 is the lowest and 10 are the highest.

Table 4.13: analysis of sensorial evaluation for cream C (see appendix C):

Marks \ Evaluation	1	2	3	4	5	6	7	8	9	10
Texture							11%	48%	38%	3%
Color	2%	2%	7%	15%	25%	27%	13%	7%	2%	
Odor	1%	2%	5%	8%	12%	20%	26%	21%	5%	
Consistency						3%	15%	50%	32%	
Absorbance							6%	50%	41%	3%
After fed(Graceness)	2%				1%	5%	21%	47%	24%	
Fading						2%	12%	52%	34%	
Shinness	6%	2%	3%	1%	8%	9%	18%	36%	17%	

Form table (4.13) it is seen that most of the consumers were satisfied with cream C. Almost 48% gave a texture mark (8), 27% gave the color mark (6), 26% gave the odor mark (7), 50% gave the consistency mark (8), 50% gave the absorbance mark (8), 47% gave Graceness mark (8) and 52% gave the feeding mark (8).

Sensorial evaluation for cream D: the volunteers have to give a mark from 1 to 10 to each evaluation where 1 is the lowest and 10 is the highest

Table 4.14: analysis of sensorial evaluation for cream D (see appendix D):

Marks \ Evaluation	1	2	3	4	5	6	7	8	9	10
Texture							7%	49%	37%	7%
Color						8%	5%	26%	41%	20%
Odor	1%		2%	4%	4%	17%	20%	33%	19%	
Consistency						2%	13%	52%	33%	
Absorbance						2%	6%	50%	39%	3%
After fed(Graceness)						5%	20%	48%	27%	
Feeding						3%	9%	52%	36%	
Shinness			2%		5%	12%	17%	42%	22%	

Form table (4.14) it is that most of the consumers were satisfied with cream D. Almost 49% gave a texture mark (8), 41% gave the color mark (9), 52% gave the consistency also mark (8), 50% gave the absorbance mark (8), 48% gave Graceness mark (8) and 52% gave

the feeding mark (8) and 42% gave shininess mark (8). For the odor, 33% of them gave mark (8), which means that the consumers were satisfied with the odor of the cream.

Have you noticed a difference on your skin (A, B, C, D)? : 92% of the volunteers answered noticed a difference on their skin feel when they used cream A, B and C. regarding cream D 10% noticed a difference on their skin feel.

Have you seen an improvement upon use (A, B, C, D)? : it is found that 84% answered 86%, 87% and 20% have seen an improvement of their skin upon use of cream A, B, C and D respectively.

Have you experienced an allergic reaction to the cream (A, B, C, D)? : Results have shown that the majority (98-99%) of the volunteers have not experienced an allergic reaction to the cream.

Have you noticed your skin nourishment? If yes please explain: Moisturized skin or smooth skin (A, B, C, D): for cream A 91% noticed their skin nourishment in their skin (from these 59% have noticed moisturized skin and 33% smooth skin). For cream B and C 90% noticed their skin nourishment (from these 53% have noticed moisturized skin and 37% smooth skin). For cream D 10% noticed their skin nourishment (from these 5% have noticed moisturized skin and 5% smooth skin).

Are you satisfied with cream (A, B, C, and D)? 91% are satisfied with cream A, 90% are satisfied with cream B and C, while 10% are satisfied with cream D. most of volunteers noticed significant difference between creams containing olive leaves extract (A, B, C) and the one without olive leaves extract (D).

The relationship between the type of skin and improvement of the skin:

26% of the volunteers who have normal skin observed an improvement upon use while 4% have not seen any improvement upon use. 25% of the volunteers who have oily skin observed improvement upon use while 7% haven't seen an improvement upon use. It was found that 34% of the volunteers who have dry skin have seen improvement upon use while 4% have not seen any improvement upon use to see if there is a relationship between type of the skin and the improvement of the skin, Chi-Square test was done for the data (see table 4.15) . It is found that there is no relationship between the type of skin and the improvement of the skin (Asy. Sig. value is 0.397 which is larger than 0.05% at 95% confidence level).

table 4.15: Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.848 ^a	2	.397
Likelihood Ratio	1.787	2	.409
Linear-by-Linear Association	.166	1	.684
N of Valid Cases	100		

The relationship between application of the cream and improvement of the skin:

It is found that 57% of the volunteers who apply the cream once a day have seen improvement upon use while 5% have not seen improvement upon use. 19% of the volunteers who apply the cream twice a day have seen improvement upon use while 3% haven't seen an improvement upon use. 9% of the volunteers who apply the cream occasionally answered have seen improvement upon use while 7% have not seen improvement upon use, Chi-Square test was done for the data (see table 4.16) . It is obtained that is a relationship between the application of cream and the improvement of the skin (Asy. Sig. value is 0.002).

Table 4.16: Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	12.744 ^a	2	.002
Likelihood Ratio	10.324	2	.006
Linear-by-Linear Association	12.095	1	.001
N of Valid Cases	100		

The relationship between the color of the cream and the satisfaction of the cream:

Chi Square test (see table 4.17) showed that there is no relationship between the color and the satisfaction (Chi Square value 0.067)

Table 4.17: Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	13.235 ^a	7	.067
Likelihood Ratio	9.052	7	.249
Linear-by-Linear Association	1.563	1	.211
N of Valid Cases	100		

Chapter Five: Conclusion

5.1 Conclusion:

- 1- Drying of olive leaves before extraction process is very important because it prevents microbial fermentation and subsequent degradation.
- 2- Simple drying of fresh olive leaves is the most suitable method because it preserves oleuropein from degradation while drying at elevated temperature (50°C) result in rapid degradation of oleuropein.
- 3- The extraction solvent, temperature, pH and type of extraction method are important parameters to recovery of oleuropein from olive leaves.
- 4- Mixtures of organic solvents (water/ethanol, water/acetonitrile, and water/methanol) with water give higher oleuropein content compared to pure solvents e.g. water, methanol, ethanol.
- 5- Temperature and pH also affect significantly the content of oleuropein extracted from olive leaves.
- 6- Oleuropein can be used as a moisturizing agent, antioxidant and anti aging for cosmetics products, such as anti aging cream or moisturizing day cream o/w emulsified non-ionic system, which is able to permeate the skin in small concentration.
- 7- Most of the volunteers have noticed significant difference between the creams containing oleuropein (A, B, C) and the one without oleuropein (D). There is a relationship between the application of cream and improvement of the skin.

5.2 Future work:

1. Evaluation of Oleuropein Shampoo.
2. Evaluation of w/o oleuropein Cream.

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Appendices:

1. Appendix A (SPSS Analysis for Cream A).
2. Appendix B (SPSS Analysis for Cream B).
3. Appendix C (SPSS Analysis for Cream C).
4. Appendix D (SPSS Analysis for Cream D).

Appendix A:

Statistics

	30	age	type	reap- ply	texture	color	odor	consis- tency	absorb- ance	Grace- ness	feeding
Valid	100	100	100	100	100	100	100	100	100	100	100
Missing	0	0	0	0	0	0	0	0	0	0	0

shinness	skinfeel	improvement	allergic	nourishment	explain	satisfied	percentage
100	100	100	100	100	92	100	94
0	0	0	0	0	8	0	6

age

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	18-30	29	29.0	29.0	29.0
	31-45	57	57.0	57.0	86.0
	46+	14	14.0	14.0	100.0
	Total	100	100.0	100.0	

type

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	normal	30	30.0	30.0	30.0
	oily	32	32.0	32.0	62.0
	dry	38	38.0	38.0	100.0
	Total	100	100.0	100.0	

reapply

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	once a day	62	62.0	62.0	62.0
	twice a day	22	22.0	22.0	84.0
	occasionally	16	16.0	16.0	100.0
	Total	100	100.0	100.0	

texture

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	6	1	1.0	1.0	1.0
	7	12	12.0	12.0	13.0
	8	47	47.0	47.0	60.0
	9	36	36.0	36.0	96.0
	10	4	4.0	4.0	100.0
	Total	100	100.0	100.0	

reapply

		Frequency	Percent	Valid Percent	Cumulative Percent
	once a day	62	62.0	62.0	62.0
	twice a day	22	22.0	22.0	84.0
	occasionally	16	16.0	16.0	100.0

color

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	3	1	1.0	1.0	1.0
	4	1	1.0	1.0	2.0
	5	13	13.0	13.0	15.0
	6	18	18.0	18.0	33.0
	7	12	12.0	12.0	45.0
	8	23	23.0	23.0	68.0
	9	26	26.0	26.0	94.0
	10	6	6.0	6.0	100.0
	Total	100	100.0	100.0	

odor

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	1	1.0	1.0	1.0
	2	2	2.0	2.0	3.0
	3	3	3.0	3.0	6.0

reapply

		Frequency	Percent	Valid Percent	Cumulative Percent
	once a day	62	62.0	62.0	62.0
	twice a day	22	22.0	22.0	84.0
	occasionally	16	16.0	16.0	100.0
	4	5	5.0	5.0	11.0
	5	7	7.0	7.0	18.0
	6	20	20.0	20.0	38.0
	7	23	23.0	23.0	61.0
	8	28	28.0	28.0	89.0
	9	11	11.0	11.0	100.0
	Total	100	100.0	100.0	

consistency

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	6	3	3.0	3.0	3.0
	7	14	14.0	14.0	17.0
	8	51	51.0	51.0	68.0
	9	32	32.0	32.0	100.0
	Total	100	100.0	100.0	

absorbance

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	7	6	6.0	6.0	6.0
	8	50	50.0	50.0	56.0
	9	41	41.0	41.0	97.0
	10	3	3.0	3.0	100.0
	Total	100	100.0	100.0	

graceness

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	3	3.0	3.0	3.0
	6	6	6.0	6.0	9.0
	7	20	20.0	20.0	29.0
	8	46	46.0	46.0	75.0
	9	25	25.0	25.0	100.0
	Total	100	100.0	100.0	

feeding

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	6	3	3.0	3.0	3.0
	7	11	11.0	11.0	14.0
	8	50	50.0	50.0	64.0
	9	36	36.0	36.0	100.0

feeding

		Frequency	Percent	Valid Percent	Cumulative Percent
	6	3	3.0	3.0	3.0
	7	11	11.0	11.0	14.0
	8	50	50.0	50.0	64.0
	9	36	36.0	36.0	100.0
	Total	100	100.0	100.0	

shinness

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	1	1.0	1.0	1.0
	2	3	3.0	3.0	4.0
	3	3	3.0	3.0	7.0
	5	6	6.0	6.0	13.0
	6	11	11.0	11.0	24.0
	7	16	16.0	16.0	40.0
	8	39	39.0	39.0	79.0
	9	21	21.0	21.0	100.0
	Total	100	100.0	100.0	

skinfeel

		Frequency	Percent	Valid Percent	Cumulative Percent
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Valid	yes	92	92.0	92.0	92.0
	no	8	8.0	8.0	100.0
	Total	100	100.0	100.0	

improvement

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	85	85.0	85.0	85.0
	no	15	15.0	15.0	100.0
	Total	100	100.0	100.0	

allergic

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	1	1.0	1.0	1.0
	no	99	99.0	99.0	100.0
	Total	100	100.0	100.0	

nourishment

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	91	91.0	91.0	91.0
	no	9	9.0	9.0	100.0
	Total	100	100.0	100.0	

explain

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	moisturized skin	57	57.0	62.0	62.0
	smooth skin	35	35.0	38.0	100.0
	Total	92	92.0	100.0	
Missing	System	8	8.0		
Total		100	100.0		

satisfied

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	91	91.0	91.0	91.0
	no	9	9.0	9.0	100.0
	Total	100	100.0	100.0	

percentage

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	25%	2	2.0	2.1	2.1
	55%	1	1.0	1.1	3.2
	70%	1	1.0	1.1	4.3
	75%	4	4.0	4.3	8.5
	80%	29	29.0	30.9	39.4
	85%	35	35.0	37.2	76.6
	90%	18	18.0	19.1	95.7
	95%	4	4.0	4.3	100.0
	Total	94	94.0	100.0	
Missing	System	6	6.0		
Total		100	100.0		

Appendix B

Statistics

		30	age	type	reapply	texture	color	odor	consistency	absorb- ance
N	Valid	100	100	100	100	100	100	100	100	100
	Miss- ing	0	0	0	0	0	0	0	0	0
Graceness	feeding	shin- ness	skin feel	improve- ment	allergic	nourish- ment	explain	satisfied	percent- age	
100	100	100	100	100	100	100	92	100	94	
0	0	0	0	0	0	0	8	0	6	

Frequency Table

30

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	female	100	100.0	100.0	100.0

age

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	18-30	31	31.0	31.0	31.0
	31-45	55	55.0	55.0	86.0
	46+	14	14.0	14.0	100.0
	Total	100	100.0	100.0	

type

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	normal	31	31.0	31.0	31.0
	oily	32	32.0	32.0	63.0
	dry	37	37.0	37.0	100.0
	Total	100	100.0	100.0	

reapply

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	once a day	62	62.0	62.0	62.0
	twice a day	22	22.0	22.0	84.0
	occasionally	16	16.0	16.0	100.0
	Total	100	100.0	100.0	

reapply

		Frequency	Percent	Valid Percent	Cumulative Percent
	once a day	62	62.0	62.0	62.0
	twice a day	22	22.0	22.0	84.0
	occasionally	16	16.0	16.0	100.0

texture

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	6	1	1.0	1.0	1.0
	7	12	12.0	12.0	13.0
	8	46	46.0	46.0	59.0
	9	37	37.0	37.0	96.0
	10	4	4.0	4.0	100.0
	Total	100	100.0	100.0	

color

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	3	1	1.0	1.0	1.0
	6	10	10.0	10.0	11.0
	7	11	11.0	11.0	22.0
	8	31	31.0	31.0	53.0
	9	38	38.0	38.0	91.0
	10	9	9.0	9.0	100.0
	Total	100	100.0	100.0	

reapply

		Frequency	Percent	Valid Percent	Cumulative Percent
	once a day	62	62.0	62.0	62.0
	twice a day	22	22.0	22.0	84.0
	occasionally	16	16.0	16.0	100.0

Odor

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	1	1.0	1.0	1.0
	2	2	2.0	2.0	3.0
	3	3	3.0	3.0	6.0
	4	5	5.0	5.0	11.0
	5	7	7.0	7.0	18.0
	6	19	19.0	19.0	37.0
	7	23	23.0	23.0	60.0
	8	27	27.0	27.0	87.0
	9	13	13.0	13.0	100.0
	Total	100	100.0	100.0	

consistency

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	6	2	2.0	2.0	2.0

	7	15	15.0	15.0	17.0
	8	52	52.0	52.0	69.0
	9	31	31.0	31.0	100.0
	Total	100	100.0	100.0	

absorbance

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	7	5	5.0	5.0	5.0
	8	50	50.0	50.0	55.0
	9	42	42.0	42.0	97.0
	10	3	3.0	3.0	100.0
	Total	100	100.0	100.0	

graceness

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	4	4.0	4.0	4.0
	6	7	7.0	7.0	11.0
	7	20	20.0	20.0	31.0
	8	44	44.0	44.0	75.0
	9	25	25.0	25.0	100.0
		Total	100	100.0	100.0

feeding

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	6	3	3.0	3.0	3.0
	7	13	13.0	13.0	16.0
	8	48	48.0	48.0	64.0
	9	36	36.0	36.0	100.0
	Total	100	100.0	100.0	

shinness

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	2	4	4.0	4.0	4.0
	3	3	3.0	3.0	7.0
	5	5	5.0	5.0	12.0
	6	13	13.0	13.0	25.0
	7	16	16.0	16.0	41.0
	8	38	38.0	38.0	79.0
	9	21	21.0	21.0	100.0

shinness

		Frequency	Percent	Valid Percent	Cumulative Percent
	2	4	4.0	4.0	4.0
	3	3	3.0	3.0	7.0
	5	5	5.0	5.0	12.0
	6	13	13.0	13.0	25.0
	7	16	16.0	16.0	41.0
	8	38	38.0	38.0	79.0
	9	21	21.0	21.0	100.0
	Total	100	100.0	100.0	

skinfeel

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	92	92.0	92.0	92.0
	no	8	8.0	8.0	100.0
	Total	100	100.0	100.0	

improvement

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	84	84.0	84.0	84.0
	no	16	16.0	16.0	100.0
	Total	100	100.0	100.0	

allergic

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	1	1.0	1.0	1.0
	no	99	99.0	99.0	100.0
	Total	100	100.0	100.0	

nourishment

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	91	91.0	91.0	91.0
	no	9	9.0	9.0	100.0
	Total	100	100.0	100.0	

explain

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	moisturized skin	59	59.0	64.1	64.1
	smooth skin	33	33.0	35.9	100.0
	Total	92	92.0	100.0	
Missing	System	8	8.0		
Total		100	100.0		

satisfied

		Frequency	Percent	Valid Percent	Cumulative Percent
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Valid	yes	91	91.0	91.0	91.0
	no	9	9.0	9.0	100.0
	Total	100	100.0	100.0	

percentage

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	25%	2	2.0	2.1	2.1
	55%	1	1.0	1.1	3.2
	75%	5	5.0	5.3	8.5
	80%	28	28.0	29.8	38.3
	85%	35	35.0	37.2	75.5
	90%	18	18.0	19.1	94.7
	95%	5	5.0	5.3	100.0
	Total	94	94.0	100.0	
Missing	System	6	6.0		
Total		100	100.0		

Appendix C:

Statistics

		30	age	type	reapply	texture	color	odor	consistency	absorbance
N	Valid	100	100	100	100	100	100	100	100	100
	Missing	0	0	0	0	0	0	0	0	0
Graceness	feeding	shininess	skin feel	improvement	allergic	nourishment	explain	satisfied	percentage	
100	100	100	100	100	100	100	92	100	94	
0	0	0	0	0	0	0	8	0	6	

Frequency Table

30

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid female	100	100.0	100.0	100.0

age

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	18-30	29	29.0	29.0	29.0
	31-45	57	57.0	57.0	86.0
	46+	14	14.0	14.0	100.0
	Total	100	100.0	100.0	

type

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	normal	30	30.0	30.0	30.0
	oily	32	32.0	32.0	62.0
	dry	38	38.0	38.0	100.0
	Total	100	100.0	100.0	

reapply

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	once a day	62	62.0	62.0	62.0
	twice a day	22	22.0	22.0	84.0
	occasionally	16	16.0	16.0	100.0
	Total	100	100.0	100.0	

Texture

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	6	1	1.0	1.0	1.0

type

		Frequency	Percent	Valid Percent	Cumulative Percent
	normal	30	30.0	30.0	30.0
	oily	32	32.0	32.0	62.0
	dry	38	38.0	38.0	100.0
	7	12	12.0	12.0	13.0
	8	47	47.0	47.0	60.0
	9	36	36.0	36.0	96.0
	10	4	4.0	4.0	100.0
	Total	100	100.0	100.0	

color

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	3	1	1.0	1.0	1.0
	4	1	1.0	1.0	2.0
	5	13	13.0	13.0	15.0
	6	18	18.0	18.0	33.0
	7	12	12.0	12.0	45.0
	8	23	23.0	23.0	68.0
	9	26	26.0	26.0	94.0
	10	6	6.0	6.0	100.0
	Total	100	100.0	100.0	
Odor					

color

		Frequency	Percent	Valid Percent	Cumulative Percent
	3	1	1.0	1.0	1.0
	4	1	1.0	1.0	2.0
	5	13	13.0	13.0	15.0
	6	18	18.0	18.0	33.0
	7	12	12.0	12.0	45.0
	8	23	23.0	23.0	68.0
	9	26	26.0	26.0	94.0
	10	6	6.0	6.0	100.0
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	1	1.0	1.0	1.0
	2	2	2.0	2.0	3.0
	3	3	3.0	3.0	6.0
	4	5	5.0	5.0	11.0
	5	7	7.0	7.0	18.0
	6	20	20.0	20.0	38.0
	7	23	23.0	23.0	61.0
	8	28	28.0	28.0	89.0
	9	11	11.0	11.0	100.0
	Total	100	100.0	100.0	

consistency

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	6	3	3.0	3.0	3.0
	7	14	14.0	14.0	17.0
	8	51	51.0	51.0	68.0
	9	32	32.0	32.0	100.0
	Total	100	100.0	100.0	

absorbance

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	7	6	6.0	6.0	6.0
	8	50	50.0	50.0	56.0
	9	41	41.0	41.0	97.0
	10	3	3.0	3.0	100.0
	Total	100	100.0	100.0	

graceness

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	3	3.0	3.0	3.0
	6	6	6.0	6.0	9.0
	7	20	20.0	20.0	29.0
	8	46	46.0	46.0	75.0

	9	25	25.0	25.0	100.0
	Total	100	100.0	100.0	

feeding

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	6	3	3.0	3.0	3.0
	7	11	11.0	11.0	14.0
	8	50	50.0	50.0	64.0
	9	36	36.0	36.0	100.0
	Total	100	100.0	100.0	

shinness

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	1	1.0	1.0	1.0
	2	3	3.0	3.0	4.0
	3	3	3.0	3.0	7.0
	5	6	6.0	6.0	13.0
	6	11	11.0	11.0	24.0
	7	16	16.0	16.0	40.0
	8	39	39.0	39.0	79.0
	9	21	21.0	21.0	100.0
	Total	100	100.0	100.0	

skinfeel

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	92	92.0	92.0	92.0
	no	8	8.0	8.0	100.0
	Total	100	100.0	100.0	

improvement

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	85	85.0	85.0	85.0
	no	15	15.0	15.0	100.0
	Total	100	100.0	100.0	

allergic

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	1	1.0	1.0	1.0
	no	99	99.0	99.0	100.0
	Total	100	100.0	100.0	

nourishment

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	91	91.0	91.0	91.0
	no	9	9.0	9.0	100.0
	Total	100	100.0	100.0	

explain

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	moisturized skin	57	57.0	62.0	62.0
	smooth skin	35	35.0	38.0	100.0
	Total	92	92.0	100.0	
Missing	System	8	8.0		
Total		100	100.0		

satisfied

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	91	91.0	91.0	91.0
	no	9	9.0	9.0	100.0
	Total	100	100.0	100.0	

percentage

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	25%	2	2.0	2.1	2.1
	55%	1	1.0	1.1	3.2
	70%	1	1.0	1.1	4.3
	75%	4	4.0	4.3	8.5
	80%	29	29.0	30.9	39.4
	85%	35	35.0	37.2	76.6
	90%	18	18.0	19.1	95.7
	95%	4	4.0	4.3	100.0
	Total	94	94.0	100.0	
Missing	System	6	6.0		
Total		100	100.0		

Appendix D:

Statistics

		30	age	type	reapply	texture	color	odor
N	Valid	100	100	100	100	100	100	100
	Missing	0	0	0	0	0	0	0
consistency	absorbance	graceness	feeding	shinness	allergic	improvement	satisfied	
100	100	100	100	100	100	100	100	100

Frequency Table

30

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	female	100	100.0	100.0	100.0

age

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	18-30	29	29.0	29.0	29.0
	31-45	57	57.0	57.0	86.0
	46+	14	14.0	14.0	100.0
	Total	100	100.0	100.0	

type

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	normal	30	30.0	30.0	30.0
	oily	32	32.0	32.0	62.0
	dry	38	38.0	38.0	100.0
	Total	100	100.0	100.0	

reapply

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	once a day	62	62.0	62.0	62.0
	twice a day	22	22.0	22.0	84.0
	occasionally	16	16.0	16.0	100.0
	Total	100	100.0	100.0	

texture

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	7	7	7.0	7.0	7.0
	8	49	49.0	49.0	56.0
	9	37	37.0	37.0	93.0
	10	7	7.0	7.0	100.0
	Total	100	100.0	100.0	

color

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	6	8	8.0	8.0	8.0
	7	5	5.0	5.0	13.0
	8	26	26.0	26.0	39.0
	9	41	41.0	41.0	80.0
	10	20	20.0	20.0	100.0
	Total	100	100.0	100.0	

odor

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	1	1.0	1.0	1.0
	3	2	2.0	2.0	3.0
	4	4	4.0	4.0	7.0
	5	4	4.0	4.0	11.0
	6	17	17.0	17.0	28.0
	7	20	20.0	20.0	48.0
	8	33	33.0	33.0	81.0
	9	19	19.0	19.0	100.0
	Total	100	100.0	100.0	

Consistency

	Frequency	Percent	Valid Percent	Cumulative Percent
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odor

		Frequency	Percent	Valid Percent	Cumulative Percent
	1	1	1.0	1.0	1.0
	3	2	2.0	2.0	3.0
	4	4	4.0	4.0	7.0
	5	4	4.0	4.0	11.0
	6	17	17.0	17.0	28.0
	7	20	20.0	20.0	48.0
	8	33	33.0	33.0	81.0
	9	19	19.0	19.0	100.0
Valid	6	2	2.0	2.0	2.0
	7	13	13.0	13.0	15.0
	8	52	52.0	52.0	67.0
	9	33	33.0	33.0	100.0
	Total	100	100.0	100.0	

absorbance

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	6	2	2.0	2.0	2.0
	7	6	6.0	6.0	8.0
	8	50	50.0	50.0	58.0
	9	39	39.0	39.0	97.0
	10	3	3.0	3.0	100.0

odor

		Frequency	Percent	Valid Percent	Cumulative Percent
	1	1	1.0	1.0	1.0
	3	2	2.0	2.0	3.0
	4	4	4.0	4.0	7.0
	5	4	4.0	4.0	11.0
	6	17	17.0	17.0	28.0
	7	20	20.0	20.0	48.0
	8	33	33.0	33.0	81.0
	9	19	19.0	19.0	100.0
	Total	100	100.0	100.0	

graceness

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	6	5	5.0	5.0	5.0
	7	20	20.0	20.0	25.0
	8	48	48.0	48.0	73.0
	9	27	27.0	27.0	100.0
	Total	100	100.0	100.0	

feeding

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	6	3	3.0	3.0	3.0
	7	9	9.0	9.0	12.0
	8	52	52.0	52.0	64.0
	9	36	36.0	36.0	100.0
	Total	100	100.0	100.0	

shinness

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	3	2	2.0	2.0	2.0
	5	5	5.0	5.0	7.0
	6	12	12.0	12.0	19.0
	7	17	17.0	17.0	36.0
	8	42	42.0	42.0	78.0
	9	22	22.0	22.0	100.0
	Total	100	100.0	100.0	

allergic

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	1	1.0	1.0	1.0
	no	99	99.0	99.0	100.0
	Total	100	100.0	100.0	

improvement

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	10	10.0	10.0	10.0
	no	90	90.0	90.0	100.0
	Total	100	100.0	100.0	

satisfied

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	10	10.0	10.0	10.0
	no	90	90.0	90.0	100.0
	Total	100	100.0	100.0	

استخلاص مادة الأوروروبين من أوراق الزيتون الفلسطينية باستخدام طرق استخلاص بسيطة واستخدامها في بعض مستحضرات التجميل

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الملخص:

في هذا البحث تم جمع أوراق الزيتون من مدينة بيت ساحر - فلسطين في منتصف شهر تشرين ثاني لاستخدامها في استخلاص مادة Oleuropein، وتم غسلها وتجفيفها على درجات حرارة مختلفة (درجة حرارة الغرفة، ودرجة حرارة عالية (50°C)) ومقارنتها مع أوراق الزيتون الجافة طبيعياً التي تم جمعها جافة من الشجرة ومع أوراق الزيتون الخضراء، حيث أظهرت النتائج أن أعلى تركيز تم الحصول عليه من أوراق الزيتون المجففة في درجة حرارة الغرفة (10 mg/g) في حين كان التركيز (1.7mg/g) من أوراق الزيتون المجففة على درجة حرارة مرتفعة (50°C) و (2.5 mg/g) من أوراق الزيتون الجافة طبيعياً، أما أوراق الزيتون الخضراء أظهرت النتائج أدنى تركيز (0.1 mg/g).

وفي هذا البحث أيضاً تم دراسة العوامل التي تؤثر في عملية استخلاص Oleuropein من أوراق الزيتون، وهي تأثير المذيبات ودرجة الحموضة ودرجة الحرارة واساليب الاستخلاص (النقع، استخلاص الساخن المتكرر Soxhlet) وقد وجد أن الماء النقي والميثانول والإيثانول ليست مذيبات جيدة لاستخلاص مادة Oleuropein من أوراق الزيتون، فقد وجد أن 80% إيثانول أعطى أعلى تركيز يتبعه 20% أسيتونايتريل. وأن درجة الحرارة لها تأثير كبير في عملية الاستخلاص واستخدام وسط حامضي يعطي تركيز أعلى من الوسط القاعدي، وأن الاستخلاص بواسطة Soxhlet أفضل من طريقة النقع.

وتم استخدام مادة Oleuropein المستخلصة في تركيبة كريم مرطب للبشرة، وتم وضعها بعدة تراكيز (0.1%, 0.4%, 1.0%) ودراسة الخصائص الفيزيائية والكيميائية والثباتية لمدة ثلاثة شهور ومقارنة النتائج مع منتج تجاري. وأظهرت النتائج ثباتية الكريم خلال الفترة المذكورة. ونم توزيع عينات من المستحضرات على مجموعة من المتطوعين بلغ عددهم 100 متطوع. وتم عمل استبيان وتوزيعه وتحليل النتائج باستخدام برنامج SPSS اصدار 0.6 حيث أظهرت النتائج أن معظم المتطوعين لاحظوا فروق ذات دلالة احصائية بين الكريمات التي تحتوي على مادة Oleuropein (عينات تحمل الرموز A, B, C) والأخرى التي لا تحتوي على مادة Oleuropein (كريم D).