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"Extraction of Corn silk and its applications in healing and cosmetics''

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List of Abbreviations (Nomenclature)

Symbols	Definition
UTI	Urinary Tract Infection
CAM	Complementary And Alternative Medicine
CS	Corn silk
TC	Total Cholesterol
TG	Total Triglyceride
TFC	Total Flavonoids Content
TPC	Total Phenolic Content
CKD	Chronic Kidney Disease
GI	The Glycemic Index
T2DM	Type 2 Diabetic Mellitus
ROS	Reactive Oxygen Species
DPPH	1-Diphenyl-2-Picryl Hydrazyl Radical
ADI	Acceptable Daily Intake
NOAEL	No-Observed-Adverse-Effect Level
DEEP	The Deprived Families Economic Empowerment Program
ICR	Institution cancer research

Abstract

The name of corn silk is used to describe hundreds of light-yellow threads that envelope an ear of mature corn. Ranging from 10-20cm in length, their purpose is to catch pollen from air.

Because of the availability of corn silk at May and September of each year in our country, corn silk was collected and picked up for use in preparation for in vitro treatment.

The silk is characterized by the fact that it has high nutritional, mineral and medicinal values. Cornsilk contains phytochemical as flavonoids, saponins, tannins and alkaloids. It also contains proteins, carbohydrates, fibres and lipids. Because of these nutritional, mineral values and phytochemicals available in corn silk, its involved in the treatment of many diseases such as urinary tract infections (UTI), Beside kidney detox, high uric acid/gout, gallstones, cat/dog incontinence, and diabetic blood sugar support. Also used in cosmetics.

Experiments was carried out on corn silk. Formulation experiments was conducted as well as producing cream, corn silk tea, tincture and tablets.

The objectives of this study are to design a suitable oral dosage form of corn silk as teabags with stevia plant for treating urinary tract infection and a cream in cosmetic field. Secondly, to perform chemical experiments in order to increase the efficiency of extraction. Thirdly, checking and investigating the active ingredients of corn silk by performing GC-MS test and finally, performing anti-oxidant activity tests.

This was done by collecting corn silk, drying it, then grinding it and using it in extraction processes using solvent types including ethanol, methanol and ethyl acetate .It is desired to get a high yield of extraction by studying the parameters which controls the contact between the solvent and the dried, grinded solids of corn silk.

The main parameters that controls the effectiveness of extraction are type of solvent, time of extraction, temperature of solution, volume of solvent added, speed of rotation and method of extraction. Filtration must be done after extraction and ethanol has been recovered using rotary evaporator under vacuum to separate ethanol solution from the solid phase to get the yield.

As a result, in our project it was found that ethanol extraction is the most suitable method to use with corn silk due to its availability, non-toxicity, low cost and high solubility because of its polarity. Compared with methanol, it was found that methanol is more toxic although it has the highest percentage of yield 18.5% (w/w), for ethanol 5.7%(w/w) but for ethyl acetate, it has the lowest percentage of yield 3.2%(w/w), also it was found to be a toxic compound.

Results show that the best time used in the mixing process in all solvent types is 4 hours because it has the highest yield. After 4 hours, there's a decrease in the curve and then stabilizes.

Four different tests gave an indication to the free radical scavenging of corn silk. These tests are ferric reducing/antioxidant power (FRAP), Cupric reducing antioxidant power (CUPRAC assay)**,** Free radical scavenging activity using DPPH (DPPH assay)**,** Free radical scavenging activity using ABTS (ABTS assay), Total phenolic content (Folin-Ciocalteau assay) and Total flavonoid content and they are performed in three types of solution. The first solution is 99 wt.% ethanol, the second one is 80 wt.% ethanol, and the last one is water. 80 % ethanol gave the highest antioxidant power than the other solvents.

Many patients who have UTI having up corn silk teabag, three times daily, expressed their feeling of using it. It overcome their fatigue, and stresses caused by diabetes and continued their working day as normal persons. Also, it overcomes their UTI.

The project was done successfully and the results are satisfactory and perfectly meet the relevant literature.

Chapter one: Introduction

1.1: Background

Complementary and alternative medicine (CAM) is a group of diverse medical and health care systems, therapies, and products that are not presently considered a part of conventional medicine(Patricia M. Barnes, 2002). Complementary medicine is a formal method of health care in most countries of the ancient world. It is expected to become more widely integrated into the modern medical system, including the medical curriculum. More than 70% of the developing world's population still depends primarily on the complementary and alternative systems of medicine (CAM). There has been increased global interest in traditional medicine and there are efforts underway to monitor and regulate herbal drugs and traditional medicine. China has been successful in promoting its therapies with more research and a science-based approach .There is no doubt that today the concept of Arabic traditional herbal medicine is a part of modern life in the Middle East, and it is acquiring worldwide respect, with growing interest among traditional herbalists and the scientific community (Hassan Azaizeh, 2008) .

Corn is one of the most widely grown cereal crops in the world and has become the third most important cereal crops other than wheat and rice(Ramessar, n.d.). The Production of corn was reported to increase from 713 million metric tons in 2006 to nearly 1021 million metric tons 2016 with an incredible growth percentage of 116 % from 1990 to 2016 worldwide. The United States of America and China have the domestic consumption of corn in the world (Gloy, 2017). The plant is characterized by the fact that each part of it has a high nutritional value.(Nurhanan Abdul Rahman, 2013)

Corn silk, which is also known as (Maydis stigma) or (Zea Mays), are a bundle of silky, long and yellowish strands appears on the top of corncob. It is an important herb used traditionally by the Chinese, and Native Americans to treat many diseases. It is also used as traditional medicine in many parts of the world such as Turkey, United States and France (Khairunnisa Hasanudin, 2012).

Corn silk(CS)is rich in phenolic compounds, particularly flavonoids. It also consists of proteins, vitamins, carbohydrates, calcium, potassium, magnesium and sodium salts, volatile oils and steroids such as sit sterol and stigma sterol, alkaloids, tannins and saponins (Nurhanan Abdul Rahman, 2013).

CS has been used as diuretic, antillithiasic, urincosuric, and antiseptic. It is used for the treatment of edema as well as for cystitis, gout, kidney stones, nephritis, and prostatitis (Thoudam Bhaigyabati, 2011).

Corn silk has been reported to exhibit positive effect on glycaemic metabolism by increasing insulin level whereby the increasing of insulin level and recovery of β-cells were known to be the mechanism involved in the glycaemic metabolism.

By going to the field of cosmetics, various cosmetic ingredients are derived from Zea mays plants. These include, silk extract, Corn Silk Extract is a slightly viscous brown liquid with a pH of 4.5 to 6.5. Scramble for 1960s make-up that's best for selfie. Corn Silk face powder makes a comeback with women claiming it gives them an ideal look for the photo craze that has swept the planet. Powdered corn silk can be used to make poultices for easing boils and inflamed skin, the powder can be added to skin products to soothe the face and to make face powders and in face masks to help detoxify the skin.

1.2: Problem statement

Nowadays there are many people suffering from several diseases. However, alternative medicine has made a trust with people at recent years, due to its effectiveness and its natural quality. By taking an example, Urinary tract infection (UTI), is the second most common infections presentation in community practice; worldwide about 150 million people were diagnosed with UTI each year.

This disease is affected by either bacterial infection or yeast infection. The main bacteria cause this type of infections is called Escherichia coli bacteria. E-Coli gets inside the urinary system through the urethra, and then begin to multiply and distribute in the bladder. Although the urinary system is designed to prevent the entry of these prokaryotes, this defensive system sometimes fails to perform its task. When such failure occurs, microbes take control and begin to multiply, causing severe and severe inflammation of the urinary tract.(Lal, 2016).

However, there are many reports showed that corn silk has been used as an alternative medicine for urinary system, due to increasing incidence of antibiotic resistance among bacterial pathogens necessitates medicinal plants as an alternate therapy in the management of UTI. The result of urinary tract infection study gives indication that there is a very effective treatment of infection which is caused by bacteria especially E-coli. The treatment of stones also effective, but need more time to get rid of all types of stones. (Ahmed Salih Sahib, 2012)

It is Also used for the treatment of edema, kidney stones, diuretic, prostate disorder as well as bedwetting and obesity. It soothes and relaxes the lining of the bladder and urinary tubules, hence reducing irritation and increasing urine secretion.(Khairunnisa Hasanudin, 2012)All of mentioned diseases and health issues are very common on Palestinian society that need to be encountered.

1.3: Purpose of the study

Finding out and Designing any form of product that convey the bioactive material to the body of patient. This can be done by direct or indirect methods.

The anticipated product teabag likewise product, tincture, or/and cream.

1.4: Objectives

Studying the healing effectiveness of the active constituents of corn silk on UTI diseases. Then preparing the proper formula, and design a suitable dosage form to apply it in a suitable manner, as well as studying its applications in cosmetics field.

Primarily, UTI will be discussed as a serious disease and how CS can be helpful in reducing its symptoms. Extraction the active ingredients of CS will be one of the most important objectives in this project by applying different methods of extraction in order to extract all components of CS, from Flavonoids to saponins to vitamins, minerals, etc. In addition, anti-diabetic effects of a polysaccharide from corn silk will be studied.

Anyway, CS has been applied in cosmetic industry, making these yellow strands as a treasure that can be beneficial at different fields.

To sum up, Corn silk is rich in phenolic compounds, vitamins, saponins, alkaloids, volatile and fixed oil. Which makes this study fruitful and promising.

1.5: Scope of the work

The preparation to this study was started at last summer. The raw material or corn silk were abundant on Palestinian market. The corn silk silky strains were collected from vegetable vendors, that are dried soon at room temperature without exposing to the sun for about two weeks with daily reciprocating, then after complete drying, it was kept for further future processing.

After drying is accomplished, a sample of dried corn silk is grinded to a fine particle by a stainless-steel grain grinder. Then the grain powder is treated with ethyl alcohol to make tincture and to found out the effective material that are solved in alcohol which is about 5.7%(w/w).

1.6: Significance

Flavonoids are a group of natural substances with variable phenolic structures. They are now considered as an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function (A. N. Panche, 2016). Corn silk is rich in these Phenolic compounds. Making this herb a very important field of study.

In addition, CS contains saponins, which are characterized by their structure containing a triterpene or steroid aglycone and one or more sugar chains. Consumer demand for natural products coupled with their physicochemical (surfactant) properties and mounting evidence on their biological activity (such as anti-cancer and anti-cholesterol activity) has led to the emergence of saponins as commercially significant compounds with expanding applications in food, cosmetics, and pharmaceutical sectors (Mazza, 2007).

Moreover, corn silk offers a healthy dose of potassium and vitamins C. The major nutrient in corn silk is potassium, which is responsible for all of the benefits that come from this part of the corn plant. It is believed that this herbal extract can act as a powerful diuretic and that probably accounts for the help that corn silk can provide for the urinary system(Vijitha T P, 2017).

Polysaccharides of corn silk may lead to the reduction of blood glucose, as well as reducing serum lipid level including total cholesterol (TC) and total triglyceride (TG).

In the field of cosmetics, many different products can be made up from corn silk including cream, powder, liquid, capsules, gel, tincture or tea. For instance, to make skin look and feel healthy, skin brightening powder of CS used, this powder contains the unique Bio-Active Complex formulated with vitamins and botanicals, excellent as a highlighter, Brightens and minimize the appearance of pores (MDPI, 2014).

1.6: Organization of the report

This report consists of eight main chapters; **Chapter one** introduces the alternative medicine with the description of corn silk and clarifies the problems that will tend to be solved. **Chapter two** shows the main constraints and standards in this study.

Chapter three shows the previous researches and studies published on this subject and included a literature review of the topics related to corn silk and general information about it, which is divided into three sections: The first section will talk about the general nutritional and medicinal values of corn silk as well as the application of nutrients in medicine and cosmetics. The second section will concern about methods of extraction of phytochemicals and gives toxicity investigation researches of the main ingredients of corn silk. The third section includes additives can be added into the corn silk to give a final product with the benefits needed.

Chapter four includes the methodology of work, summarizes what have been done which is concern about two sections: extraction of the active ingredient from corn silk and the other section is increasing the efficiency of extraction and optimizing the most suitable conditions of extraction.

While **chapter five** concerns about the experimental methods. Taking into consideration that it divided into three section. First, the exctraction of ethanol of corn silk. This section contains three parts: Pre experiment preparation, ethanol extraction under optimum conditions and recovery of active ingredients using rotary evaporator under vacuum pressure. Secondly, measurments of anti-oxidant activity.

Chapter six includes results and discussion and it disccuss the optimization conditions for extraction like using different types of solvents, temperature, speed of rotation, time of mixing and method of extraction. The second part, determination of total phenolic compounds in cornsilk. While the final part talks about the anti-oxidant activity.

In **chapter seven** there was a brief conslusion of the main results.

Finally, **chapter eight** contains references.

Chapter Two: Constraints, standards

There are many difficulties have been encountered during the study and during the experimental parts. Study difficulties include choosing the safest and the most suitable extraction method especially when they deal with medicinal field. Methanol for example is a toxic primary alcohol causes vomiting, blindness, kidney failure, and it can be fatal with a small amount. Even though methanol extraction has been reported to have the highest anti-oxidant activity among all solvents used, there should be awareness in dealing with this type of extraction.

Dosage of CS in the form of tea bag is another serious problem, hence there is one machine for tea packaging in Tiasir town in district of Tubas. So, CS tea bag packaging is done manually by discharging tea bags and refilling with CS blends. This needs more energy and time.

In addition, using the sugar as a sweetener for the corn silk bags is unhealthy especially for those patients who are suffering from diabetes. Stevia plant has been chosen to be an alternative sweetener. So, this plant was added to corn silk tea and it was purchased from Palestinians farm in Qalqilya.

Chapter three: Literature Review

3.1 General Nutritional and Medicinal Values

3.1.1 General uses of corn silk

Corn silk is the yellowish strands that appears in the top of corncob. These strands can be divided into two parts: Mature corn silk, and Immature corn silk. Mature strands are usually obtained from the fully ripened corncobs while the immature strands are obtained from the unripenedones.(N.A. Rahman, 2013)

The General uses of corn silk can be divided into two main parts: the first part is in the medicine field, and the second part is in cosmetics field. The medical uses of corn silk are several due to its abundance in phytochemicals and minerals that play an important role in the benefits of CS.

- Medical values

CS is reported to have anti-inflammatory, anti-diabetic, anti-obesity and anti-oxidant properties that give it several medical uses from the treatment of Urinary tract infection to kidney stones, fatigue, and Diuresis, kaliuresis effect, and in controlling of diabetes(Khairunnisa Hasanudin, 2012). In addition, it is reported to be effective in weight loss. That is all, due to the presence of phytochemicals that is considered as an antiseptic chemical that are derived from CS. As well as, the presence of minerals that give additional nutritional values.

In another research, the phytochemical analysis of the benzene, chloroform, ethanol, ethyl acetate, methanol and petroleum ether extracts of corn silk showed positive results for the presence of flavonoids, alkaloids, phenols, steroids, glycosides, carbohydrates, amino acids, terpenoids and tannins. Methanolic extract of corn silk gave the maximum extraction of phytochemicals than any other extracts. Methanolic extract was followed by ethanolic extract for the presence of phytochemical constituents.

- Cosmetics field

Various cosmetic ingredients are derived from Zea mays (Corn) plants. These include, silk extract. Many different products can made from CS like cream, powder, liquid, capsules, gel, tincture or tea.

For instance, to make skin look and feel healthy, skin-brightening powder of CS is used. This powder contains the unique Bio-Active Complex formulated with vitamins and botanicals, excellent as a highlighter, Brightens and minimize the appearance of pores.

Dermatologist tested after 3 days of CS extract on Melan-A cells showed that corn silk extract at the 100ppm concentration decreased melanin production by 37.2% without cytotoxicity.

Powdered CS can be used to make poultices for easing boils and inflamed skin, the powder can be added to skin products to soothe the face and to make face powders and in face masks to help detoxify the skin.

The skin color of the human body is determined by melanin, carotenoids, hemoglobin, and bilirubin, among which melanin is the most important factor. Although melanin protects the skin, the hyper-production of melanin pigment can cause melasma, freckles, and dark spots. To prevent or improve skin darkening due to melanin hyper-production, skin pigment-suppressing agents using kojic acid, arbutin, or licorice extract have been developed, but various problems such as adverse side effects and weak efficacy have been observed.

CS has the inhibitory effect on melanin production in Melan-A cells by measuring melanin production and protein expression. In addition, corn silk extract aqueous solutions were applied on the human face with hyper pigmentation twice a day for 8 weeks, and skin color was measured to check for any adverse reactions and examine the degree of skin pigment reduction. The results of cell viability and melanin production after 3 days of sample treatment on Melan-A cells showed that corn silk extract at the 100ppm concentration decreased melanin production by 37.2% without cytotoxicity.

3.1.2 Phytochemicals of corn silk

Phytochemicals: They are plant chemicals. Phytochemicals are defined as bioactive non-nutrient plant compounds in fruits, vegetables, grains, and other plant foods that have been linked to reducing the risk of major chronic diseases. They are otherwise called as the secondary metabolites. The phytochemicals vary in distribution within the plant parts, as well as in their occurrence within plant species.

Corn silk contains phytochemicals as flavonoids, saponins, tannins and alkaloids. It also contains proteins, carbohydrates, fibres and lipids. By going to the mineral contents of CS, these are the most important minerals of CS (Ca, Mg, K, Na, Cu, Fe, Mn and Zn). Phytochemicals and minerals of mature and immature corn silks were obtained according to a scientific research paper titled"Nutritional compositions and anti-oxidative capacity of the silk obtained from immature and mature corn"written by Nurhanan Abdul Rahman and W.I. Wan Rosli.(N.A. Rahman, n.d.)

In another research, the phytochemical analysis of the benzene, chloroform, ethanol, ethyl acetate, methanol and petroleum ether extracts of corn silk showed positive results for the presence of flavonoids, alkaloids, phenols, steroids, glycosides, carbohydrates, amino acids, terpenoids and tannins. Methanolic extract of corn silk gave the maximum extraction of phytochemicals than any other extracts. Methanolic extract was followed by ethanolic extract for the presence of phytochemical constituents.(Thoudam Bhaigyabati, 2011).

Flavonoids are a widely distributed group of plant phenolic compounds, which are effective as antioxidants. The flavonoids are categorised in different classes as alkaloids, terpenoids and phenolics. Flavonoids carry out a number of protective functions in the human body. Many flavonoids have evolved as bioactive compounds that interfere with nucleic acid or proteins and show antimicrobial or insecticidal and pharmacological properties.

Flavonoids are therefore of interest in medicine as therapeutics and at the same instance in agriculture as pesticides. Flavonoids have also been recognised for their antimicrobial activity and many researchers have isolated and identified the structures of flavonoids having properties of antifungal, antiviral and antibacterial activity. Because of this property, many flavonoids are now being used extensively in the fields of nutrition, food safety and health.

Flavonoids can prevent injury caused by free radicals in various ways and one way is the direct scavenging of free radicals. Flavonoids are oxidised by radicals, resulting in a more stable, less-reactive radical. In other words, flavonoids stabilise the reactive oxygen species by reacting with the reactive compound of the radical. Because of the high reactivity of the hydroxyl group of the flavonoids, radicals are made inactive. (A. N. Panche, 2016).

Saponins are glycosides with foaming characteristics. Saponins consist of a polycyclic aglycone attached to one or more sugar side chains. The aglycone part, which is also called sapogenin, is either steroid (C27) or a triterpene (C30). The foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part. The non-sugar part of saponins have also a direct antioxidant activity, which may results in other benefits such as reduced risk of cancer and heart diseases.(Phytochemicals, 2016).

Tannins are complex chemical substances derived from phenolic acids (sometimes called tannic acid). They are classified as phenolic compounds, which are found in many species of plants. They are large molecules that bind readily with proteins, cellulose, starches, and minerals. These resulting substances are insoluble and resistant to decomposition. The tannins in corn silk have been medically proven to help prevent urinary tract infections by reducing the ability of the bacteria *E. coli* from adhering to cells lining the urinary tract.(USDA, 2014)

3.2Nutrients, minerals and flavonoids compositions

3.2.1 Nutrients compositions

The silks were extracted with ethanol using the Soxhlet extraction method to determine the polyphenol and ABTS radical scavenging capacity. From this study, the highest content of total polyphenol of immature silks was exhibited by ethanol extract (92.21 mg GAE/g). In the ABTS free radical assay method, all immature silk extracts had higher percentage of inhibition compared to the mature silks. The ethanol extract of immature (EC_{50} = 0.478 mg/ml) and mature silk (EC_{50} = 0.799 mg/ml) exhibited the strongest antioxidant capacity followed by the water and ethyl acetate extract.(Nurhanan AbdulRahman, 2014).

The silks of immature and mature corn were evaluated for their variations in nutritional compositions, mineral content and antioxidant capacity.

Table 1: Nutritional compositions of mature and immature corn silk

Nutritional compound	Immature silks	Mature silks
Moisture (fresh)	89.31	84.42
Moisture (Oven dried)	4.15	3.90
Crude lipid (% dry basis)	1.27	0.66
Crude protein (% dry basis)	12.96	8.95
Ash (% dry basis)	5.28	5.51
Carbohydrate (% dry basis)	27.80	29.74
Total dietary fiber (g/100g)	48.50	51.24

Immature silks are rich in protein and lipids. While mature silks are richer in Carbohydrate and total dietary fibber than the immature silks. (N.A. Rahman, n.d.)

3.2.2 Minerals compositions

By going to the minerals of corn silk. There is a significant variation between mature and immature corn silks in the mineral content. Immature silks have a higher level in calcium, magnesium, Copper and zinc minerals than mature silks. While the mature is rich in potassium, sodium, Iron and manganese. The following table shows a comparison between Mature and immature corn silks in mineral levels. (N.A. Rahman, n.d.)

Table 2: Minerals compositions in mature and immature corn silk

Minerals	Immature silk, µg/g CS	Mature silk, µg/g CS
Calcium, (Ca)	1087.08	707.04
Magnesium, (Mg)	1219.17	361.50
Potassium, (K)	26281.67	35671.67
Sodium, (Na)	190.67	266.67
Copper, (Cu)	5.6	4.12
Iron, (Fe)	2.17	4.50
Manganese, (Mn)	32.17	35.57
Zinc, (Zn)	46.37	35.92

3.2.3 Flavonoids compositions

A recent study showed that the total flavonoids (TFC) content of the butanol fraction of CS extract is in good correlation with the total phenolic content (TPC).Butanol fraction of CS is significantly higher in TPC and TFC.(Khairunnisa Hasanudin, 2012)

Table 3 : Total flavonoids content in corn silk determined by butanol extraction.

Process	Phenolic compound	wt %
Butanol extraction	Gallic acid equivalent	164.1 µg Gallic Acid Equivalent (GAE)/g DCS
Butanol extraction	Rutin equivalent	69.4 µg Rutin Equivalent (RE)/g DCS

The upper (dark brown) parts of corn silk (mature) had higher amount of total phenolics than the lower parts (immature) of CS.

Table 4: Total phenolic content in corn silk

Consistent	wt. % in Upper part	wt. % in lower part
total phenolics	180 µg GAE/g F.W	151.33 µg GAE/g F.W
total anthraquinones	17.22 µg/g F.W	8.61 µg/g F.W
total flavonoids	119.47 µg/g F.W	101.66 µg/g F.W

A flavonoid, 3'-methoxymaysin and reduced derivatives of maysin have been isolated and identified from CS of several corn inbreeds.

The compounds isolated include

- 2"-O-α-L-rhamnosyl-6-C-quinovosylluteolin,
- 2"-O-α-L-rhamnosyl-6-C-fucosylluteolin.
- 2"-O-α-L-rhamnosyl-6-C-fucosyl-3'-methoxyluteolin.

Five other flavonoid derivatives were isolated from CS ethanol extract (80%) and identified as

- 2''-O-α-L-rhamnosyl-6-C-3''-deoxyglucosyl-3'-methoxyluteolin
- 6,4'-dihydroxy-3'-methoxyflavone-7-O-glucosides
- ax-5''-methane-3'-methoxymaysin.
- ax-4''-OH-3'-methoxymaysin
- 7,4'-dihidroxy-3'-methoxyflavone
- 2-O-α-L-rhamnosyl-6-C-fucoside.

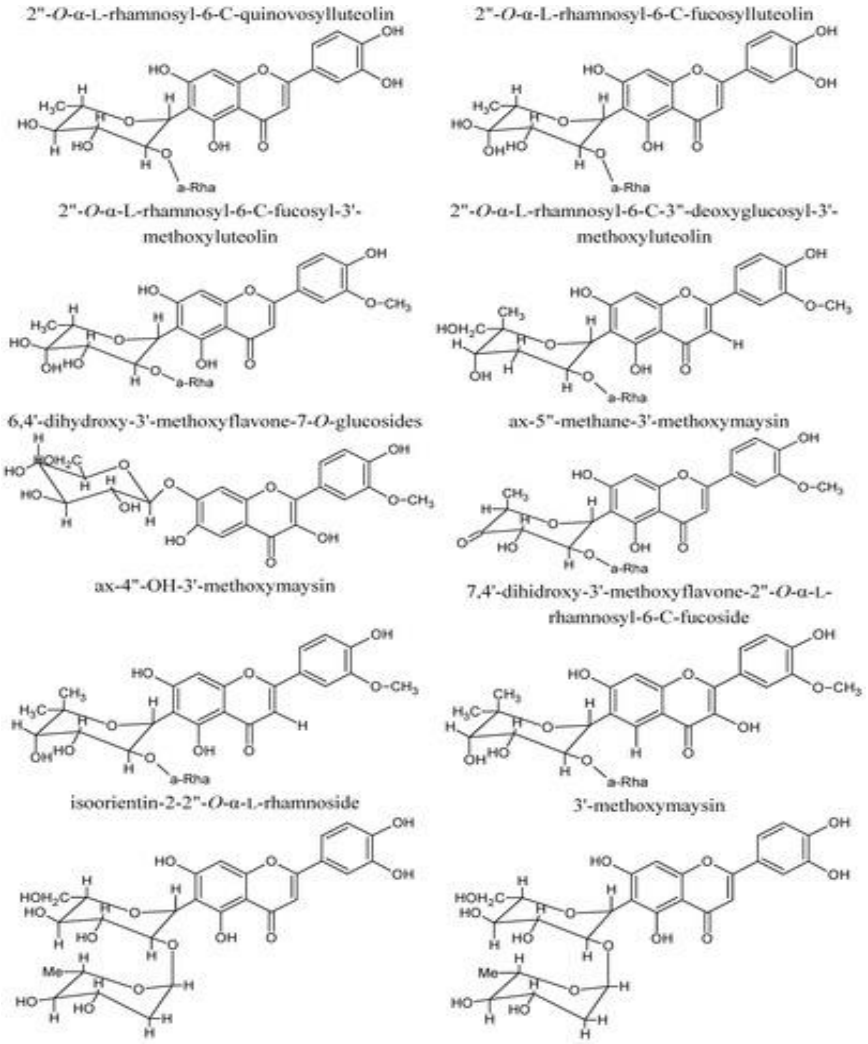


Figure 1: Flavonoids structures in corn silk.

3.3 Healing effectiveness of corn silk

3.3.1 Treatment of Urinary Tract Infection

A urinary tract infection tract also may be called cystitis or a bladder infection is what happens when bacteria (germs) get into the urinary tract (the bladder) and multiply. The result is redness, swelling and pain in the urinary. Bladder infections or urinary tract infections (UTI) are very common in women but less so in men, a UTI in a man may be caused by an enlarged prostate gland, which can block the flow of urine.

Most UTIs stay in the bladder, the pouch-shaped organ where urine is stored before it passes out of the body. If a UTI is not treated promptly, the bacteria can travel up to the kidneys and cause a more serious type of infection, called pyelonephritis (pronounced pie-low-net-rightists).

Pyelonephritis is an actual infection of the kidney, where urine is produced. This may result in fever and back pain. Children have though less likely often than adults. Girls, especially between the ages of 4 and 8, are more likely to have UTIs than boys. Because it's easy to overlook symptoms of a UTI in children, parents should look for the following signs of a possible UTI.(Anon., 2010) The main symptoms which usually are easy to treat are burning, itching and constant urge to urinate.

The use of CS herbal medicine in treatment of chronic and acute UTI is not a new approach certainly. The main property of this herb that is used for this treatment is that it has a variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids; these metabolites have been found invitro to have specific antimicrobial properties.

CS can also be taken as a tea to sooth and treat the symptoms of UTI. Corn silk is best used in combination with other stronger antiseptic herbs to treat bladder infections but it will provide effective symptom relieve from burning and pain associated with UTI. (Vijitha T P, 2017)

3.3.2 Treatment of CKD (Chronic Kidney Disease)

CKD is basically a common kidney disease, where patients will have gradual reduction of kidney function over time and it includes conditions that damage your kidneys and decrease their ability. If the disease gets worse, wastes and extra fluids will build up in blood and make the body feel sick. Over time, the patient experiences a host of complications like high blood pressure, anemia, weak bones, poor nutritional health and never damage. In addition, this disease also increases the risk of having heart and blood vessels diseases. For its treatment, corn silk is usually prescribed in the form of corn silk tea. Corn silk tea has the function of increasing the urine output, which can help remove the toxins and wastes out, hence reducing creatinine level. In addition, it also helps remove the excess fluid out, which can help relieve the swelling. High blood pressure, being the most prominent symptom, is reduced with the help of corn silk tea.(Vijitha T P, 2017)

3.3.3 Treatment of Nephrotoxicity

Nephrotoxicity is toxicity in the kidneys. It is a poisonous effect of some substances, both toxic chemicals and medications, on function. The symptoms which usually are need to treat are excess urea concentration in the blood (azotaemia), anaemia, increased concentration of hydrogen ions in the blood (acidosis), excess body fluids (over hydration), and high blood pressure (hypertension).Grinded powder of corn silk and binahong leaves and the extract was evaporated using rotary vacuum evaporator to obtain viscous extract that was referred to as ethanol extract. This is how to use corn silk for treatment. Corn silk and binahong leaves could improve kidney function in rat model of kidney failure. Combination of half dose of each extract showed significant improvement in functioning of the kidney.(Vijitha T P, 2017)

3.3.3Anti-diabetic effect

The Glycaemic Index (GI) is a relative ranking of carbohydrate in foods according to how they affect blood glucose levels. Hyperglycaemia occurs when blood sugar levels are too high. People develop hyperglycaemia if their diabetes is not treated properly. Hypoglycaemia sets in when blood sugar levels are too low. Diabetes is a common metabolic disease with far-reaching

health effects. In type 1 diabetes, the body only produces very little insulin, or none at all. In type 2 diabetes, not enough insulin is released into the bloodstream, or the insulin cannot be used properly.

A recent study aimed to investigate the physicochemical properties and anti-diabetic effects of a polysaccharide obtained from corn silk. PCS was isolated and the physicochemical properties were characterized. The hypoglycaemic effects were determined using the high-fat diet and streptozocin induced type 2 diabetic mellitus (T2DM) insulin resistance mice. The results showed that PCS2 was a heteropolysaccharide with the average molecular weight of 45.5 kDa. PCS2 was composed of d-galactose, d-mannose, d-(+)-glucose, d-(+)-xylose, l-arabinose and l-rhamnose. PCS2 treatment significantly reduced the body weight loss, decreased blood glucose and serum insulin levels, and improved glucose intolerance ($P < 0.05$). The levels of serum lipid profile were regulated and the levels of glycated serum protein, non-esterified fatty acid were decreased significantly. The activities of superoxide dismutase, glutathione peroxidase and catalase were notably improved. PCS2 also exerted cyto-protective action from histopathological observation. These results suggested that PCS2 could be a good candidate of functional food or medicine for T2DM treatment (Wenzhu Zhaoa, 2012).

Another study showed that, the ingestion of CSFs with a dose under 500 mg/kg had no observed adverse effect on normal mice and it had significant anti-diabetic potential, accompanied with anti-oxidant and anti-hyper-lipidemic activities(Yan Zhang, 2015).

3.3.4 Anti-Oxidant activity

Free radicals are atoms, molecules, or ions that have an unpaired valence electron. They are unstable which can damage body cells. They often occur as the result of normal metabolic processes. For example, when human body uses oxygen, it creates free radicals as a by-product and the damage caused by those free radicals is called "oxidative stress."

Oxidative stress is an excessive production of reactive oxygen species (ROS) outstripping antioxidant defence mechanism, has been implicated in the pathophysiological conditions that affect the cardiovascular system, and can cause severe damage to biological macromolecules and deregulation of normal metabolism.

However, Antioxidants are free-radical scavengers which provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species and subsequent lipid peroxidation, protein damage and DNA strand breaking.

Anyway, there was a recent study on the anti-oxidant activity of corn silk constituents using several solvents of extraction.

1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for the determination of free radical scavenging activity of the various extracts. The indication was changing the colour of DPPH from deep violent to white yellow. Solvents used for extraction were Methanol, Ethanol, Benzene, Chloroform, Ethyl acetate and petroleum ether.

The methanol extract of corn silk with 100µg concentration gives higher percentage (95.6%) of free radicals scavenging activity than the other extracts. Low percentage (44.2%) of free radical scavenging activity was noted in chloroform extract of the corn silk.

Another investigation has been done in this study to evaluate the total anti-oxidant activity by phosphor-molybdenum method using the same previous solvents. Methanol extract of corn silk exhibited the strongest antioxidant activity (85.2 mg/ml) among all the other extracts, while ethyl acetate extract yielded the lowest (45.5 mg/ml). The total antioxidant activity may be attributed to the presence of Phenolic and flavonoids constituents in all the fractions.(Ebrahimzadeh, et al., 2008)

3.3.5 Anti-obesity effect

Obesityis a chronic disease caused by multiple factors such as consumption behavior, life style, genetics, and medication. As the result, the body fat and weight excessively increase, and obesity is defined when body mass index >30 (kg/m^2) that can lead to other metabolic syndromes such as dyslipidaemia, ischemic stroke, heart failure, and cancer that can cause mortality. Flavonoids including apigenin, genistein, catechin, quercetin and anthocyanins were reported as anti-obesity agents involving regulation of adipocyte life cycle: anti-adipogenesis and lipolysis activity.

The anti-obesity potential of CSEs involving anti-adipogenesis and lipolysis induction is manifested in association with the composition of flavonoids and phenolics as the functional ingredient. The quercetin derivatives, the major components in the sweet CSEs, are believed

to play major roles in anti-obesity activity involving inhibition of preadipocyte proliferation and differentiation as well as induction of adipocyte lipolysis(else, 2016).

3.4 Toxicity investigation

The interest in using herbal medicines has increased over the years. Being natural and traditionally used make users, think herbal medicines are safe and harmless. Thus, it is important to carry out toxicity studies and determine the safety of herbal products.

According to a scientific research paper titled with "Acute and Subacute Toxicity Evaluation of Corn Silk Extract", written by AeWha Ha and others. The main objective of this paper was to determine the acute and subacute toxicity of corn silk extract in ICR mice. In addition, investigating the safety of corn silk extracts including high maysin, and the applicability of corn silk extracts containing high maysin as a functional food material.

Acute toxicity is defined as the unwanted effect(s) that occurs either immediately or at a short time interval after a single or multiple administration of such substance within 24 hours.

While **Sub-acute toxicity** is defined as adverse effects occurring after multiple or continuous exposure between 24 h and 28 days.

Investigations were applied in ICR mice. ICR is an abbreviation related to the Institute of Cancer research. From ICR, the Swiss Albino mice (Commonly used) was discovered. From that time, it is known as ICR mice. ICR mice mean an commonly use outbred mice. Outbred mice mean the mice that is produced by using father and mother mice whom are collecting not within family or same mother(Ji Eun Kim, 2017).

The investigations gave positive results. It is found that, when corn silk extract is consumed in excess, there are some side effects such as allergic reactions and stimulation of uterine contraction in rabbits due to unknown toxic substances. Moreover, a recent study has reported there is no toxicity to Wistar rats even after they are administered 8% (w/w) of corn silk extract tea for 90 days. Another study evaluated the toxicity of corn silk extract administered to Wistar rats at 100, 200, and 400 mg/kg body weight for 28 days and found no haematological toxicity (Ae Wha Ha, 2018).

3.5 Corn Silk safety and Acceptable daily intake

Acceptable daily intake (ADI) is the amount of a substance that people can consume in food or beverages on a daily basis during their whole life without any appreciable risk to health.

"Sub chronic toxicity study of corn silk with rats "is a title for a scientific paper shared in the Journal of Ethno pharmacology which is written by Cuina Wang and else. The main aim of this research was to investigate the safety of corn silk by a sub chronic toxicity test performed in Wistar rats.

Raw corn silk is used in this study, because some processing can alter its components, may affect the activity of many substances, and furthermore may affect the potential adverse effect of corn silk.

Monitoring body weight gain and food consumption in feeding studies with rats, ruminant, pigs and poultry can be a sensitive indicator of overall animal health. Feeding of corn silk at level up to 8.0% in feed to rats for 90 days did not affect the body weights and feed intake. Likewise, no differences were observed in body weights and feed intake. Females consumed greater amounts of corn silk on a g/kg body weight basis than males. For example, in 8.0% females, the average consumption of corn silk was 10.308 g/kg/day, while 9.354 g/kg/day in males at the same nominal level.

The results showed a lack of toxicologically significant adverse effects following oral administration of corn silk to Wistar rats at doses up to 8.0%.

Based on the present study, the no-observed-adverse-effect level (NOAEL) of corn silk is at least 8.0% ,which corresponds to a mean daily corn silk intake of approximately 9.354 and 10.308 g/day/kg body weight for males and females, respectively(Cuina Wang, 2011).

3.6 Alternative sweetener: Stevia plant

3.6.1 Historical use and constituents of the plant

The Stevia plant is native to South America and was first consumed there over 200 years ago when the indigenous people used leaves of the plant to sweeten beverages or chewed them for their sweet taste. Stevia is a naturally sourced, zero-calorie sweetener that has been used as a natural sugar substitute and flavouring ingredient for hundreds of years. The plant leaves, often called ‘‘sweet herb,’’ were dried and used to sweeten teas and medicines or simply chewed as a sweet treat. It has been reported to be between 30 and 320 times sweeter than sucrose.

Stevia is cultivated mostly in Paraguay, Kenya, China, and the United States and within many other parts of the world, including Vietnam, Brazil, India, Argentina, and Colombia. Fortunately, Stevia plant is successfully cultivated in Palestinian. Lama Omar and Lara Bdeir have accomplished this project, which is supported by the Deprived Families Economic Empowerment Program (DEEP) that belongs to the Palestinian Ministry of Social Development.

The sweet-tasting components of Stevia are called steviol-glycosides. They have a wide variety of naturally occurring substances in which a carbohydrate portion, consisting of one or more sugars or combined with a hydroxyl compound. Glycosides is a construction consisting of two parts, an aglycone (genin) unit, which is mainly lipophilic, and a glycone unit which is hydrophilic and composed of one or more sugar components. The glycone and aglycone portions can be chemically separated by hydrolysis in the presence of acid and can be hydrolysed by alkali.

Stevia plant consists of 11 different steviol glycosides. Rebaudioside A, RebaudiosideB, RebaudiosideC, Rebaudioside D, Rebaudioside E, Rebaudioside F, Rubusoside, Steviolmonoside, Steviolbioside, Dulcoside A. Rebaudioside A, Rebaudioside B and Stevioside Respectively, have the most sweeting strength relative to Sucrose. While, Rubusoside has the lowest one among them.

3.6.2 Stevia Metabolism

Steviol glycosides pass through the upper gastrointestinal tract. Gut bacteria in the colon hydrolyse-steviol glycosides into steviol by snipping off their glucose units. Steviol is then absorbed through the portal vein and primarily metabolized by the liver, forming steviol-glucuronide, which is primarily excreted in the urine.

Research shows that there is no accumulation of Stevia (or any component or by-product of Stevia) in the body and that it passes through the body during metabolism. Energy from fermentation of glucose units (usually assessed as 2 kcal/g) is so low that it is minimal and so, effectively, Stevia can be said to provide zero calories. (High-purify Stevia leaf extract is not metabolized, so it provides zero calories).

3.6.3 Stevia safety

Several regulatory authorities have rigorously evaluated more than 200 peer-reviewed studies on animals and humans examining the safety of high-purity steviol glycosides. Based on this evidence, JECFA has established an ADI that applies to adults and children. The ADI is expressed as steviol equivalents of 4 mg/kg of body weight per day.

Stevia has been shown to be safe in more than 200 studies, and JECFA has established an ADI of 4 mg/kg body weight per day, expressed as steviol equivalents, to guarantee this safety to consumers.

Chapter four: Methodology

4.1 Research methodology

4.1.1Extraction of the active ingredients from corn silk

Solid-liquid extraction is the solid phase that containing solute is dispersed in solvent and mixed. The solute is then extracted from solid phase to the solvent and the solid phase in removed by suction filtration.

Extraction uses the property of solubility to transfer a solute from one phase to another phase. In order to perform an extraction, the solute must have a higher solubility in the second phase than in the original phase.

Extraction of the active ingredients from corn silk is done by mixing ethanol solution with dried, grinded corn silk. Depending on the polarity of the active ingredients of corn silk which is rich in phenolic compounds that have the hydroxyl group

Ethanol is used to be as the safest and the most suitable solvent. It is applied on 20 grams grinded corn silk and discussed in Chapter Six.

4.1.2 Increasing the efficiency of extraction and optimizing the most suitable conditions of extraction

The conditions of mixing are optimized and discussed in Chapter Six to provide the safest process and for getting the maximum yield of extraction for a 20 grams sample of corn silk be as the following:

1. Volume of solvent added in ml to the weight of dried corn silk in grams was 7:1
2. Type of mixer impeller is pitch impeller
3. Speed of rotation equals 350 rpm
4. Time of mixing equals 4 hours

4.1.3 Gas chromatography mass spectroscopy test

The phytochemical investigation of ethanolic extract was performed on a GC-MS equipment. GC-MS is concerned with the determination of phytochemicals present in the corn silk extract and the concentration of each component. As well as the chemical structure and the molecular weight of each one.

GC-MS is preferred to be used over HPLC test because of the volatile nature of the active ingredients of corn silk since HPLC test is used to analyze volatile and non-volatile compounds.

GC-MS begins with the gas chromatograph, where the sample is volatized. This effectively vaporizes the sample (the gas phase) and separates its various components using a capillary column packed with a stationary (solid) phase. The compounds are propelled by an inert carrier gas such as argon, helium or nitrogen. As the components become separated, they elute from the column at different times, which is generally referred to as their retention times.

GC-MS test gave positive results for the presence of poly phenolic compounds that is defined as flavonoids, tannins, alkaloids and saponins. The various components present in the entire ethanolic extract of corn silk were detected by the GC-MS test as counted below.

1. Triphenylphosphine oxide
2. Hexane-dioic acid, dioctyl ester
3. Decanoic acid, decyl ester
4. Hexa-decanoic acid, ethyl ester
5. 4,4,5,6-Tetramethyltetrahydro-1,3-oxazin-2-thione
6. 1-Undecyne
7. 1,12-Tridecadiene
8. E,E-1,9,17-Decasatriene
9. Pentadecanoic acid, 2,6,10,14-tetramethyl- methyl ester

The following figure shows the **separation chromatogram** for ethanolic extract of corn silk. Experimental conditions: sample weight 20 gram; temperature 37 °C; time of extraction 4 hours.

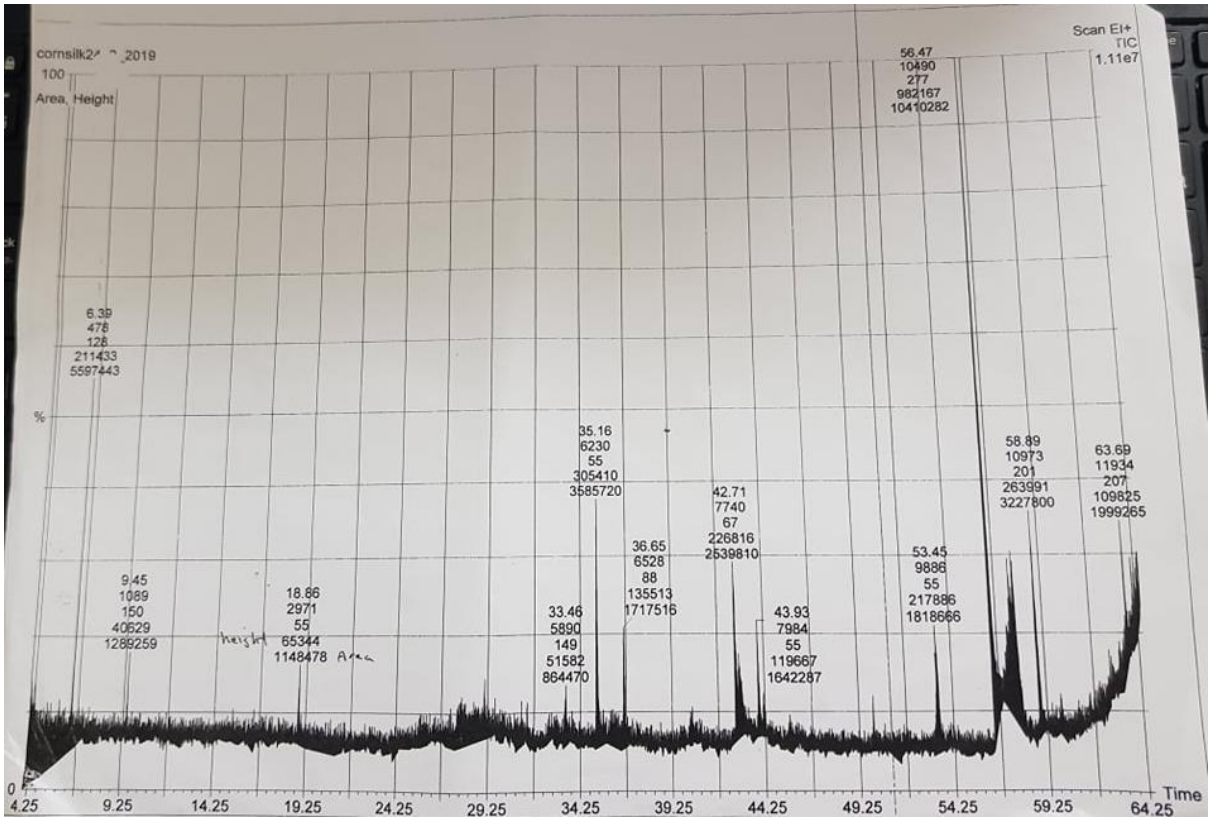


Figure 2: Separation chromatogram of ethanolic extract of corn silk

4.1.4 Phenolics and flavonoids content in corn silk

Phenolic compounds are large class of secondary metabolites produced by plants to defend themselves against pathogens. Phenolics or polyphenols have received considerable attention because of their physiological function, including their antioxidant activities and free radical scavenging abilities, which improves its beneficial implications for human health.

Flavonoids are water soluble polyphenolic molecules containing 15 carbon atoms. And free radical scavengers. They are belonging to the polyphenol family. flavonoids have the ability to prevent oxidative cell damage, also it has strong anticancer activity. They can lower the risk of heart disease. As antioxidants, flavonoids from these plants provide anti-inflammatory activity.

In this project total phenolic and total flavonoids content were determined with the corporation of Dr.Fuad Al-Rimawi from Al-Quds University. The experiments are discussed in the experimental section and the results are shown and discussed in Chapter Six.

4.1.5 Antioxidant activity and free radical scavenging tests

Free radicals are atoms, molecules or ions with unpaired electrons, which are highly active to chemical reactions with other molecules. In the biology system, the free radicals are often derived from oxygen, nitrogen and sulfur molecules.

As discussed in Chapter three these free radicals are parts of groups of molecules called reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulfur species (RSS). For example, ROS includes free radicals such as superoxide anion ($O_2^{-\bullet}$), per hydroxyl radical ($HO_2^{\bullet}$), hydroxyl radical (·OH), nitric oxide (NO), and other species such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl).

In this project, ethanolic extract of corn silk showed a strong result in free radicals scavenging by its phenolic compounds. Four different tests are made to check the antioxidant power of the active ingredients. 99 wt.% ethanol extraction and 80 wt.% ethanol extraction in addition to water solutions are used and compared. The procedures and results are shown and discussed in the experimental section in Chapter five and Chapter Six.

Free radical scavenging and antioxidant strength determined by the following tests:

1. Free radical scavenging activity using DPPH
2. Cupric reducing antioxidant power
3. Free radical scavenging activity using ABTS
4. ferric reducing antioxidant power

Chapter Five: Experimental methods

4.1Ethanol extraction of corn silk

5.1.1 Pre experiment preparation

At this stage, a few different pre-experiments are carried out as the following:

1. Corn silk raw material is collected and dried for 10 days with continuous reciprocating to avoid rotting of raw material.
2. CS is subjected to a stainless-steel grinder in order to increase the contact area between solid material and solvent.
3. Corn bags are prepared by discharging tea bags and filling them manually.
4. CS label is designed for the trade mark

5.1.2 Ethanol extraction under optimum conditions

1. 20 grams of dried, grinded corn silks collected for extraction
2. 400 ml of 99.8wt% ethanol solution is added to the grains and covered to avoid Ethanol evaporation.
3. Mixing of ethanol solution and solid material is accomplished for 4 hours
4. Heater is added to increase the efficiency of extraction; solution temperature was measured with thermometer to be 37°C
5. The extract phase is filtrated by suction filtration and taken into a 1 litter flask for ethanol and solid content recovery

5.1.3 Recovery of active ingredients using rotary evaporator under vacuum pressure

1. Ethanol extract phase is poured in 1 litter flask and weighted to evaluate the percentage of yield after evaporation
2. Speed of rotation is adjusted at 4 rpm.
3. Dry ice is added to water container for performing condensation, and the vacuum pump is performed for safety regulation.
4. Ethanol is vaporized and the remains is weighted to be 1.45 grams
5. Non-Volatile active ingredients concentration is determined as the following:

$$wt\% = \frac{Mass\ of\ extracted\ ingredients\ (g)}{Mass\ of\ sample\ (g)} * 100\% = \frac{1.45}{20} * 100\% = 7.25\ \%$$

5.2 Measurements of antioxidant activity

5.2.1 FRAP assay

The antioxidant activity of the extracts was determined using a modified method of the assay of ferric reducing/antioxidant power (FRAP) of Benzie and Strain (1999) [13]. Freshly prepared FRAP reagent (3.0 mL) was warmed at 37°C and mixed with 40 µl of the extract and the reaction mixtures were later incubated at 37°C. Absorbance at 593 nm was read with reference to a reagent blank containing distilled water which was also incubated at 37 °C for up to 1 hour instead of 4 min, which was the original time applied in FRAP assay. Aqueous solutions of known Fe^{+2} concentrations in the range of 2-5 mM were used for calibration, and results were expressed as mmol Fe^{+2} /g.

5.2.2 Cupric reducing antioxidant power (CUPRAC assay)

The cupric ion reducing antioxidant capacity of the extracts was determined according to the method of Apak et al. (2008) [14]. 100 µl of sample extract was mixed with 1ml each of 10 mM of cupper chloride solution, 7.5 mM of neocuproine alcoholic solution (99.9% ethanol), and 1 M (pH 7.0) of ammonium acetate buffer solution, and 1 ml of distilled water to make final volume 4.1 ml. After 30 min, the absorbance was recorded at 450 nm against the reagent blank. Standard curve was prepared using different concentrations of Trolox. The results were expressed as µmol Trolox/g.

5.2.3 Free radical scavenging activity using DPPH (DPPH assay)

DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable DPPH radical, and the procedure was done according to Brand-Williams et al. (1995) [15]. A 3.9 mL aliquot of a 0.0634 mM of DPPH solution in methanol (95%) was added to 100 µl of each extract. The mixture was vortexed for 5-10 sec. The change in the absorbance of the sample extract was measured at 515 nm for 30 min till the absorbance reached a steady state. Standard curve was prepared using different concentrations of Trolox. The results were expressed as µmol Trolox/g.

5.2.3 Free radical scavenging activity using ABTS (ABTS assay)

A modified procedure using ABTS (2, 2-azino-di-(3-ethylbenzothialozine-sulphonic acid)) as described by Pellegrini et al. (1999) was used [16]. The ABTS stock solution (7 mM) was prepared through reaction of 7 mM ABTS and 2.45 mM of potassium per-sulphate as the oxidant agent. The working solution of $ABTS^{+\cdot}$ was obtained by diluting the stock solution in 99.9% ethanol to give absorption of 0.70 ± 0.02 at 734 nm. 200 µl sample extract was added to 1800 µl of $ABTS^{+\cdot}$ solution and absorbance readings at 734 nm were taken at 30 °C exactly 10 min after initial mixing (A). The radical-scavenging activity of the test samples was expressed as Trolox equivalent antioxidant capacity TEAC (µmol Trolox/g sample).

5.3 Total phenolic content and total flavonoids content measurements

5.3.1 Total phenolic content (Folin-Ciocalteu assay)

Total phenolics were determined using Folin-Ciocalteu reagents. Plant extracts or gallic acid standard (40 µl) were mixed with 1.8 mL of Folin-Ciocalteu reagent (prediluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min, and then 1.2 mL of sodium bicarbonate (7.5%, w/v) was added to the mixture. After standing for 60 min at room temperature, absorbance was measured at 765 nm. Aqueous solutions of known gallic acid concentrations in the range of 10 - 500 mg/L were used for calibration. Results were expressed as mg gallic acid equivalents (GAE)/ g sample.

5.3.2 Total flavonoids content

The determination of total flavonoids was performed according to the colorimetric assay of Kim et al. (2003). Distilled water (4 mL) was added to 1 mL of the extract in a test tube. Then, 0.3 mL of 5% sodium nitrite solution was added, followed by 0.3 mL of 10% aluminum chloride solution. Test tubes were incubated at ambient temperature for 5 minutes, and then 2 mL of 1 M sodium hydroxide were added to the mixture. Immediately, the volume of reaction mixture was made to 10 mL with distilled water. The mixture was thoroughly mixed using test tube shaker and the absorbance of the pink color developed was determined at 510 nm. Aqueous solutions of known catechin concentrations in the range of 50 - 100 mg/L were used for calibration and the results were expressed as mg catechin equivalents (CEQ)/ g sample.

Chapter Six: Results and Discussion

6.1 Optimization for the conditions of extraction

It is desired to get a high yield of extraction by studying the parameters which controls the contact between the solvent and the dried, grinded solids of corn silk.

The main parameters that controls the effectiveness of extraction:

1. Type of solvent.
2. Time of extraction.
3. Temperature of solution.
4. Volume of solvent added.
5. Speed of rotation.
6. Method of extraction.

6.1.1 Type of solvents

Depending on the Literature survey several types of solvent used and studied on corn silk to achieve the safest and the most suitable method of extraction. Solvent should have crucial characteristics in order to achieve the safest extraction of the polyphenolic compounds from corn silk. As this project deals with the medical and dermal health of the human body it is essential to take lethal and dermal toxicity into consideration.

The most important specifications that should be taken into consideration while choosing the most suitable solvent are mentioned as the following:

1. **Non-toxicity:** corn silk tincture can be used as drops in many drinks in the treatment of urinary tract infection, or it can be as corn bags in the principle of tea bags. So, it is essential for the solvent to be safe and non-toxic. In addition, it is used as a cream in kind of cosmetics field, therefore it should not have side effects on the skin as redness or allergy to skin.
2. **Availability:** the solvent should be abundant and available in our country
3. **Low cost:** it is undesired to have solvents with high cost. Even though it was effective in the extraction

4. **Miscibility and polarity:** solvent should have the high polarity in order to extract the active ingredients of corn silk which is polar and contain the hydroxyl group
5. **Yield:** it is defined as the amount of solute extracted by solvent divided by the mass of sample. While corn silk contains essential phytochemicals solvent should be effective in the extraction
6. **Volatility**: the solvent should be volatile in order to achieve the recovery of the active ingredients using rotary evaporator under vacuum

Screening and scoring method are used to estimate the most suitable solvent to be used in the laboratory for the extraction. The positive sign means an advantage or "good", negative sign means "unsuitable or bad" zero means "same as". The net score for each alternative provides a relative ranking

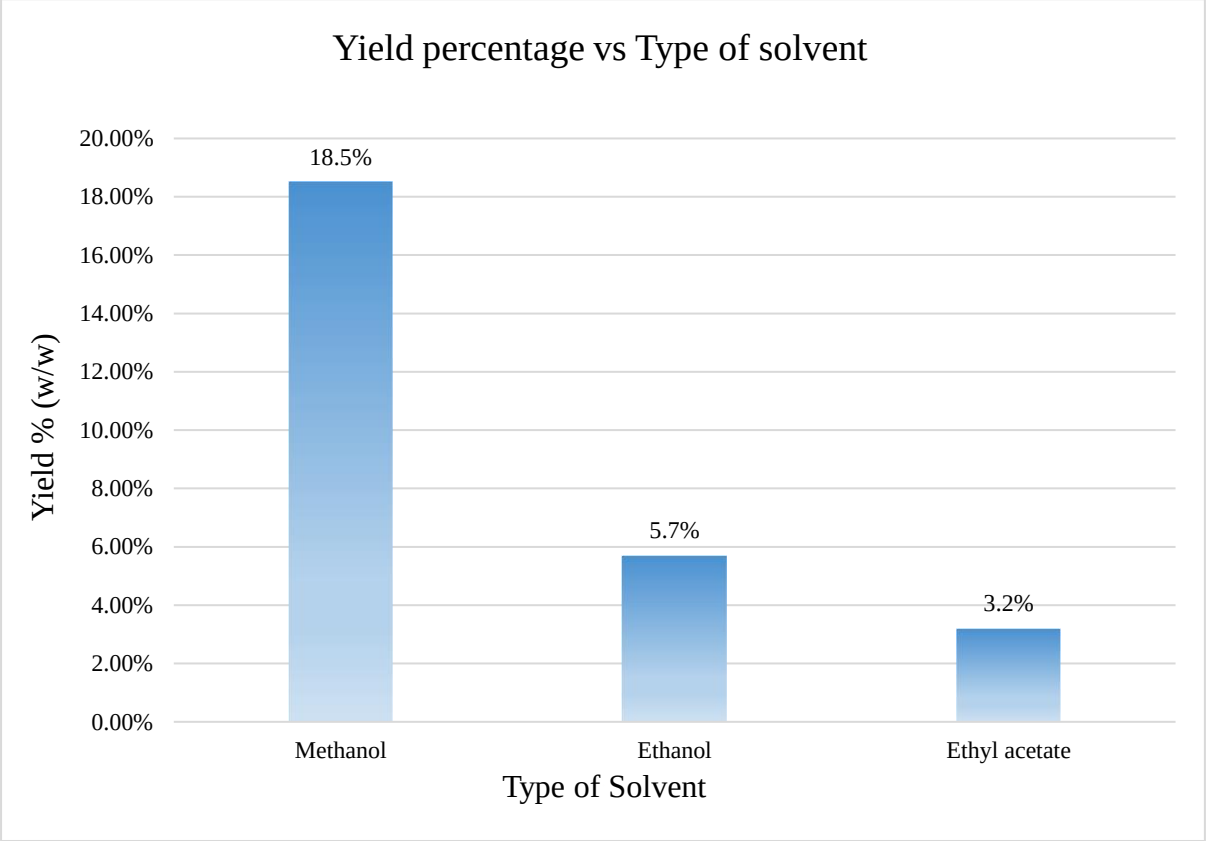
Table 5: comparison of solvents.

Criterion	Benzene	ethanol	methanol	Ethyl acetate
Toxicity	-	+	-	-
Flammability	-	-	-	-
Solubility in corn silk	0	+	+	0
cost	-	+	+	+
Availability	-	+	+	+
Volatility	+	+	+	+
Total	-3	+4	+2	+1

As benzene is highly flammable and hazardous to be used in such experiments, 99.9 wt.% ethanol, 99.95 wt.% methanol and 96 wt.% ethyl acetate are tested for the selection of solvent that provide us with highest yield and antioxidant activity.

Table (6) shows the relation between types of solvent used in the extraction VS yield percentage as weight basis. Extraction conditions were adapted for 20 grams corn silk sample, temperature 20 °C, Speed of mixing 350 rpm, time of extraction 4 hours, volume of solvent added 400 ml.

Table 6: Yield percentage vs Type of solvent



Despite Methanol extraction gave the maximum yield 18.5% of extraction for the same conditions, it should not be used because of its toxicity. Ethanol solution gave 5.7 % yield percentage which is considered to be acceptable. While ethyl acetate gave the minimum percentage. Optimum solvent is chosen to be **ethanol**.

6.1.2 Time of extraction

The contact time between solid particles of corn silk and the solvent is studied for methanol and ethanol solvents. The time range is taken from 2 hours to 5 hours. It is desired to have the optimum time of extraction for ethanol extraction to get the highest yield.

Methanol extraction is also studied to study the behaviour of extraction during time.

Table 7: Yield percentage vs Time, methanol extraction.

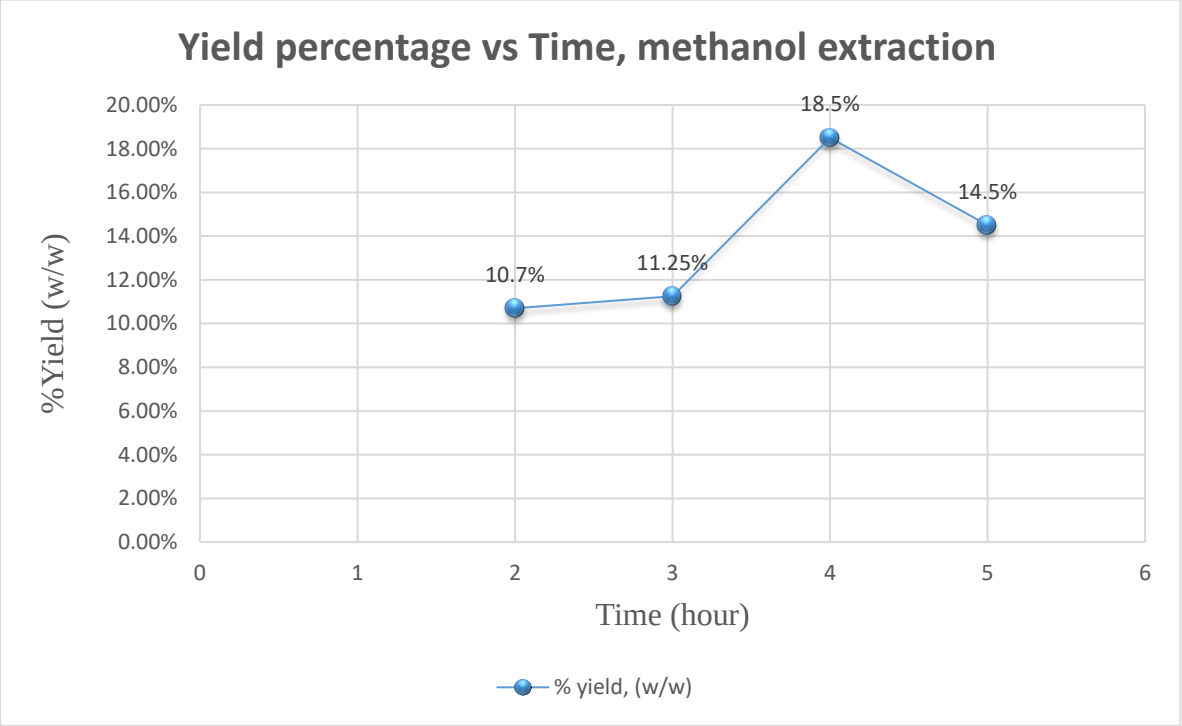
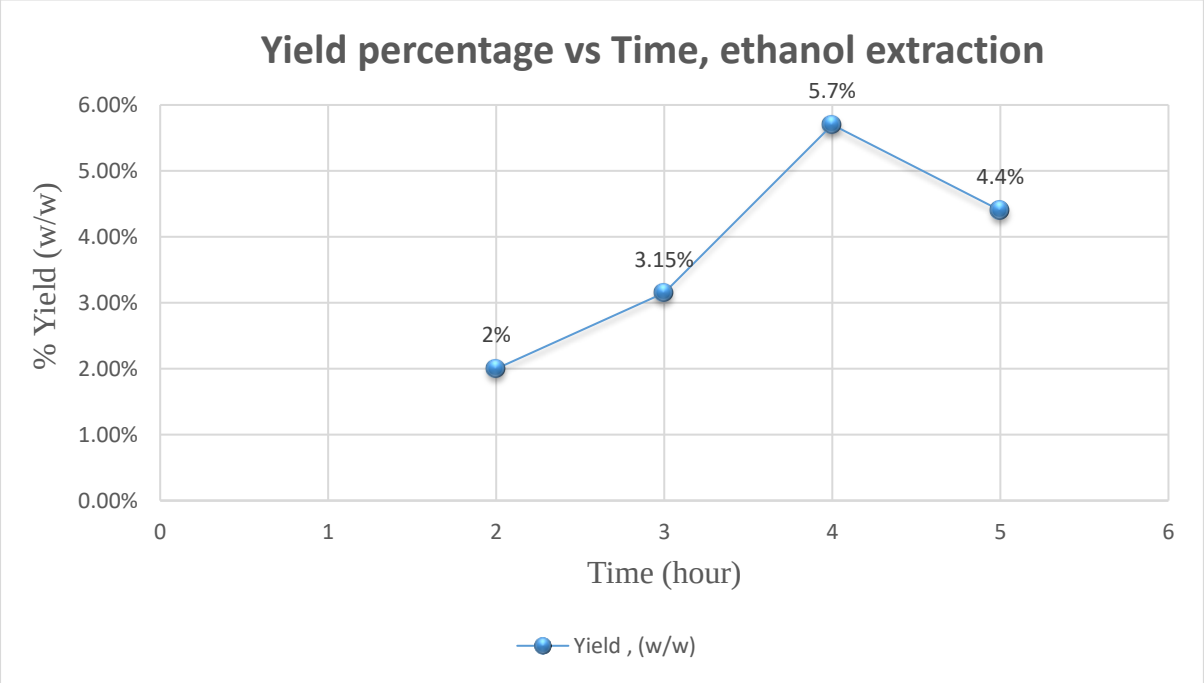


Table 8: Yield percentage vs Time, ethanol extraction.



Yield percentage versus time curves for both ethanol and methanol gave the optimum time of mixing at 4 hours. It is obvious that there may be experimental errors for yield value 5 hours. Or it may be decomposition in corn silk after 4 hours.

6.1.3 Temperature of solution

Source of heat was applied on the sample of ethanol and methanol extraction. A temperature of 37 °C was chosen like the human body temperature, it was measured by thermometer with the importance of covering the beaker with parafilm to avoid the evaporation of solvent. An extraction yield increased sharply from 18.5% at 20 °C for methanolic extraction to 24.5%. In addition, ethanolic extraction yield increased from 5.17% to 7.25%.

6.1.4 Speed of rotation

Using a very high speed of rotation because of mixer impeller used. Pitch impeller is used in the mixing of solution and it required a high speed of rotation about 350 rpm.

6.1.4 Method of extraction

Solid-liquid extraction can be achieved by three major methods:

1. Continuous solid liquid extractor: which is used for large amount of solid material
2. Regular mixing in a beaker: which is the method used in this project used for small amount of corn silk
3. Soxhlet extractor: which is hard to be applied on corn silk because it is used for the extraction of essential oils from seeds in very small amount.

6.2 Determination of phenolic compounds present in corn silk

After choosing the optimum conditions of extraction GC-MS test is applied on the sample and gave the following results

Table 9: GC-MS test results.

Name	Retention time	height	area	Retention index	%Area	%Height
4,4,5,6-Tetramethyltetrahydro-1,3-oxazin-2-thione	9.45	40629	1289259	1089	3.724	1.511
Decanoic acid, decyl ester	18.86	65344	1148478	2971	3.317	2.430
Hexadecanoic acid, ethyl ester	35.16	305410	3585720	6230	10.358	11.360
Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester	36.65	135513	1717516	6528	4.961	5.040
1-Undecyne	42.71	226816	2539810	7740	7.336	8.436
1,12-Tridecadiene	43.93	119667	1642287	7984	4.744	4.451
E,E-1,9,17-Decasatriene	43.93	119667	1642287	7984	4.744	4.451
Hexanedioic acid, dioctyl ester	53.463	217886	1818666	9886	5.253	8.104
		1230932	18969743			

These results are taken from the chromatogram graph by GC-MS analysis. Retention time represents the time taken for a component presents in corn silk to pass through a chromatography column. It is calculated as the time from injection to detection. Where the retention index attempt to give an indication for the component present.

The Height and the area of each peak compares the behaviour of each component according to a reference peak for the same component. %Area represents the concentration of each component in corn silk.

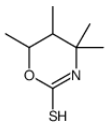
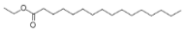
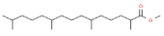
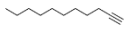
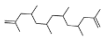
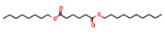
Name	Formula	Molecular weight (g/mol)	Chemical structure	Chemical class	Function
4,4,5,6-Tetramethyltetrahydro-1,3-oxazin-2-thione	C8H15NOS	173		alkaloids	antibacterial
Decanoic acid, decyl ester	C20H40O2	312		Aliphatic carboxylic acid	antioxidant
Hexadecanoic acid, ethyl ester	C18H36O2	284		fatty acid	hydrating the skin
Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester	C20H40O2	312		fatty acid	hydrating the skin
1-Undecyne	C11H22	152		alkaloids	antibacterial
1,12-Tridecadiene	C13H24	180		alkaloid	antibacterial
E,E-1,9,17-Decasatriene	C22H40	304	**	**	Unknown
Hexanedioic acid, dioctyl ester	C22H42O4	370		dicarboxylic acid	antioxidant

Table 9: corn silk components.

6.3 Antioxidant activity

Four different tests gave an indication to the free radical scavenging of corn silk. These tests are performed in three types of solution. The first solution is 99 wt.% ethanol, the second one is 80 wt.% ethanol, and the last one is water. 80 % ethanol gave the highest antioxidant power than the other solvents as summarized in the table (11).

Table 10: antioxidant activity tests results.

	TPC** (mg/g)	TFC (mg/g)	FRAP (mmol/g)	CUPRAC (mg/g)	DPPH (µmol/g)	ABTS (µmol/g)
Ethanol (99 %)	43.2	14.5	1.5	152.5	177.8	4
Ethanol (80 %)	94.3	22.1	2.5	243.3	210.1	4.2
Water	77.2	9.3	1.1	112.5	154.2	1.5

***DW: dry weight**

Total phenolic content (TPC as mg Gallic acid/g DW*), total flavonoids contents (TFC as mg catechin/g DW), FRAP (mmol Fe^{+2}/g DW), CUPRAC (mg Trolox/g DW), DPPH (µmol Trolox/g DW), ABTS (µmol Trolox/g DW) of corn silk extracts.

Chapter Seven: Conclusion

- The objective of this project was studying of the healing effectiveness of the active constituents of corn silk on UTI diseases. Then preparing the proper formula, and designing a suitable dosage form to apply it in a suitable manner, as well as studying its applications in cosmetics field.
- Corn silk is indeed a miracle plant in corn with enormous potentials yet to be fully explored in medicinal and cosmetics applications.
- This study has tried to reviews more information about corn silk, description of the plant and multipurpose uses of corn silk as a cream, tea, tablets and tincture.
- This project was considered to design a suitable oral dosage form of corn silk as teabags with stevia plant for treating urinary tract infection and a cream in cosmetic field. Chemical experiments were performed in order to increase the efficiency of extraction. The active ingredients of corn silk were checked and investigated by performing GC-MS test and finally, the anti-oxidant activity tests were performed.
- The active ingredients of corn silk were shown and found by GC-MS test.
- Anti- oxidant activity tests were used; four different tests gave an indication to the free radical scavenging of corn silk. These tests are ferric reducing/antioxidant power (FRAP), Cupric reducing antioxidant power (CUPRAC assay)**,** Free radical scavenging activity using DPPH (DPPH assay)**,** Free radical scavenging activity using ABTS (ABTS assay), Total phenolic content (Folin-Ciocalteu assay) and Total flavonoid content and they are performed in three types of solution. The first solution is 99 wt.% ethanol, the second one is 80 wt.% ethanol, and the last one is water. 80 % ethanol gave the highest antioxidant power than the other solvents.
- This project was done by collecting corn silk, drying it under sun, then grinding it and using it in extraction processes using solvent types including ethanol, methanol and ethyl estate. Doing filtration and then recovering of solvents using rotary evaporator under vacuum.
- The acceptable daily intake for corn silk is evaluated to be at least 8.00%(w/w). Which corresponds in a mean daily corn silk of approximately 9.354 and 10.308 g/day/kg body weight for males and females, respectively.
- For future work, it is recommended to study and make tablets from corn silk. Perhaps making an extractor device.

Chapter eight: References

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Appendices:

Figure 3: Dried corn silk.

Figure 4: Grinding corn silk by stainless steel grinder.

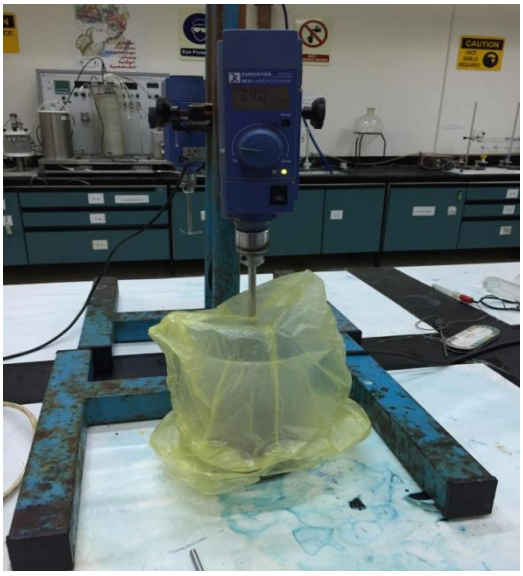

Figure 5: mixing corn silk tincture.

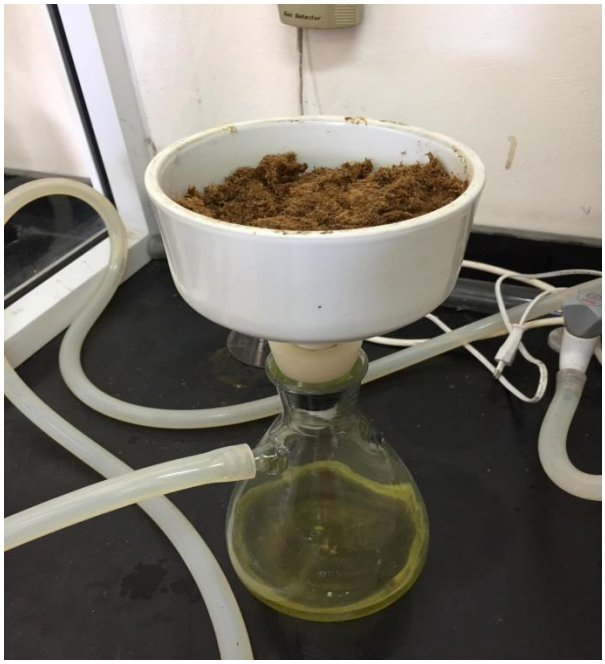

Figure 6: Filtration of corn silk tincture by suction filtration.

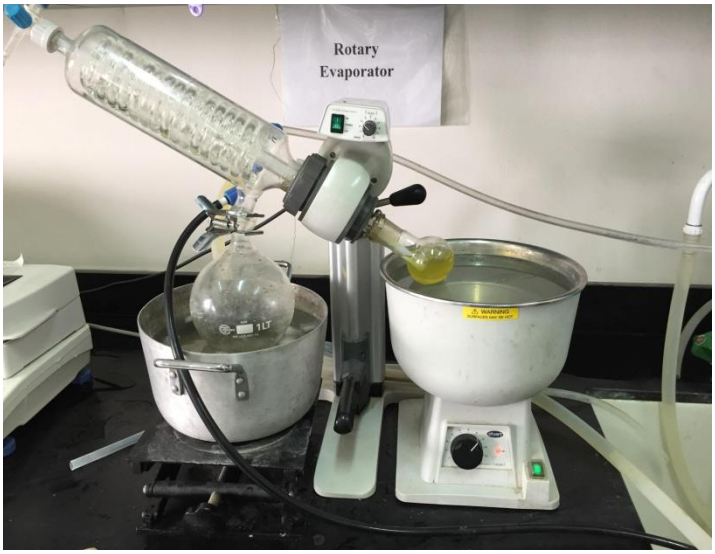

Figure 7: Extraction of active ingredient by rotary evaporator.

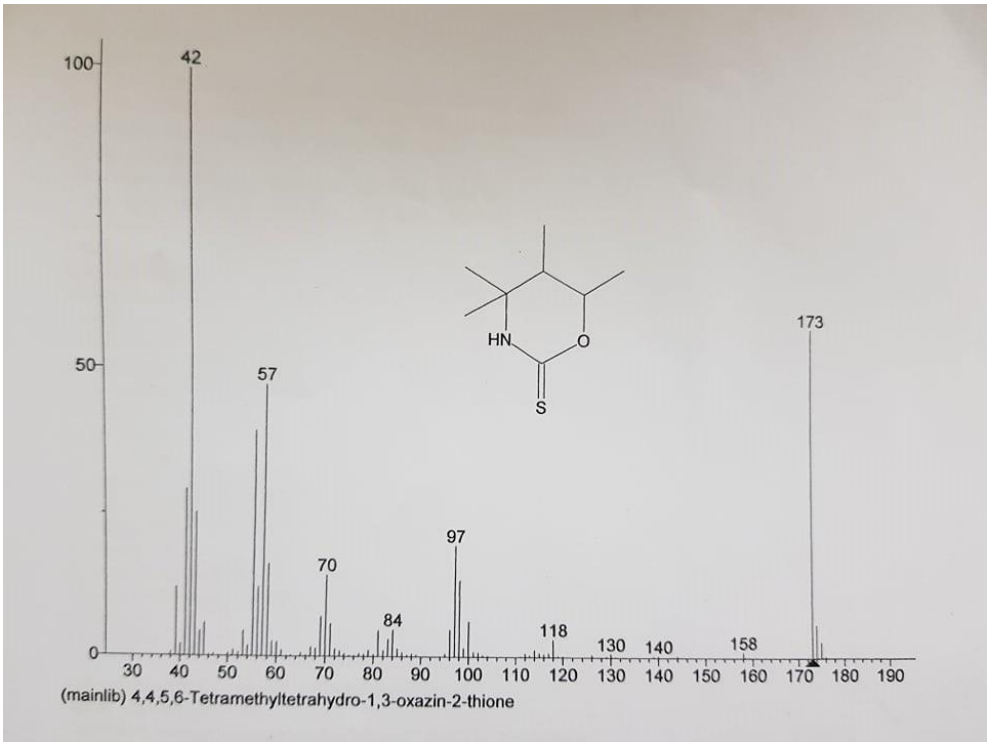

Figure 8:chromatograph of 4,4,5,6-Tetramethyltetrahydro-1,3-oxazin-2-thione