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Favorable attributes and biological effects of Hibiscus Sabdariffa

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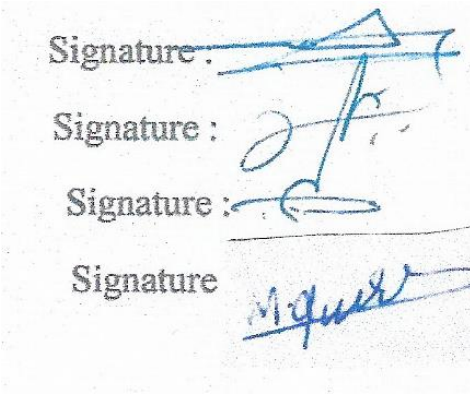

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2022/1444هـ

Dedication:

I dedicate this thesis first and foremost to my dear parents. Second, I want to thank my brothers Ahmad, Soheb, Mahmoud, and Moustafa for their perseverance and unwavering support by dedicating this work to them. Thirdly, this thesis is dedicated to my sister Tartial, my friends, and all Palestinian people all over the world

Declaration:

I certify that this Master's thesis is the result of my work and that it (or any section of it) has not been submitted to another university or organization for a higher degree.

Signature: 

Iman Shafout

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Abstract

The hibiscus Sabdariffa calyx extract was studied for its antioxidant activity and some biological effects. Three different solvents were used to extract the hibiscus sabdariffa calyx (Ethanol 99%, ethanol 35% and ethyl acetate. Antioxidant capacity was determined in these three different hibiscus extracts by DPPH assay. The extract exhibited inhibition of the DPPH activity in three extracts reaching 87.85% in ethanol 99% extract, 69.64% in ethanol 35% extract and 81.6% in ethyl acetate extract. By using Gallic acid as a standard, the Folin-Ciocalteau method was used to determine the total phenolic content. The total phenolic content was found to be 95.37 ± 0.3 mg gallic acid/ g in ethanol 35% extract, 55.8 ± 0.3 mg gallic acid/ g in ethanol 99%, and 48.73 ± 1.5 mg gallic acid /g in Ethyl acetate extract. The total flavonoids content was estimated by the aluminum chloride method. The total flavonoids content was found to be the highest amount with ethanol 35% extract which reached 64.8 ± 0.88 mg/g, while it was 24.59 ± 1.7 mg/g and 20.1 ± 0.9 mg/g in ethyl acetate, respectively. The hibiscus extract was examined using an HPLC device at a wavelength of 280 nm, and the result was that it contained a group of compounds: Gallic acid, chlorogenic, vanillic acid, caffeic acid, syringic acid, and sinapic acid. The main enzyme in the mevalonate pathway, which creates cholesterol, is 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase. When HMG-CoA reductase is inhibited, the liver produces less cholesterol. Statins, a class of synthetic medications, are frequently used to treat hypercholesterolemia. Natural HMG-CoA reductase inhibitors of plant origin are required because statin side effects exist. In this study, the anti-HMG-CoA reductase activity of Hibiscus sabdariffa ethanolic extracts was examined. The therapy reduced LDL cholesterol by 41.7±0.87%. Meanwhile the hibiscus sabdariffa microemulsion reduced the LDL by 24% Although the concentration of the active substance is 12.9% of the first concentration. The anti-glycation synthesis of end products was assessed using an in vitro glucose-bovine serum albumin (BSA) assay.. The obtained results show that Concentrations of (10.7, 8, 6.7, 5.3, and 2.6). mg/mL of Hibiscus sabdariffa ethanolic extract could inhibit AGE-formation by 17%, 14%, 6%, 5.68%, - 5% respectively.

In the presence of positive controls (Gentamicin (10 mg/disc) and Penicillin) when using the disc diffusion method (10 units), the hibiscus sabdariffa extract showed a good ability to inhibit the action of four different types of bacteria (MRSA, E-coli, S. aureus, and Pseudomonas). The results of the extract's inhibition on MRSA at concentrations of 100% and 75% were 26±3mm and 8.3±0.6mm, respectively. The results of the extract's inhibition on E-coli were the concentrations of 100% and 75% were 20.3 ± 1.1 mm and 7 ± 1 mm respectively while the inhibition effect of +ve control Gentamicin was 15 mM Hibiscus extract had an inhibitory effect on S. aureus where the of inhibition for 100% and 75% were 34.3 ± 0.6 mm and 11 ± 1 mm which is higher than the effect of +ve control penicillin 15 mm. The effect of the extract inhibition on Pseudomonas at a concentration of 100% and 75%, respectively, 24.3 ± 0.6 mm and 9.7 ± 1.1 mm, while the effect of +ve control Gentamicin reached 29 mm. In this experiment, a phase diagram was worked out, and two important areas appeared. The microemulsion area centered at the top of the drawing is close to the highest percentage of the surfactant and the crystal liquid area. The preliminary results of the current investigation indicate that the H. sabdariffa extract could lower cholesterol levels by reducing the activity of the enzyme HMG-CoA reductase.. In addition, the Hibiscus extract has antioxidants, phenols, and flavonoid compounds. The hibiscus has also an antibacterial effect.

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List of Abbreviations, Symbols and Terminology:

Abbreviation	**Definition**
HS	Hibiscus sabdariffa
HSC	Hibiscus sabdariffa calyx
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
HMG-CoA	3-hydroxy -3-methyl-coenzyme A
HMGR	3-hydroxy-3-methyl-glutaryl-coenzyme A reductase
LDL	Low density lipoprotein
S.aureus	Staphylococcus aureus
E.coli	Escherichia coil
LPS	Lipopolysaccharides
MRSA	Methicillin-resistant Staphylococcus aureus
HBA1c	Hemoglobin A1c
LDL-C	Low density lipoprotein cholesterol

Abbreviation	Definition
IPM	Isopropyl Myristate
DDPH	2,2-Diphenyl-1-picrylhydrazyl propane hydrochloride
HPLC	High-performance liquid chromatography
DW	Distilled water
KH_2PO_4	Monopotassium phosphate
$ALCL_3$	Aluminum chloride
$NaHPO_4$	Disodium phosphate
AB	Assay buffer
(MHA)	Mueller Hinton Agar
AGEs	Anti-glycation End products
BSA	bovine serum albumin

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Chapter (one)
Introduction

1. Introduction

Hibiscus Sabdariffa (Roselle) is an annual herb belonging to the family Malvaceae. It grows in tropical and subtropical regions of Asia, Africa, and South America such as India, Malaysia, the Philippines, the Caribbean, Indonesia, Australia, Hawaii, Brazil, Argentina, Spain, Egypt, and Sudan. The name hibiscus varies from country to country, for example in addition to Roselle, in English, Indian sorrel, karkadé, quimbombó chino, and another name (Mahadevan .N et al., 2009)

1.1 Plant description:

It is an annual plant with an erect, primarily branching stem that can reach a height of 3.5 meters. The leaves are alternate, glabrous, and long-petiolate, palmately divided into 3-7 lobes, and have serrated margins. The flowers are large, short-peduncled; red to yellow with a dark center, and the capsules are 5 cm long and 5.3 cm wide. The root is a deep penetrating taproot. (Shruthi, V. H. et al, 2016). Fig 1 shows the Hibiscus sabdariffa plant.

FIG 1: HIBISCUS SABDARIFFA PLANT (AFRICANPLANTS.SENCKENBERG.DE)

In soil rich in organic matter and vital nutrients, the Hibiscus Sabdariffa needs between 4 and 8 months to reach maturity. In the first three to four months, the plant needs an ideal rainfall of roughly 130-250 mm, although Hibiscus sabdariffa is extremely sensitive to fluctuations in day length. Due to this photoperiodism, planting times must be determined based on day length rather than the need for rain. (Mohamed, B. B et al 2012).

1.2 Traditional Use of Hibiscus sabdariffa:

Calyces Hibiscus sabdariffa (CHS) fresh or dried calyces are used to make herbal beverages, hot and cold beverages, fermented beverages, wine, jam, jellied confections, ice cream, chocolates, flavorings, puddings, and cakes (Ismail, A.et al., 2008). While in Sudan and Nigeria, the fleshy calyces are cooked with sugar to create a beverage known as "Karkadé" or "Zoborodo," the fleshy calyces are used in Egypt to make "karkade tea" and fermented drinks. This beverage is known as Jamaica in Mexico, also known as "agua de Jamaica" or "té de Jamaica." In The United States, Germany, and England hibiscus flower is traded and used as an important ingredient in industrially produced teas and beverages (Plotto et al, 2004). In the West Indies, the calyces can also be used as a coloring and flavourings ingredient in rum, and the leaves are cooked along with chicken or fish. Also, the leaves are widely consumed as affordable vegetables in Myanmar for poor people (Ismail, A. et al, 2008; Islam, M. et al, 2016). While the cooked leaves are used as vegetables in Malaysia, the leaves are consumed green or dried in Sudan together with onions and groundnuts (Ismail, A. et al, 2008). Roselle leaves are either fried with fish in Bangladesh or boiled with fresh or dried fish to produce a paste with garlic, onion, and chilies. Along with prawn stock, Hs leaves are also used to make a popular soup or meal (Islam, M. et al, 2016). The seeds are used in meals in Africa, including fatty soups and sauces, after being roasted or ground into a powder. The seeds' oil is also used in China and West Africa It is used as a source for the garment industry, fishing nets, and rope industry in countries such as India, However, research is still ongoing to show promising technical characteristics when utilized as source material for the manufacturing of high-quality paper as well as a replacement for synthetic or mineral fibers in composite materials (Sáyago-Ayerdi, S.G. et al., 2007; Mahadevan. N et al., 2009).

1.3 Phytochemistry:

Gossypetine, hibiscetine, and sabdaretine are flavonoids found in dried calyces. Alkaloids, sitosterol, cyanidin-3-rutinose, delphinine, anthocyanin, galactose, pectin, protocatechuic acid, citric acid, quercetin, stearic acid, and wax17 are also present in it. Delphinine, cyanidin 3-monoglucoside (chrysanthemum), and delphinine are also detected in minor amounts3. From flower buds, three neutral polysaccharides made of arabinans and arabinogalactans that are water soluble have been identified. Acid and pectin are abundant in calyces. Crude protein and minerals like sodium, potassium, aluminum, magnesium, calcium, and phosphorus have been found in calyces after analysis revealed their presence. Additionally found in calyces are mucin, calcium citrate, ascorbic acid, gossypetine, and hibiscus chloride. (Mahadevan, N. et al. 2009)

Gossypetine, hibiscetine, and sabdaretine are flavonoids found in dried calyces. Additionally, it has sitosterol, anthocyanin, citric, and alkaloids. The seeds' level of dietary fiber (39.5–42.6%), protein (18.8–22.3%), and fat (19.1–22.8%) was determined to be high. The minerals phosphorus, magnesium, calcium, lysine, and tryptophan were discovered to be present in significant amounts in the seeds. 70% of the fatty acids in seed oil are unsaturated, with 44% of those being linoleic acid. In addition to cellulose, pentosans, and starch, seeds also contain

nitrogen. (Ismail, Amin, et al. 2008). It has been observed that seed oil contains steroids and tocopherols19–21. Quercetin, 3-O-rutinoside, corchoionoside C, 2, 3-dihydro-2-(4'-hydroxy-3'-methoxyphenyl 3-D-glucopyranosylmethyl-7-hydroxy-5- benzofuranpropanol, and trans-carveol-6-O-glucopyranoside were extracted from 70% aqueous ethanol extract of leaves). (Maganha, E. G. et al. 2010).

1.4 Medicinal properties:

Hs is a treatment for kidney and bladder stones in traditional therapies. It is also utilized as an aphrodisiac, anticancer, antioxidant, antiseptic, sedative, digestive, purgative, emollient, demulcent, hypocholesterolemic, antispasmodic, diuretic, uricosuric, mild laxative, and antihypertensive agent. (Maganha, E. G. et al. 2010; Shruthi, V. H. et al. 2016)

1.4.1 Antioxidant:

Antioxidants are chemicals that stop the oxidation of other molecules from harming cells. A chemical reaction called oxidation involves the transfer of electrons from one molecule to an oxidizing substance. Free radicals are known to be created during oxidation reactions. These free radicals are extremely reactive entities that have one or more unpaired electrons in their outermost shell. The chain reaction begins as soon as they form. The antioxidant reacts with these free radicals to stop the chain reaction by eliminating the intermediate free radicals and obstructing additional oxidation reactions by oxidizing themselves. (Mamta, Misra, K. et al., 2014). Enzymatic antioxidants and non-enzymatic antioxidants are the two types of antioxidants. Catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase are examples of primary enzymes that act as antioxidants, as well as glucose-6-phosphate dehydrogenase and glutathione reductase, which act as secondary enzymes. (Ndhlala, A. R. et al. 2010). This study focus on Non-enzymatic antioxidants. The Non- enzymatic antioxidants divide into lipid-soluble (vitamin E) biomolecules or water-soluble (phenolic compounds) (Ndhlala, A. R. et al. 2010).

Hibiscus Sabdariffa contains a lot of antioxidant compounds such as polyphenols, carotenoid, protein, Gallic acid, and flavonoids which play an important effect as a safe antioxidant like an all-natural source of plant antioxidant. (Al-Hashimi, A. G. et al 2012).

1.4.1.1 Phenols:

Polyphenols are a heterogeneous phytochemicals compounds class constituting the most abundant class of antioxidants compound. Their chemical and physical characteristics, which in turn depend on their molecular structures, govern their antioxidant activities, which in turn regulate metabolism. (Mamta, Misra, K. et al., 2014; Ndhlala, A. R. et al., 2010)

1.4.1.2 Flavonoids:

Significant classes of natural products are flavonoids; in particular, they are a non-enzymatic plant class with a polyphenolic structure that is abundant in fruits, vegetables, and some drinks

like Hibiscus sabdariffa (Ndhlala, A. R. et al., 2010). Depending on the attached carbon rings, their degree of unsaturation, and their degree of oxidation, flavonoids are categorized into various subgroups (Fig. 2). These subgroups include the catechins, flavones, flavonols, flavanones, flavanonols, chalcones, and anthocyanins that are found in hibiscus. (Zha, J. et al.2019)

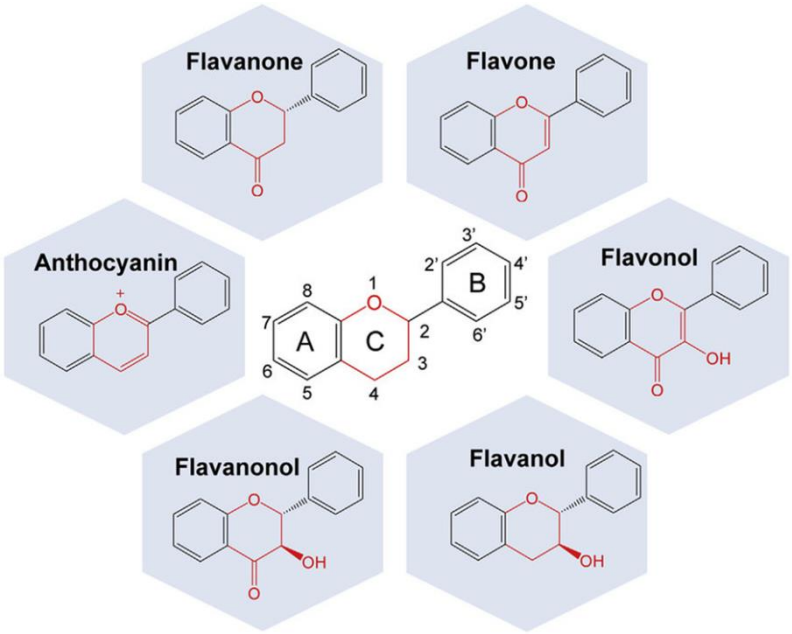


FIG (2) FLAVONOIDS SUBGROUPS ZHA, J. ET AL.)
2019)

In this study, the inhibitory effect of hibiscus extract on the HMG-COA enzyme was investigated

1.4.2: HMG-CoA reductase inhibitory activity

A primary cause of cardiac issues, including myocardial infarction, is hypercholesterolemia, which results in atherosclerosis. High levels of plasma cholesterol, particularly those of low-density lipoprotein (LDL) and triglycerides are what cause hypercholesterolemia, which can result in obesity, diabetes, and cancer. The rate-limiting enzyme in the production of cholesterol is the (HMG-CoA) reductase, which is responsible for converting HMG-CoA to mevalonate. The equation for cholesterol biosynthesis is shown in fig. 3. Inhibition of HMG-CoA reductase successfully lowers cholesterol levels in humans and most animals by activating sterol regulatory element-binding protein, which upregulates (HMG-CoA) reductase and LDL receptor (Baskaran

G. et al 2015).

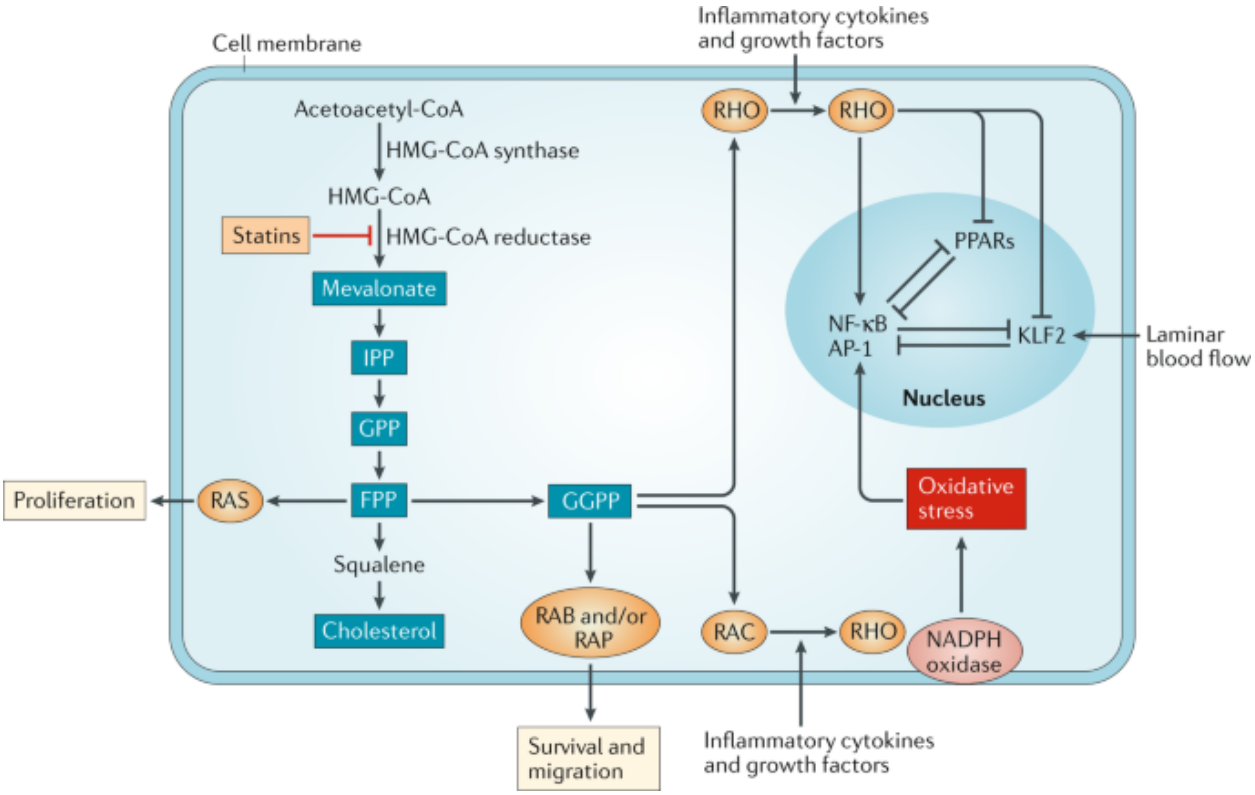


FIG (3) CHOLESTEROL BIOSYNTHESIS EQUATION(PARIHAR.S.P 2018)

1.4.3The Antiglycation and Diabetes complications:

AGEs are a broad family of biomolecules that are made when lipid, nucleic acid, or protein substrates are combined with reducing sugars like glucose and fructose in non-enzymatic processes. In hyperglycemia, glycation damages cells by decreasing intracellular and extracellular protein activity. In the case of insulin, this decreases the molecule's bioactivity and frequently impacts the biomolecule's half-life. Some AGEs, such as N-(carboxymethyl) lysine (CML), interact with the AGE receptor (RAGE), inducing NF-KB activation, taking part in the development of atherosclerosis, triggering inflammatory processes that contribute to macro- and microvascular complications of diabetes, chronic diseases, and a decrease in the effectiveness of the immune system as a whole. When reducing sugars are conjugated to biomolecules, several oxidative changes take place.

1.4.5 Antibacterial bio compound:

An antibacterial agent prevents bacteria from proliferating and growing. These substances combat germs and either destroy them or prevent them from growing. All antibiotic medications, chlorine, and heat have antibacterial qualities. (Higginbotham, K. L. et al.2014)

Hibiscus sabdariffa contains a lot of compounds that affect antibacterial such as cardiac glycosides, flavonoids, Saponins, and alkaloids. It exhibited antibacterial activities. (Mercedes, M. C.et al, 2013)

1.6 Microemulsion :

Microemulsions are macroscopically single-phase fluids made up of water and oil and separated by a monomolecular layer of amphiphilic molecules. They are also thermodynamically stable. Reverse micelles, swelling oil-in-water (o/w) micelles, and irregular bicontinuous microstructures with low to zero mean curvature are only a few of the several microstructures that they can adopt (Paul, B. K. et al., 2007).

Content of microemulsion:

1- Oil phase: (lipophilic part)

2- Water phase: (hydrophilic part)

3- Surfactants are hydrophobic and hydrophilic amphiphilic compounds. The hydrocarbon tail is hydrophobic. Due to the similarity of the hydrophobic tails, surfactants are often categorized according to their polar head. The term "non-ionic" refers to a surfactant whose head group carries no charge. It is referred to either anionic or cationic depending on whether the head group is negatively or positively charged. Zwitterionic surfactants are those that have both positive and negative groups.4- Co-surfactants are short-chain amines or alcohols. Apart from lowering the interfacial tension these also help in changing the curvature of the reverse micelles(Paul, B. K. et al., 2007).

1.6.1 Application of microemulsion:

Microemulsion be can used in enhanced oil recovery, fuels, coatings, and textile finishing, as lubricants, as cutting oils and corrosion inhibitors, and as liquid membranes. It is also used in detergency, cosmetics, agrochemicals, food, pharmaceuticals, environmental remediation, and detoxification, in analytical applications, media synthesis (microemulsion gel technique). (Paul, B. K. et al 2001).

1.7 Objectives and Goals:

The main objective of this study is to the effectiveness of hibiscus in reducing LDL in the blood.

Additionally, there are other objectives needed to be studied and achieved:

- Determination of the antioxidant activity of Hibiscus sabdariffa extract.
- Determination the total phenolic content (TPC).

- Determination the total flavonoids content (TFC).
- Check the antibacterial activity of Hibiscus sabdariffa extract.
- Preparing a microemulsion from hibiscus extract.
- Check the effect of HS extract on Anti-glycation End products.
- HPLC analysis of the standards of polyphenolic compounds and flavonoids

Chapter (Two)
Literature Review

2. Literature review:

2.1 Medical effect:

Scientists all over the world have recently begun to focus more on studying plants and their vital compounds in the hopes of using them as a natural alternative to chemicals, such as hibiscus plants, which have been the subject of numerous studies on the vital compounds present in them, such as antioxidants. Traditional medicine for kidney and urinary bladder stones is said to be Hs. It's also utilized as an antibiotic, antifungal, hypocholesterolemic (used to lower total cholesterol and LDL-C cholesterol while raising HDL-C cholesterol), antispasmodic, diuretic, uricosuric, mild laxative, and lowering blood pressure.(Maganha, E.G.et al 2016)

2.1.1 Antioxidant:

(Formagio, A. S. N. et al. (2015)) studied the phenolic and flavonoid content, as well as the antioxidant and antitumoral activity of methanolic extracts of the leaves and calyx of HS grown with poultry manure and organizer in three different conditions. The TPC of each extract was determined using the Folin-Ciocalteu reagent, as well as the flavonoids of aluminum chloride. A free radical scavenging assay based on 2, 2-diphenyl-1-picrylhydrazyl (DPPH.) was used to assess the antioxidant parameters. A colorimetric antitumor assay based on sulforhodamine B. The highest flavonoid and phenolic contents were found in leaf extracts (104.52 and 389.98 mg g–1, respectively) and calyx extracts (148.35 and 474.09 mg g–1, respectively) from organosuper- plowed plants, though these Values were similar to those found in the control plants and did not substantially differ

In the same context, (Liuqing, Y., Ying, G. et al. (2012) , The antioxidant potential of dried roselle (Hibiscus sabdariffa L.) calyx and fruit extracts in distilled water-ethanol (30, 60, and 95 percent) was assessed using the (DPPH), hydroxyl, and 2-2'-and-bis-(3-ethyl-benzothiazole-6-sulfonic acid) (ABTS) radical tests. According to the findings, the extract in 95% ethanol had the least amount of radical-scavenging activity, whereas the extract in 30% ethanol had the most. According to correlation studies between antioxidant activities and total phenol, anthocyanin, and total saccharide contents, the 30 percent ethanol extract's higher activity can be attributable to its higher total phenol content (20.25 mg GAE/g). The relative R2 values were 0.9615, 0.9089, and 0.9771. The 30% ethanol extract therefore has the potential to be a powerful natural antioxidant with good free radical scavenging properties.

The same result was reported by (Al-Hashimi, A. G. et al 2012) In the study, aqueous and alcoholic extracts of Hibiscus sabdariffa L. were found to have antioxidant activity, reducing power, and ferrous ion chelation. For the aqueous and alcoholic extracts, the total phenolic content was 77.2 mg/g and 87.7 mg/g, respectively. Both the alcoholic roselle extract and the synthetic antioxidant BHT (75.67%) had the same levels of antioxidant activity. With a rate of 222.60%, the alcoholic extract demonstrated the greatest ability to reduce. At a concentration of 5 mg/ml, the chelating of ferrous ions in aqueous and alcoholic extracts was 73.97 and 32.29%, respectively.

2.2 HMG-CoA reductase inhibitory activity

Cholesterol is one of the most dangerous diseases of this era. Therefore, in this research, we studied the effect of hibiscus in reducing the amount of LDL in the blood. Many similar studies have been conducted in this field; including study done by (Vilasinee Hirunpanich Sato, et al 2005) the study measured the antioxidant properties of the dried HSc aqueous extracts. by using rat in vitro LDL has been measured . As a result, it showed that the dried HSc extracts have significant antioxidant activity in (Cu2+)-mediated LDL oxidation in vitro (p 0.05). The extracts had a dose-dependent inhibitory effect on LDL oxidation at doses ranging from 0.1 to 5 mg/ml. More so than 100 M of vitamin E, 5 mg/ml of roselle prevented the development of TBARs. Finally, this research offers a quantitative understanding of the strong antioxidant action of Hs in vitro. Knowledge of the strong antioxidant activity of Hs in vitro.

In the same field, a study was conducted by (Chen, J.-H. et al 2013). This study aims to learn more about the high flavonoid content, and polyphenolic extract from Hibiscus sabdariffa leaf's anti-atherosclerotic effects. In vitro testing established HLP's inhibitory effect on LDL oxidation and lipid peroxidation. In oxidized-LDL (ox-LDL)-induced macrophage J774A.1 cells, HLP demonstrated potential in lowering foam cell production and intracellular lipid accumulation at non-cytotoxic concentrations. the data revealed that HLP up-regulated the LXR/ABCA1 pathway, which in turn stimulated cholesterol clearance from macrophages and delayed atherosclerosis. Molecular evidence showed that the effects of HLP might be mediated via the liver-X receptor (LXR)/ATP-binding cassette transporter A1 (ABCA1) system. Aimed to research the anti-atherosclerotic impact of hibiscus, and the results revealed that HLP may someday be developed as an anti-atherosclerotic agent.

2.3 Antiglycation and Diabetes complications:

A study by L. Beaulieu et al. examines the inhibitory effect of the Cree traditional medicine Wiishichimanaanh (Vaccinium Vitis-idea) on the formation of advanced glycation end products. Various works for testing the formation of AGEs were conducted for various plant types, including Vaccinium Vitis-idea. Incubation media with BSA (1.0 mg/mL), glucose (100 mM), fructose (100 mM), and either V. vitis-idea berry extract or vehicle (80% Ethanol: 20% H2O, v/v) in 100 mM sodium phosphate buffer were generated. The AGEs test used an 80% ethanolic extract. To ascertain concentration-dependent reactions, ten different concentrations of the berry

extract (0.39-200 g/mL) were created. Quercetin (2.5 g/mL) served as the positive control. After that, the incubation medium was shaken and incubated for 7 days. at 37 ºC in the dark. Using a fluorometer and excitation and emission wavelengths of 355 nm and 460 nm, respectively, the production of fluorescent AGEs was quantified. According to the findings, Vaccinium Vitis-idea extract could, in a dose-dependent manner, suppress the synthesis of glycated end products by up to 80%. With the extract tested samples' IC50 at 13.5 g/mL.

2.4 Antibacterial:

One of these studies (Higginbotham, K. L.et al 2014) Aqueous Hibiscus extracts from the "Mi Costenita" brand were sterilized by membrane filtration (0.22-mm pore size) or autoclaving (121uC, 30 min.) and tested for antimicrobial activity against the foodborne pathogens in a microbiological medium and ultra-high temperature-processed milk with different fat percentages. In a microbiological media, autoclaved and cider extracts from the Escherichia coli O157:H7 strain ATCC 43894 was more active than filtered extracts. A 20 mg/ml filtered extract had the same effect on E. coli as the control, but autoclaved extracts reduced viable cells by 3 to 4 log CFU/ml. After 24 hours at 60 mg/ml, both extracts rendered cells inactive. S. aureus numbers for both strains were down (about 2.7).

In the same field (Mercedes, M. C. et al 2013) Salmonella typhimurium and Salmonella choleraesuis were tested for resistance to calyx extracts from five different cultivars of Hibiscus sabdariffa. Salmonella cultures were treated with calyx extracts made from methanol, ethanol, and water. Both Salmonella serotypes were resistant to all extracts' antibacterial properties. The "Alma Blanca" cultivar, in which anthocyanin production has been all but reduced, showed the strongest antibacterial effects.

Chapter (Three)
Methodology

3. Methodology

3.1 Chemicals and Reagents:

Hibiscus sabdariffa calyces powder, Ethanol 99%, Ethanol 35%, Ethylacetat, D.W, Folin – Cocteau reagent (10 times diluted).Sodium Carbonate solution (Na2Co3,Gallic acid, Aluminum chloride (AlCl3)2%, sodium acetate (lm), Methanol, Quercetin (reagent), DPPH, Ascorbic acid, Bacterial cultures (plate count agar), Antibiotic control(Pinsellin, Gentamicin), Tween 80, Tween 20, IPM, Bovine Serum Albumin (BSA), Monopotassium phosphate (KH2PO4), Disodium phosphate (Na2HPO4), Sodium chloride (NaCl), distilled water, Fructose, Glucose, HMG-CoA Reductase Assay Kit, ultrapure water ,Materials were purchased from Sigma Aldrich:

3.2 Materials and devices:

Analytical balance, Test tube, Test tube rack, Vortex, Centrifuge, Volumetric flask, Beakers, Micropipette, Shaking water bath, Spectrophotometer(UV2550, Shimadzu, Kyoto, Japan), HPLC, Incubator, Autoclave,6 mm filter paper discs, Sterile Petri dishes, Sterile swabs, Forceps, Benchtop sanitizer, Alcohol burner, Ethanol for flame sterilizing, marker, Rotary evaporator from IKA WEREK RV06-ML, Spatula, soxhlet apparatus(mrc model FA-46) fluorscan .

3.3. Extraction:

Hibiscus was extracted in three different types of solvents (ethanol 99%, ethanol 35%, and ethyl acetate), by (type FA-46) Soxhlet apparatus for 3 hours at a temperature of 75ºC and the ratio was 1:9 (wt./vol) plant powder. The Hs extract was filtered by MN615.110 mm filter paper after extraction. After filtration, rotary evaporator (IKA WERKE RV06-ML) was used concentrated the crude ethanolic extract HS at a temperature of 65ºC. The Percentage yield of dried sticky, mainly obtained through evaporation was determined by the following equation:

Percentage yield $= \frac{Wt\ of\ dried\ extracts\ x\ 100}{Wt\ of\ powder\ taken}$

3.4The Antioxidant Assay:

3.4.1The DPPH assay:

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay described by suman and others (Chandra, S. et al ,2014) 1 ml of the sample and 6 ml of methanol were combined with 3 ml of 4% DPPH methanolic solution. At room temperature, the solution was incubated for 30 minutes. At a

wavelength of 517 nm, the reaction mixtures' absorbance was measured against a blank (EMC-61PC-UV Spectrophotometer). The formula below was used to determine the proportion of the DPPH scavenging effect. Effect of DPPH scavenging (%)/%

Inhibition=A0 -A1 /A0 × 100

Where A0 = absorbance of control.

A1 = absorbance of standard.

3.4.2 TPC assay:

The total phenolic content of the extract was evaluated with the Folin-Ciocalteu method (Azlim Almey, A. A. et al, 2010). The Folin-Ciocalteu reagent reduces samples that contain polyphenols, resulting in a complex that is blue in hue. The Gallic acid sample (1 ml, 1000 ppm) was combined with sodium carbonate (75 g/L), a tenfold diluted Folin-Ciocalteu reagent, and 5 mL of the sample. The quantitative phenolic determination was carried out using a UV spectrophotometer at 765 nm following a30-minute incubation at 25°C (EMC-61PC-UV Spectrophotometer). A calibration curve for gallic acid with concentrations of 20, 30, 40, 50, 60, 70, and 80 g/ml was used to determine the phenolic content of the extracts. By plotting the value of absorbance vs. concentration, ethanoic gallic acid is calculated as g/mL. Then use the calculation below to get the TPC:

TPC=C×VM

Where c: the concentration of the sample, v the volume of solution, M the sample mass.

3.4.3 TFC assay:

The total flavonoid content has been determined using the aluminum chloride colorimetric technique (Chandra, S. et al, 2014). The calibration curve for the standard was made using 5% quercetin. The standard quercetin solutions were created using distilled water and repeated dilutions (10 - 50 - 100 - 200 g / ml). 5.6 ml of distilled water, 3 ml of methanol, 200 l of 2% aluminum chloride solution, and 1 ml of sodium acetate solution were combined with 1 ml of diluted standard quercetin solutions or extracts. the solution was let to sit for 30 minutes at room temperature. The reaction mixtures' absorbance was then measured at 420 nm against a blank (EMC-61PC-UV Spectrophotometer) the TFC determination by the equation below:

TFC=v ×cm

Where c is the concentration of the solution, v is the volume of solution, and m is the sample mass.

3.4.4 RP-HPLC analysis of flavonoids:

For the separation, measurement, and identification of these chemicals, the flavonoid concentration in natural extracts is routinely examined using the high-performance liquid chromatography (HPLC) technique. HPLC, a very efficient tool, was used to identify the crude raw extracts of the extract plant components. In 20 ml of water, 0.5447g of crude extract was dissolved. D.W. solvent was used to test crude extracts. The product is produced when the concentration reaches 27.235 mg/ml. In HPLC with a UV detector, a C18 column (15 cm with a UV detector). 4-micron-sized particles) and a water mobile phase with a 254-nm wavelength: (50:50) methanol standard with a concentration of 0.01 g per 10 ml (v/v) and a flow rate of 1.0 ml per minute.

3.5: HMG-CoA Reductase:

Spectrophotometric measurements were used to determine the HMGR inhibitory activity of the Hs plant. A reaction mixture including the Hs extract (5 µg), 920 µl of assay buffer (pH 7.2), 60µ l of HMG-CoA substrate, 20 µl of NADPH, and 5 µl of HMG-CoA reductase was added to the Hs extract. At 340 nm, absorbance was measured every 15 seconds for 5 minutes while the reaction was incubated at 37 °C. As a positive control, pravastatin was utilized. The following formula was used to compute the HMGR inhibition (%).Inhibition $\%=(\Delta Absorbance\ control - \Delta Absorbance\ test|\Delta Absorbance\ control) \times 100\%$

3.6 AGE inhibition assay:

The procedure was carried out as previously described (Harris, C. S. et al., 2011) with the following modifications:

Preparation of Incubation media:

A 100 mM sodium phosphate monobasic monohydrate buffer at pH 7.4 was created. A stock solution of bovine serum albumin (BSA) (1 mg/ml) was created in 100 mM sodium phosphate monobasic monohydrate buffer (pH 7.4). A stock solution of the (100 mM glucose/100 mM fructose) mixture was made in 100 mM sodium phosphate monobasic monohydrate buffer (pH 7.4).

Samples Preparation:

To determine concentration-dependent responses, 5 different Hibiscus sabdariffa ethanolic extracts concentrations were diluted by ethanol 99 percent, ranging from 2.5 mg/ml to 12.5 mg/ml for each extract.

Samples:

Hibiscus sabdariffa samples were made in Eppendorf and cultured in an incubator shaker at 37°C for 7 days with 100 µl of BSA, 100 µl of sugar solution, 300 µl of phosphate buffer (pH 7.4), and 500 µl of each of the five concentrations.

Control:

With five different doses ranging from 2.5 mg/ml to 12.5 mg/ml, 100 ml of BSA, 100 ml of sugar solution, 300 l of phosphate buffer (pH 7.4), and 500 l of the Quercetin standard (Q4951) were made in Eppendorf and incubated in an incubator shaker at 37°C for seven days.

Negative control:

500 ml of 99% ethanol, 300 ml of phosphate buffer (pH 7.4), 100 ml

of BSA, and 500 l of sugar solution were produced in Eppendorf and incubated for 7 days in an incubator shaker at 37 °C.

Using a fluorimeter (Nutrition and Health Research Institute, Al-Quds University) and excitation and emission wavelengths of 455 nm and 375 nm, respectively, the formation of fluorescent Antiglycation end products (AGEs) in each sample was measured after 7 days of incubation. The experimental treatments and the negative control had their fluorescence values blanked against BSA, phosphate buffer, and the appropriate extract blanks in order to remove baseline fluorescence. The experimental treatments included BSA, sugar, and either extract or pure standard. Using the corrected fluorescence readings (F) for the negative control (F negative control) and experimental treatments (F experimental corrected), the percentage of inhibition of AGE formation was determined as follows:

% inhibition= (F negative control – F experimental corrected) / F negative control)X100%

3.7 Antibacterial assay:

The antibacterial test utilized agar disc diffusion (Murray et al., 1999). Penicillin and the common antibiotics Gentamicin (10 micrograms/disc) served as positive controls for the bacteria under investigation (10 micrograms/disc). Antibacterial efficacy was determined by measuring the size of the zones of inhibition that surrounded the disc when applied against the tested microorganisms. While the D.W was used to be negative control

Escherichia coli, Staphylococcus aureus, (MRSA), and Pseudomonas aeruginosa clinical isolates were donated by the College of Health Professions Department at the University of Al-Quds.

medium:The Department of Microbiology, Faculty of Sciences and Technology, Al Quds University, provided the Mueller-Hinton Agar.

The proper amount of food energy, the right temperature, and the right humidity were all requirements for the development of bacteria. We need to create these conditions using culture media in order to develop these cells in the lab. Solid culture media in Petri dishes is Muller Hinton agar. The bacterial inoculum was generated in broth and then Mueller Hinton Agar (MHA) was made and sterilized using an autoclave for around 30 minutes. For the following step, the media was then placed in sterile Petri dishes. For 10 to 15 minutes, they were allowed to solidify. To stop moisture from gathering on the medium's surface, plates were kept in a plastic bag and stored upside down.

The culture of bacteria was diluted and then measured using a spectrophotometer set to 625 nm to be 108 CFU/ml similar to that of the MacFarland nephlometer .after that the bacteria were injected into the nutritional broth and incubated at 37°C for 24 hours (optical density 0.08 to 0.1).With the use of a cotton swab, the inoculum was spread evenly across the MHA surface once it had set.

Using the disc diffusion method, the antibacterial activity of four distinct bacterial strains was examined. For around 30 minutes, the disc (5mm in diameter) was autoclaved to sterilize it.

Muller Hinton agar plates were filled with the generated bacterial species. A reference antibiotic disc was applied on the surface of MHA as a positive control. Then, 50 microliters of plant extracts (at various concentrations) and 50µ l of a negative control (solvent) were impregnated onto sterile discs. The plates were then kept at 37°C for a further 24 hours.

3.8 (Phase Behavior):

3.8.1 Microemulsion (Tween 80- 20)

Pseudo-ternary phase diagrams for oil, water, surfactant, and co-surfactant mixture were determined utilizing titrating by the mixture of distilled water and hibiscus extract to approach to build it.

The phase diagram of systems including Hibiscus extract in water, tween 80 and tween 20 in a 1:1 ratio as surfactants, and IPM as the oil phase.

Ten test tubes with various oil to surfactant ratios were placed at a temperature of 25°C (shown in table 1) These samples were titrated with water phase, which was added until the end limit was reached, as shown in table (2), and was then vigorously stirred after each addition of the aqueous phase on a vortex mixer. When necessary, a 5-minute centrifuge was used to speed up the separation process between each addition.

TABLE (1) OIL PHASE TO SURFACTANT WEIGHT RATIOS.

# of tube	The Oil phase (mg) (IPM)	Surfactant (mg) phase Tween 80- Tween 20
1	0.9	0.1
2	0.8	0.2
3	0.7	0.3
4	0.6	0.4
5	0.5	0.5
6	0.4	0.6
7	0.3	0.7
8	0.2	0.8
9	0.1	0.9
10	0	1

TABLE (2): THE AMOUNT OF WATER ADDED EACH TIME IN TITRATION.

No of Addition	The addition Mass (mg)	percentage of Addition (%)
1	0.03	2.9
2	0.03	5.6
3	0.03	8.25
4	0.03	10.714
5	0.03	13.04
6	0.03	15.25
7	0.03	17.35
8	0.03	19.35
10	0.03	23.07
11	0.03	24.8
12	0.03	26.47
13	0.03	28.05
14	0.03	29.577
15	0.03	31.03

No of Addition	The addition Mass (mg)	percentage of Addition (%)
17	0.03	33.77
18	0.03	35.06
19	0.03	36.30
20	0.03	37.5
21	0.12	41.66
22	0.12	45.65
23	0.12	48.97
24	0.06	50.49
25	0.12	53.27
26	0.06	54.5
27	0.06	55.75
27	0.06	56.89
28	0.06	57.98
29	0.12	60
30	0.12	61.83
31	0.12	63.50
32	0.12	65
33	0.12	66.44
34	0.24	68.944
35	0.12	70
36	0.66	75
37	1.5	81.81
38	1.5	85.71
39	3	90
40	6	93.75
41	6	95.45
42	6	96.42
43	6	97.05
44	12	97.8
45	15	98.36
46	30	98.9
47	30	99.1
48	30	99.33
49	30	99.44
50	120	99.6
51	>150	100

3.9: Cream

Formula for Hand Cream.

TABLE (3): HAND CREAM FORMULA

phase	Ingredients	Wight (g)
A	Stearic Acid	8
A	Acetyl stearyl alcohol	1
A	Isopropyl Myristate	4
A	Glyceryl Monostearate	2
A	lanolin	1
B	Glycerin	8
B	Triethanolamine	1
B	Sorbitol	6
B	Methyl paraben	0.130
B	Water	60
B	HS extract	2
C	Perfume q.s	

Procedure:-

Heat Part A to 40 Cº. Heat Part B to 40Cº and add B to A with constant agitation. At 400Cº add perfume and agitate. Discontinue agitation and package.

Chapter (four)
Results and Discussion

4. Result and Discussion:

4.1 : Extraction :

The final weights of the extracted Hs for three different solvents (99% ethanol, 35% ethanol, and ethyl acetate) were 3.50 ± 0.20 g, 2.351 ± 0.17 g, and 2.957 ± 0.31 g with percentages of 14%, 9.404%, and 11.68%. The amount extracted using Ethanol 99% is higher because ethanol is able to dissolve polar and non-polar compounds more than ethyl acetate and ethanol 35%, which contains a high percentage of water. This result is consistent with the results obtained by Chinedu et al. (2011) who used the maceration method instead of Soxhlet, with the results being a methanolic extract of 27.3 ± 0.33%, an ethanolic extract of 12.8 ± 0.12%, an acetone extract of 5.9 ± 0.09%, and an aqueous extract of 4.0 ± 0.12%.

4.2 Antioxidant result:

4.2.1 DPPH Assay

The antioxidant result of Hibiscus sabdariffa extraction in three different solvent (ethanol 99% , Ethyl acetate , and ethanol 35%) shown in table (4).

TABLE (4) RADICAL SCAVENGING FROM DPPH ASSAY FOR ETHANOL 99%, ETHYLACETAT , ETHANOL 35% HSC EXTRACT

Sample	calculation of % Radical Scavenging from DPPH Assay			
	Absorbance Measurement Data			
	Concentration (mg/ml)	control	sample	%RSA
Ethanol 99% extract	12	1.2912	0.156867±0.028	87.85
Ethyl acetate extract	12	1.2912	0.2542±0.022	81.6
Ethanol 35% extract	12	1.2912	0.3919±0.0248	69.64

The result shows that the extract containing 99% ethanol has the highest percentage of antioxidants. While the percentage was 87.85, the percentage of ethyl acetate extract was 81.6, and ethanol 35% was 69.64, and the reason for this is that the antioxidant compounds are two types of rods and non-polar and therefore ethanol 99% has the ability to dissolve a greater number of antioxidants than the less polar ethyl acetate and ethanol 35% because it contains a high percentage of water and thus less ability to dissolve non-polar compounds, and this result is consistent with the results that came from both (Al-Hashimi, A.G. et al., 2012), where the amount of antioxidants increases with increasing the concentration of ethanol alcohol.

4.2.2 Total phenol content assay:

The result of TPC of the different solvent ethanol 99% , Ethylacetat , and ethanol 35% shown in table (5)

TABLE (5) TPC AND TFC OF HIBISCUS SABDARIFFA CALYCES EXTRACT OF THREE DIFFERENT SOLVENT

Name of solvent	TFC(mg/g)	TPC (mg/g)
Ethanol 99%	24.59±1.7	55.816±0.319
Ethylacetate	20.1±0.9	48.73±1.54
Ethanol 35%	64.8±0.88	95.37±0.311

TPC: total phenol content, TFC: total flavonoids content

The results of hibiscus showed a high percentage of phenols, reaching 95.37±0.311mg/g in 35% ethanol extract and 55.816±0.319 mg/ g in 99% ethanol extract, and the lowest amount was in ethyl acetate, reaching 48.73±1.54 mg/g. This result is consistent with results of (Formagio, A. S. N. et al., 2013); . Liuqing, Y., Ying, G et al., 2012) where the lower of ethanol concentration, the higher the amount of phenol extracted. The reason for this is that phenol is divided into several types, including polar, which are water-soluble and non-polar. Acetate is weaker than ethanol as a solvent.

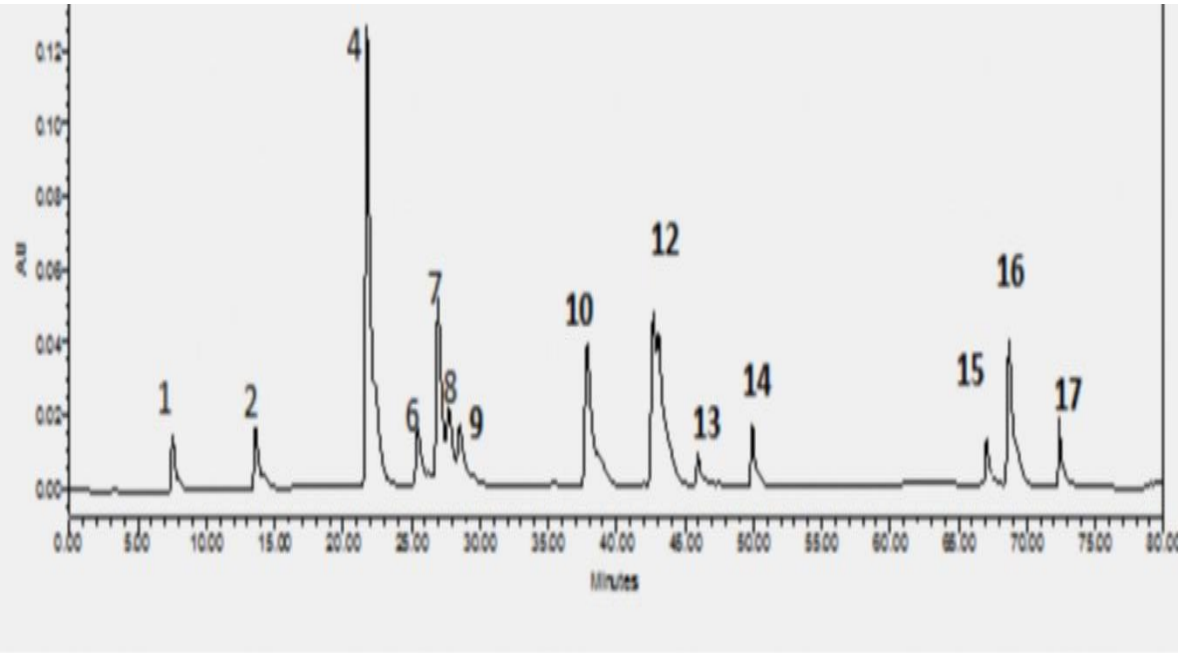
4.2.3 : Total flavonoids result and discussion :

The table (5) show the results of assaying the amount of flavonoids present in hibiscus extracted in three different solvents: ethanol 99%, ethanol 35% and ethyl acetate.

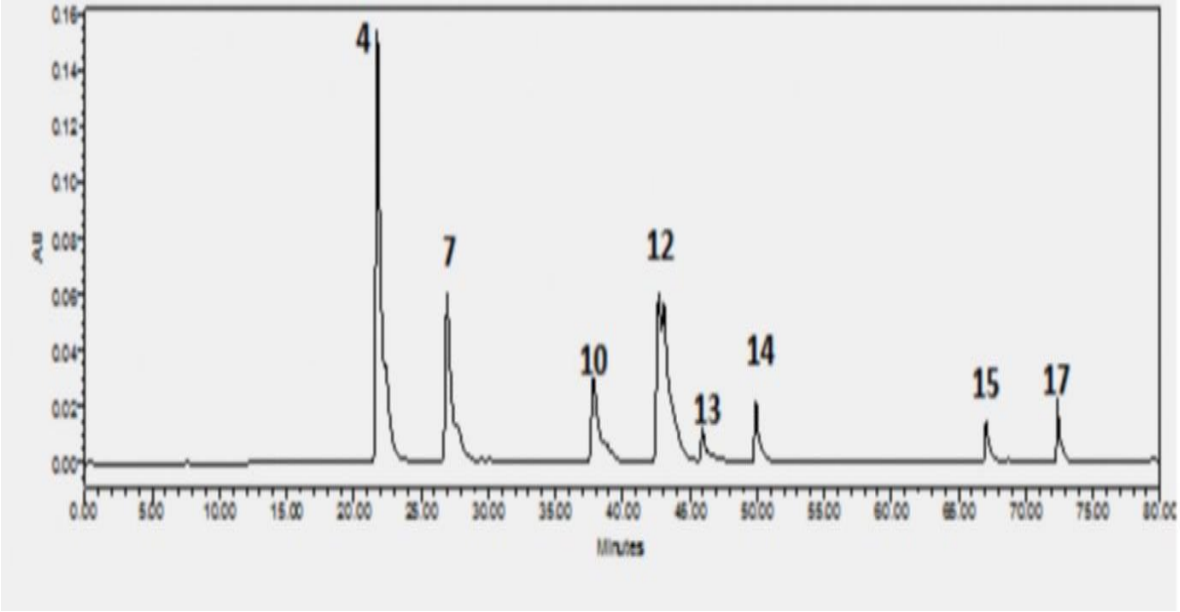
Natural plants are a rich source of flavonoids, and hibiscus showed high levels of flavonoids, reaching 64.8±0.88mg/g in 35% ethanolic extract, 24.59±1.7mg/g in 99% ethanolic extract, and 20.1±0.9mg/g in ethyl acetate g. The results of the experiment indicate that the lower the alcohol concentration and the greater the amount of water in it, the greater the number of flavonoids in the hibiscus extract.

4.2.4 HPLC analysis of the standards of polyphenolic compounds and flavonoids

Using the RP-phase procedure outlined above, the mixture containing 17 standards in the continuously flowing mobile phase stream that carries the sample into the HPLC column (20 L) into the HPLC chromatograph. Since each compound has a unique wavelength of maximum absorption, different wavelengths were employed with the photodiode array detector (Table 6). The chromatograms of the standards mixture at various wavelengths are shown in Figure 4 (300 nm (a), 323 nm (b), 270 nm (c), and 290 nm (d)). Figure 3.1 (a-d) makes clear that the 17 compounds may be separated using various wavelengths. Table (6) lists the standards' retention times together with the standards' maximum wavelengths of absorption.



(a)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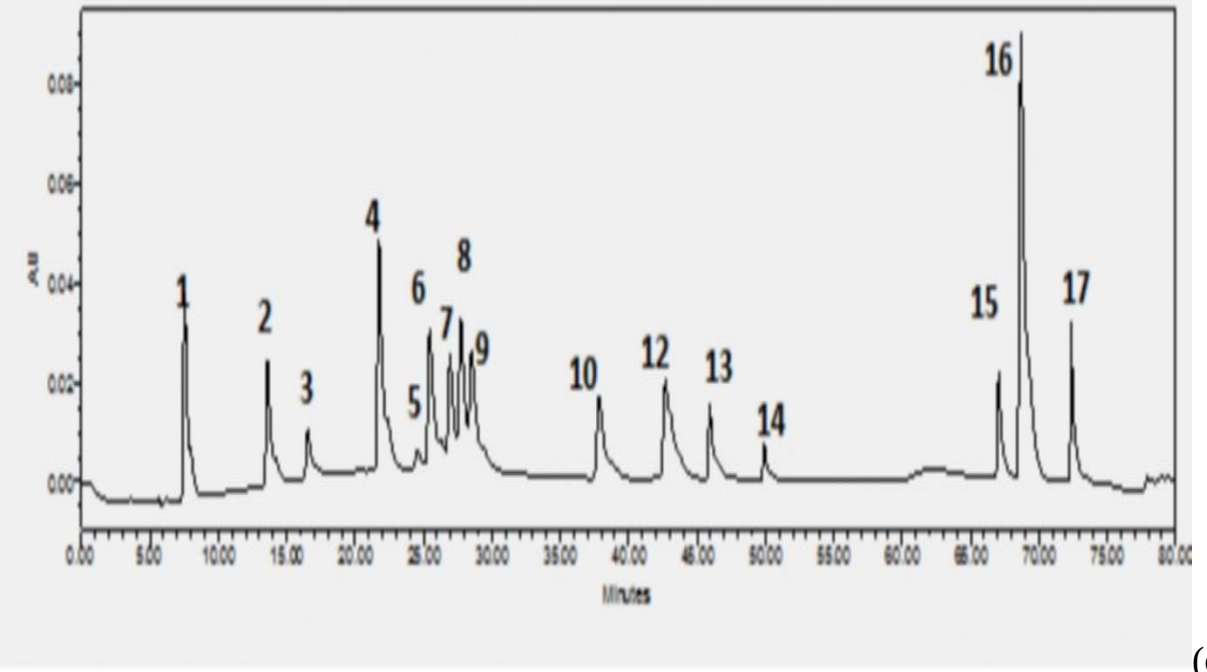
(b)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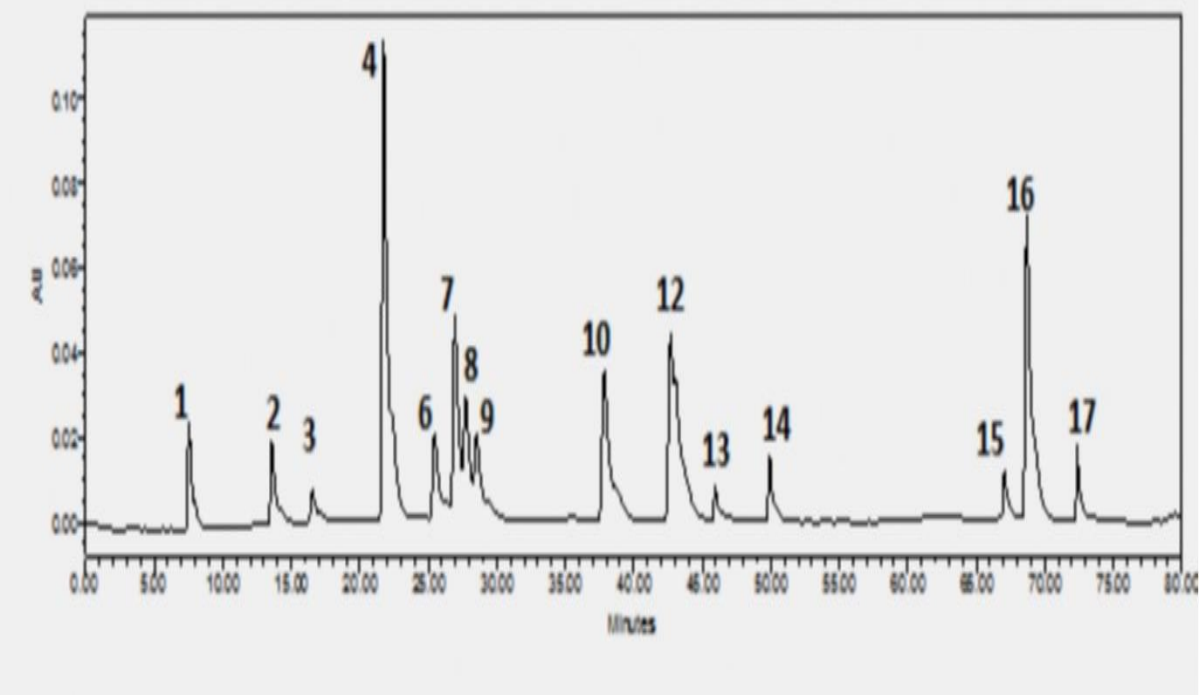
(c)



(d)

Figure 4 RP-HPLC analysis of polyphenolic and flavonoid standards yielded the following HPLC chromatograms: 300nm(a), 323nm(b), 270nm(c), and 290nm9d

TABLE (6) A LIST OF THE STANDARD CHEMICALS THAT WERE TESTED USING THE RP-HPLC METHOD, TOGETHER WITH THEIR RETENTION TIMES AND MAXIMUM ABSORPTION WAVELENGTHS

Standard #	The name of Standard	Retention time (min)	Wavelength (nm)
1	Gallic acid	8.26	271
2	3,4-Dihydroxybenzoic acid	13.87	259
3	3,4-Dihydroxyphenylacetic acid	16.57	280

Standard #	The name of Standard	Retention time (min)	Wavelength (nm
5	4-hydroxyphenylacetic acid	24.55	274
6	Vanallic acid	25.42	260
7	Caffeic acid	26.92	322
8	Syringic acid	27.73	274
9	Isovanallic acid	28.55	259
10	p-Coumaric acid	37.82	309
11	Ferrulic acid	42.68	322
12	Sinapic acid	43.1	323
13	Rutin	45.99	255
14	Verbascoside	49.98	329
15	Quercetin	67.04	364
16	Trans-cinnamic acid	68.69	275

4.2 HPLC analysis of plant extracts

Using the technique created for the standards, the plant extracts were examined. The chromatogram extract is displayed at 280 nm in Figure 5). Comparing the peak retention durations in the sample chromatogram of the extract with those in the standard allowed researchers to find and name the following polyphenolic compounds:

1. Gallic acid

2. chlorogenic acid

3. vanillic acid

4. caffeic acid

5. syringic acid

6. sinapic acid

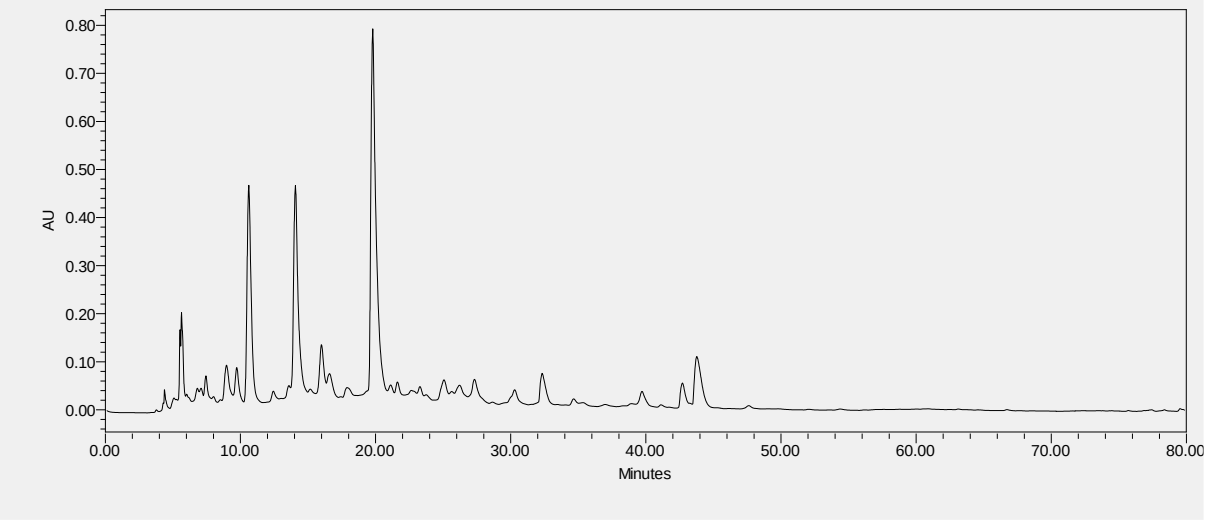


Fig (5) the chromatogram extract at 280 nm

4.3: HMG-CoA Reductase:

The results of the HMG-COA assay showed that hibiscus extract can inhibit the cholesterol production process by a percentage 41.7±0.87% , and this indicates the presence of active substances in the chemical composition of this plant capable of inhibiting the action of a percentage of the action of the drug used in the market, which is Pravastatin , and this result is consistent with (Tzu-LiLin.et.al) where it was Conducting a bed on a group of volunteers, as a result of which the hibiscus was able to reduce the proportion of this volunteers

The result of the microemulsion showed that the percentage of the resulting inhibition was 24 % of the active substance, which was 12.9% of the concentration of the first sample, in which the inhibition reached 41.7±0.87%, and this indicates that the microemulsion has a greater effectiveness.

4.4 AGE inhibition assay:

Hibiscus showed a weak ability to inhibit AGE , noting that hibiscus in its main components contains glucoside. The results for five different concentrations (10.7 , 8 , 6.7 , 5.3 , and 2.6 mg/ml) were 17.%, 14%, 6% , 5.68 % , - 5% . Where the highest result was 17% and the lowest result was -5%, and the results of the third and fourth concentrations were close. This finding

could be related to Afiune et al., 2017 one in which an experiment was conducted on a group of pregnant and non-pregnant mice, and the results revealed that this flower was only beneficial to pregnant mice with diabetes and their offspring.

4.5 Antibacterial result and discussion:

Four clinical pathogens were used to test Hibiscus sabdariffa calyx ethanol extract's antibacterial effectiveness (Escherichia coli, Staphylococcus aureus, MRSA, and Pseudomonas aeruginosa). utilizing the disc diffusion technique with positive controls (Gentamicin (10 g/disc) and Penicillin (10 units). A table in the study displays the zones of inhibition.

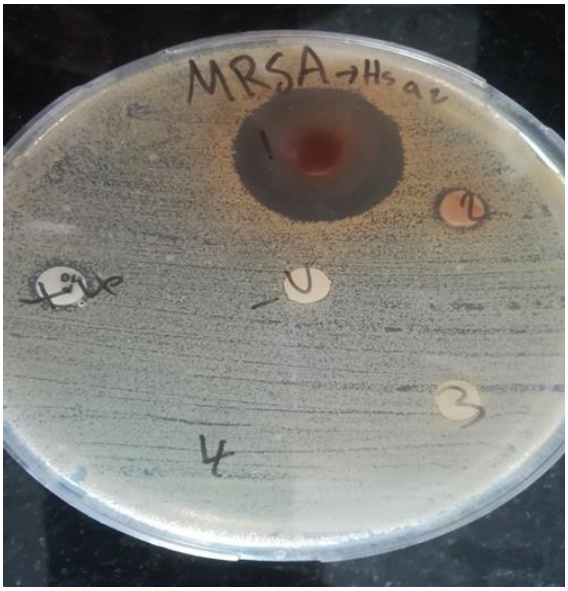
The result of antibacterial test of Hibiscus sabdariffa extract on MRSA bacteria shown in table (7)

TABLE (7) EFFECTS OF ANTIBIOTIC OF HS ETHANOIC EXTRACT ON MRSA

	Zone of inhibition (mm, diameter) Plant extract				
Microorganism	Concentration (100%)	Cons (75%)	Cons (50%)	+ve control Pincellin	-ve control D.W
MRSA	26±3mm	8.3±0.6mm	-	-	-

According to (https://www.cdc.gov/mrsa/community/index.html) This type of bacteria is resistant to Pincellin The experiment has shown this information. as shown the high capacity of the Hibiscus extract to inhibit MRSA, where the zone inhibition diameter was 26 ±3mm, While the inhibition of diluted hibiscus extract 75% in D.W was 8 ±0.6mm .and there is no effect to the dilution 50%

This result prove that hibiscus Contains active ingredients inhibit on this type of bacteria (fig (6) show zone inhibition of HS extract on MRSA)



Fig(6) : MRSA's zone of inhibition by Hs

The result of antibacterial test of Hibiscus sabdariffa extract on E-Coli bacteria shown in table (8)

TABLE (8) EFFECTS OF ANTIBIOTIC OF HS ETHANOIC EXTRACT ON E-COLI

	Zone of inhibition (mm, diameter) Plant extract				
Microorganism	Cons (100%)	Cons (75%)	Cons (50%)	+ve control Gentamicin	-ve control D.W
E-coli	20.3±1.1mm	7±1mm	-	15 mm	-

sabdariffa calyx extracts exhibited an antimicrobial effect against e -coli where the zone of inhibition diameter of concentrated extract was 20.3±1.1mm while the inhibition zone diameter of diluted solution (75%) was 7mm and there is no effect of diluted (50%)

This result correspond with (Hashim El Kamali, H. et al 2006) result where the research study show The ability to inhibit e coli this result similar with (Higginbotham, K. L et al 2014)

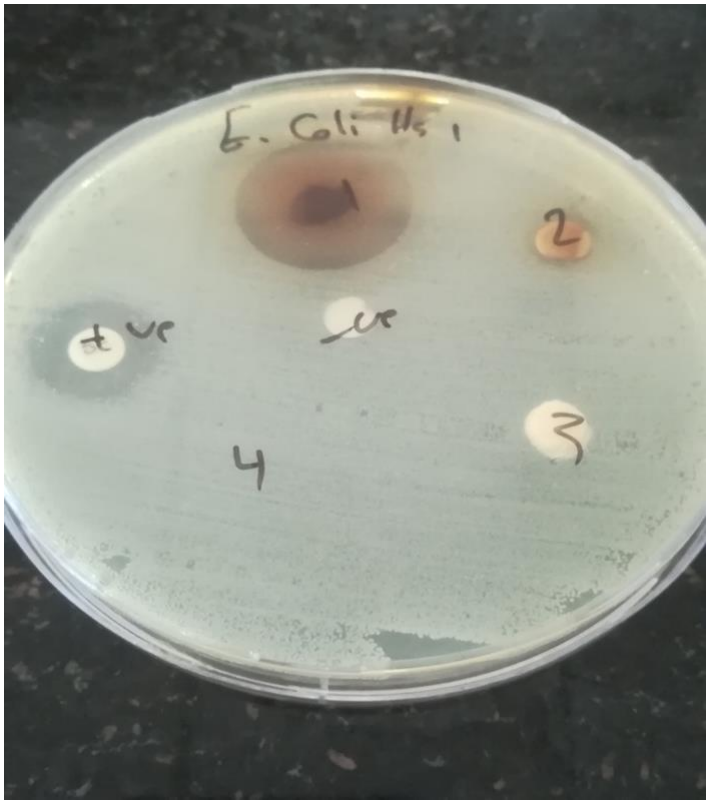


Fig (7) E. coli's zone of inhibition by Hs

The result of antibacterial test of Hibiscus sabdariffa extract on Staphylococcus aureus bacteria shown in table

TABLE (9) EFFECTS OF ANTIBIOTIC OF HS ETHANOIC EXTRACT ON STAFF AUREUS

Zone of inhibition (mm, diameter) Plant extract					
Microorganism	Con (100%)	Con(75%)	Con (50%)	+ve control penicillin	-ve control D .W
Staff aureus	34.3±0.6mm	11±1mm	-	15 mm	-

HSC extracts exhibited an antimicrobial effect against s. aureus where high inhibition zone diameter Up to 34.3±0.6mm by concentrated extract and 11mm in diluted solution (75%) , no effect was shown by (50%) dilution solution while the inhibition of positive control penicillin was 15 mm which is lower than the inhibition of concentrated hibiscus extract which means hibiscus has a high antibacterial compound

This result similar (Higginbotham, K. L. et al. , 2014) and (Hashim El Kamali, H. et al. , 2006) those research studies show high ability of inhibition by hibiscus which extracted in different solvent ethanol methanol and ethyl acetate .

Fig (8) S. aureus's zone of inhibition by Hs

The result of antibacterial test of Hibiscus sabdariffa extract on Pseudomonas bacteria shown in table (10)

TABLE (10) EFFECTS OF ANTIBIOTIC OF HS ETHANOIC EXTRACT ON PSEUDOMONAS

Zone of inhibition (mm, diameter) Plant extract					
Microorganism	Con (100%)	Con(75%)	Con (50%)	+ve control Gentamicin	-ve control D .W
Pseudomonas	24.3±0.6mm	9.7±1.15mm	-	29 mm	-

HSC extracts exhibited an antimicrobial effect against Pseudomonas where high inhibition zone diameter Up to 24.3±0.6mm by concentrated extract and 9.7±1.15mm in diluted solution (75%) , no effect was shown by (50%) dilution solution while the inhibition of positive control Gentamicin which is higher than the inhibition of concentrated hibiscus extract .

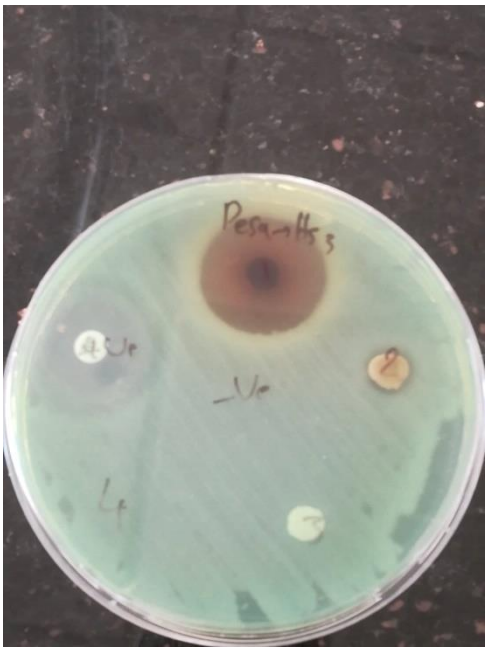


Fig (9 Pseudomonas's zone of inhibition by Hs

4.6 phase diagram:

Fig (10) show the result of phase diagram

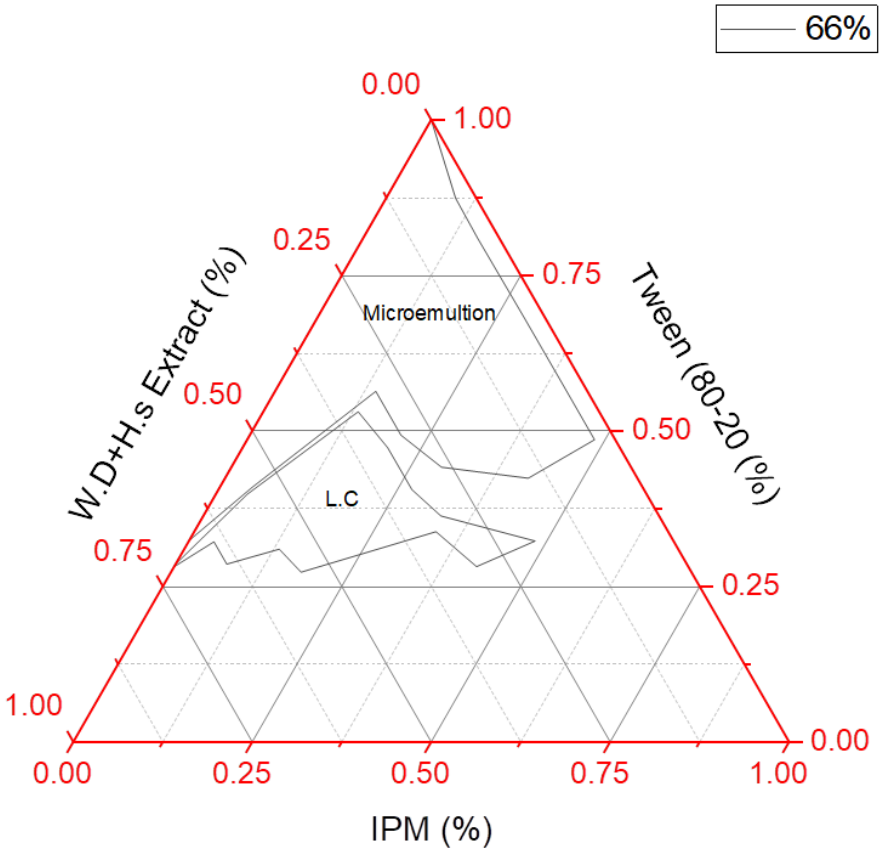


Fig (9) phase diagram of Hibiscuses

The drawing shows two regions, the microemulsion region, which is clearly visible in the upper part of the drawing, in which the surfactant concentration is highest, and the liquid crystal region

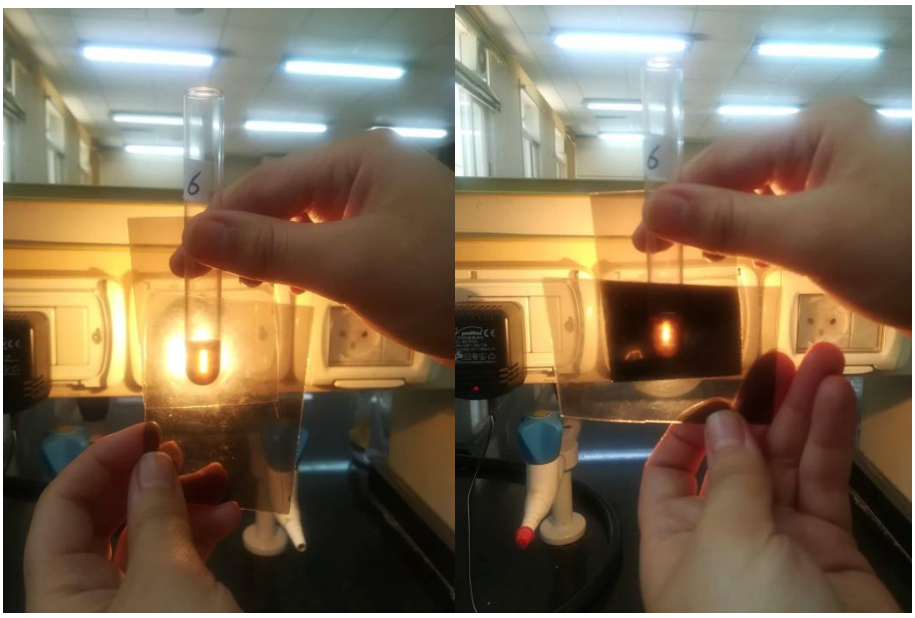


Fig (10) liquid crystals

4.7 : Cream

Softening hands cream. Apply easily and quickly. Leave no tacky film. Not interfere with normal hand perspiration. Be antiseptic. Have a pleasant smell. Have a stable color.

Fig (11) show the cream of hibiscuses

Fig (11) Hibiscuses Hand cream

5.Conclusion :

The present study offers preliminary evidence that suggests the H. sabdariffa extract can reduce cholesterol levels by reducing the activity of HMG-CoA reductase. In addition, the Hibiscus extract has high percent of antioxidants compounds Which makes it one of the plants capable of eliminating free radicals, which means that it may be anti-cancer, It has also high percent of phenols, and flavonoid compounds. Hibiscus showed a high ability to eliminate some types of bacteria, which means that it contains chemical compounds capable of eliminating bacteria.

Recommendations:

The results of the study encourage thinking about studying more pharmacological effects of hibiscus, and thus thinking about making a dietary supplement of hibiscus that helps mitigate the bad effects of drugs on the market.

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الصفات والتأثير الحيوي للكركديه

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ملخص :

في السنوات الاخيرة بدأ العالم بالتوجه الى استبدال الادوية الكيميائية بالمواد الطبيعية للتقليل من الاثار الجانبية المصاحبة لاستخدام هذه الادوية، فأصبحت البحوث العلمية تركز على دراسة التأثير الدوائية للعديد من النباتات. وفي هذه الدراسة تم اختيار نبات الكركديه لدراسة التأثيرات الدوائية والحيوية لهه النبتة.

تم دراسة التأثير المضاد للأكسدة لثلاث مستخلصات لنبات الكركديه في ثلاث انواع مختلفة من المذيبات (ايثانول ٩٩ % ، ايثانول ٣٥% ، ايثل اسيتات) باستخدام طريقة DPPH ، وكانت النتيجة قدرة عالية للكركديه كمضادة اكسدة وصلت الى 87.85% ،69.64% ، 81.6% في المستخلصات الثلاث على التوالي . وباستخدام طريقة Folin-Ciocalteau تم تحديد محتوى المستخلصات الثلاث من الفينول حيث وصل 55.816 ± 0.319ملغ/غرام جاليك اسيد ، 95.37 ± 0.311 ملغ/ غرام جاليك اسيد ، 48.73 ± 1.54 ملغ/ غرام جاليك اسيد في المستخلصات الثلاث على التوالي . وباستخدام طريقة aluminum chloride colorimetricتم تحديد محتوى المستخلصات الثلاثة من مركبات الفلافينويد الفينولية حيث كانت 24.59 ± 1.7ملغ / غرام ، 64.8 ± 0.88 ملغ/ غرام ، 20.1 ± 0.9 ملغ/غرام في المستخلصات الثلاث عل التوالي . تم ايضا دراسة تأثير مستخلص الكركديه الايثانولي في تخفيض نسبة الكولسترول الضار في الجسم (LDL) عن طريقة دراسة تأثير المستخلص في تثبيط عمل الانزيم الذي يحفز الكبد على انتاج الكوليسترول HMG-CoA reductase والذي يحول HMG-CoAالى mevalonate الذي يحفز الكبد . وقد اظهر الكركديه قدرة وصلت الى 41.7±0.87% من التأثير الدوائي pravastatinفي تثبيط انتاج الكولسترول . كما وتم دراسة تأثير الكركديه في خفض نسبة السكر في الدم مخبريا باستخدام glucose-bovine serum albumin (BSA) assay حيث استخدمت خمس تراكيز مختلفة من الكركديه (10.7, 8, 6.7, 5.3, 2.6)ملغ/ مليلتر وكانت النتيجة 17%, 14%, 6%, 5.68%, - 5% في الخمس تراكيز على التوالي . كما وتم دراسة تأثير المستخلص الايثانولي للكركديه في تثبيط نشاط اربع انواع من البكتيريا (MRSA, E-coli, S. aureus, and Pseudomonas)وتمت مقارن النتائج مع تأثير كل منPenicillin ، Gentamicin، وقد استخدمت طريقة disc diffusion methodوكانت النتائج وصل تأثير التثبيط لمستخلص الكركديه على MRSA الى 26±3 ملم في تركيز 100 % بينما وصل الى 8.3±0.6 ملم في تركيز 75 % بينما لم يظهر تركيز 50 % اي تأثير . ووصل تأثير التثبيط على E-coli الى ± 1.1ملم في تركيز 100 % و 7 ± 1ملم في 75 % بينما لم يظهر تركيز 50 % اي تأثير . وفيS. aureus وصل تأثير التثبيط الى 34.3 ± 0.6 ملم في تركيز 100 % و 11 ± 1ملم في 75 % ولم يظهر 50 % اي تأثير ، وفي Pseudomonasكان تأثير 100 % 24.3 ± 0.6ملم وتأثير 75 % 9.7 ± 1.15ملم ولم يظهر 50 % اي تأثير . وقد تم في هذه الدراسة ايضا عمل مخطط phase diagram حيث ظهرت منطقتين رائسين هما منطقة الميكرو ملشن في اعلى المخطط بالقرب من التركيز الاعلىsurfactant ، ومنطقة. liquid crystal

بحسب هذه الدراسة نستنتج ان للكركديه فوائد حيوية عديدة كونها تحوي على كمية كبيرة من مضادات الاكسدة والعديد من المركبات الفينولية و الفلافينويد ، واظهرت الدراسة ان للكركديه قدرة على خفض مستوى الكولسترول في الجسم ولها تأثير قليل على السكر في الدم بالإضافة الى قدرة عالية على تثبيط عمل بعض انواع البكتيريا