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THEME

Phytochemical Analysis and Biological Activity of *Hibiscus sabdariffa* L. Extracts.

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Abstract

This study aims to evaluate the phytochemical content and biological activity of *Hibiscus sabdariffa* L. on four extracts using solvents of different polarities: hexane, butanol, ethanol, and water. Quantitative analyses were conducted to estimate the quantitative content of flavonoids, tannins, and total phenols in each extract, and we also performed biological activity tests to evaluate the plant's biological efficacy.

The results showed that the ethanolic extract recorded the highest estimation in total phenolic content (186.3±30.20 mg GAE/g), while the butanolic extract excelled in flavonoids (63.3±0.87 µg QE/mg). The hexane extract recorded the highest estimation in terms of tannins (19.74±0.19 µg CE/mg).

As for the biological activities, the ethanolic extract showed the best antioxidant activity according to the DPPH test (58.02±0.35 µg/mL), while the FRAP test recorded the strongest effectiveness of the aqueous extract. The ethanolic extract also recorded the highest (47.21±9.61) in the phosphomolybdic reductive capacity test. On the other hand, in the anti-hemolysis test, both the aqueous and ethanolic extracts showed the highest efficacy (~600 µg/mL) and in the anti-inflammatory test, while the ethanolic extract (15.94±1.22) exhibited the highest sun protection factor (SPF).

These results clearly demonstrate the significant impact of solvent type on the extraction of biologically active compounds, and confirm that the ethanolic and butanolic extracts are the most effective options in terms of chemical content and biological activity.

Keywords:

Hibiscus sabdariffa L., plant extracts, phenolics, flavonoids, antioxidant activity, SPF, DPPH, FRAP, anti-inflammatory, anti-hemolysis.

Summary in Arabic (ملخص)

تهدف هذه الدراسة إلى تقييم المحتوى الكيميائي النباتي والنشاط البيولوجي لنبات *Hibiscus sabdariffa* L. على أربع مستخلصات باستخدام مذيبات مختلفة القطبية و هي: الهكسان، البوتانول، الإيثانول، والماء. وقد تم إجراء التحاليل الكمية لتقدير المحتوى الكمي من الفلافونويدات، التانينات، والفينولات الكلية لدى كل مستخلص وقمنا أيضا بإختبارات النشاطية البيولوجية لتقييم فعالية النبات البيولوجية،

أظهرت النتائج أن المستخلص الإيثانولي سجل أعلى تقدير في محتوى الفينولات الكلية (mg GAE/g 30.20±186.3) أما في الفلافونويدات فقد تفوق المستخلص البوتانولي (μg QE/mg 0.87±63.3)، بينما سجل المستخلص الهكساني أعلى تقدير من حيث التانينات (μg CE/mg 0.19±19.74).

أما فيما يتعلق بالأنشطة البيولوجية، أظهر المستخلص الإيثانولي أفضل نشاط مضاد للأكسدة حسب اختبار DPPH (58.02±0.35 μg/mL))، أما اختبار FRAP فقد سجل المستخلص المائي أقوى فعالية كما سجل المستخلص الإيثانولي أعلى (9.61±47.21) في اختبار القدرة الإرجاعية فوسفوموليبدينيوم. من جهة أخرى، أما في اختبار anti-hemolysis فقد سجل المستخلصان المائي والأيثانولي اعلى فعالية (١~600 μg/mL) واختبار anti-inflammatory، بينما تميز المستخلص الإيثانولي (1.22±15.94) بأعلى معامل حماية من الشمس (SPF)

توضح هذه النتائج التأثير الواضح لاختلاف نوع المذيب على استخلاص المركبات الفعالة بيولوجيًا، وتؤكد أن المستخلص الإيثانولي والبوتانولي يمثلان الخيارين الأكثر فاعلية من حيث المحتوى الكيميائي والنشاط البيولوجي.

الكلمات المفتاحية:

Hibiscus sabdariffa L.، الفينولات، الفلافونويدات، النشاط المضاد للأكسدة، مضاد للالتهاب.

Summary in French (Résumé)

Cette étude vise à évaluer le contenu chimique végétal et l'activité biologique de la plante *H. sabdariffa L*. sur quatre extraits en utilisant des solvants de polarité différente, à savoir : l'hexane, le butanol, l'éthanol, et l'eau. Des analyses quantitatives ont été effectuées pour estimer la teneur en flavonoïdes, tanins et polyphénols totaux de chaque extrait, et nous avons également réalisé des tests d'activité biologique pour évaluer l'efficacité biologique de la plante.

Les résultats ont montré que l'extrait éthanolique a enregistré la plus haute estimation en termes de contenu total en phénols (186,3±30,20 mg GAE/g), tandis que pour les flavonoïdes, l'extrait butanolique a surpassé (63,3±0,87 µg QE/mg). En ce qui concerne les tanins, l'extrait hexanique a enregistré la plus haute estimation (19,74±0,19 µg CE/mg)

En ce qui concerne les activités biologiques, l'extrait éthanolique a montré la meilleure activité antioxydante selon le test DPPH (58,02±0,35 µg/mL), tandis que le test FRAP a enregistré la plus forte efficacité avec l'extrait aqueux. L'extrait éthanolique a également enregistré la plus haute valeur (47,21±9,61) dans le test de capacité réductrice phosphomolybdène. D'autre part, dans le test anti-hémolyse, les extraits aqueux et éthanoliques ont enregistré la plus haute efficacité (~600 µg/mL) et dans le test anti-inflammatoire, tandis que l'extrait éthanolique (15.94±1.22) a montré le plus haut indice de protection solaire (SPF).

Ces résultats montrent l'impact évident de la variation du type de solvant sur l'extraction des composés biologiquement actifs, et confirment que les extraits éthanolique et butanolique représentent les deux options les plus efficaces en termes de contenu chimique et d'activité biologique.

Mots-clés :

Hibiscus sabdariffa L., extraits végétaux, phénols, flavonoïdes, activité antioxydante, SPF, DPPH, FRAP, anti-inflammatoire, anti-hémolytique.

Nomenclature

Symbol:

°C Degree Celsius is unit of temperature

µg Micrograms

µL Microlitre

L Liter

mg Milligrams

MgO Magnesium oxide

mL Milliliter

Abbreviations:

DPPH• 1,1-diphenyl-2- picrylhydrazyl

DW Dry weight

EC_{50} The effective concentration

IC_{50} The half maximal inhibitory concentration

MCF-7 Michigan cancer foundation-7

SPF Sun protected factor

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INTRODUCTION

Introduction

The importance of medicinal plants in promoting sustainable human health is well recognized. These plants possess therapeutic and healing properties that can be found in one or more of their parts. The global use of medicinal plants is on the rise, as they are increasingly employed in the prevention and treatment of various health conditions. Their affordability, especially when compared to synthetic industrial medications, makes them a viable alternative in many regions (Akinyemi et al., 2018).

Medicinal plants serve as a vast reservoir of bioactive compounds, primarily due to their secondary metabolites, which exhibit remarkable chemical diversity and a wide range of biological activities (Buchanan et al., 2000). According to the World Health Organization (WHO), approximately 80% of the global population relies on traditional plant-based preparations as a primary form of healthcare (Mostafa et al., 2018).

One such widely used medicinal plant is *Hibiscus sabdariffa* L., a member of the Malvaceae family. It is cultivated for both dietary and therapeutic purposes. Infusions made from *H. sabdariffa* are traditionally recommended for managing cholesterol levels, blood pressure, and various microbial infections. The plant also exhibits antiseptic and cardioregulatory properties. In addition to its medicinal applications, it is commonly consumed in the form of vegetables, beverages, and jams (Lepengue et al., 2011).

H. sabdariffa is traditionally used in multiple forms, including hot and cold beverages, flavoring agents, and herbal medicines, and has various food industry applications (Da-Costa-Rocha et al., 2012). It is rich in phytochemical compounds and demonstrates a broad spectrum of pharmacological activities such as antioxidant, antihypertensive, hypocholesterolemic, immunomodulatory, hepatoprotective, renoprotective, diuretic, anti-obesity, antiurolithic, antidiabetic, antimicrobial, and anticancer properties all without significant genotoxic effects (Patel et al., 2012). In Sudan, *H. sabdariffa* is a widely consumed traditional beverage, used for treating ailments such as respiratory tract infections, colds, fevers, hypertension, and malaria (Khalid et al., 2012).

In this context, the present study aims to investigate the phytochemical profile of *Hibiscus sabdariffa* L. flowers cultivated in the Tamanrasset region of southern Algeria. Although this plant is widely used in traditional medicine and consumed as a beverage across various cultures, there remains limited information regarding the chemical composition of its

different solvent-based fractions, particularly from this specific geographic region. To address this gap, a sequential extraction was carried out using solvents of increasing polarity-hexane, butan-1-ol, ethanol, and water-to separate and analyze the distribution of bioactive compounds. This fractionation approach not only helps to identify the types of secondary metabolites present in each extract but also provides insight into how solvent polarity influences the extraction efficiency of various phytochemicals.

CHAPTER I

About Hibiscus sabdariffa L.

The plant *Hibiscus sabdariffa* L., commonly known as roselle or hibiscus, belongs to the Malvaceae family (Hopkins et al., 2013).

It is widely cultivated around the world in tropical and subtropical regions, including China, Thailand, Indonesia, Egypt, Sudan, Saudi Arabia, Taiwan, Vietnam, Nigeria, and Mexico, among others (Montalvo et al., 2022).

This plant is often used in traditional medicine due to its richness in phytochemicals such as polyphenols, especially anthocyanins, polysaccharides, and organic acids, making it highly promising for modern therapeutic applications (Hopkins et al., 2013).
The main uses of (*H. sabdariffa*) cups are cooking, a source of dyes in cosmetics and food applications, and therapeutic in folk medicine for treating various diseases (Montalvo et al., 2022).

Hibiscus tea (*H. sabdariffa*) (Malvaceae) is widely used around the world as a beverage and as a treatment for high blood pressure and high cholesterol levels (Riaz et al., 2018).

1. Systematic and Classification of roselle:

Hibiscus sabdariffa L. is a tetraploid species (2n = 4x = 72) whose chromosomes are related to the diploid (2n = 2x = 36) (*Hibiscus cannabinus* L.). It belongs to the phylum of flowering plants (spermaphyte) with more than 200 species native to tropical and subtropical regions around the world (Wilson and Menzel, 1964; Mclean, 1973; Cissé *et al.,* 2008).

Morphological and agronomic characterization makes it possible to distinguish two varieties of the *sabdariffa* species (variety *sabdariffa* and variety *altissima*) whose phenotypes are complementary (Sié *et al*., 2009; Cissé *et al*., 2009; Singh *et al*., 2017; Ankrah *et al*., 2018)., In the following table is the classification of the plant Hibiscus sabdariffa L.

Table (1): Classification of the plant *Hibiscus sabdariffa*. L

Rank	**Classification**
Root	Root
Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida

Order	Malvales
Family	Malvaceae
Genus	Hibiscus
Species	L.Hibiscus sabdariffa

2- Synonyms and Nomenclatures

The species *H. sabdariffa*, widely recognized for its economic and medicinal significance, has been referred to by numerous synonyms due to historical classifications and taxonomic revisions. According to World-Flora-Online-database , its synonyms include *Abelmoschus cruentus* Walp., *Furcaria sabdariffa* Ulbr., *Hibiscus acetosus* Noronha, *Hibiscus cruentus* Bertol., *Hibiscus digitatus* Cav., *Hibiscus digitatus* var. kerrianus DC., *Hibiscus fraternus* L, *Hibiscus gossypiifolius* Mill., *Hibiscus masuianus* Is Wild. & T. Durand, *Hibiscus palmatilobus* Baill., *Hibiscus sanguineus* Griff., *Hibiscus subdariffa* Rottler, *Sabdariffa digitata* Kostel., and *Sabdariffa rubra* Kostel. (Kowti, R et al., 2020).

These nomenclatures reflect the rich history of botanical exploration and highlight the plant's broad geographic distribution and varied morphological characteristics that contributed to its reclassification over time.

English: Roselle, Sorrel, Red Sorrel, Jamaican Sorrel

French: Oseille de Guinée, Karkadé

Spanish: Flor de Jamaica, Rosa de Jamaica

Arabic: كركديه (Karkadeh)

Hindi: Lal Ambari

Chinese: 洛神花 (Luò shén huā)

German: Afrikanische Malve

Portuguese: Vinagreira, Quiabo-roxo

Thai: กระเจี๊ยบแดง (Krajiab Daeng)

Filipino (Tagalog): Gumamela, Rosas de Jamaica

Malaysia: Asam paya, Asam susur, Ribena Malaysia (Kowti et al., 2020).

3- Botanical Description of Hibiscus *sabdariffa* L.:

Hibiscus sabdariffa L. is a plant that grows from a deep taproot system and can reach a height of more than 3.5 meters. It is vigorous, sparsely branched, and very fibrous, with smooth red cylindrical stems and an edible red or pale-yellow calyx. It has a deep-penetrating

taproot, epigeal germination, rounded cotyledons measuring 2.5 cm × 3 cm, and leafy in appearance (Shruthi., 2016).

Figure (1): Flowers of *H. sabdariffa*.

3.1. Stems: The stems are woody, cylindrical, and typically red to purple (Lavanya et al., 2019).

3.2. Leaves: The upper leaves of *H. sabdariffa* are simple, while the lower ones are deeply lobed with 3 to 5 or even 7 lobes. The margins are toothed, and the leaves are generally arranged alternately along the stem. They measure 7.5 to 12.5 cm in length and are green-red, reddish-violet, with reddish veins (Shruthi., 2016).

3.3. Calyces: The calyx is typically red, composed of 5 large sepals with a collar called the epicalyx made up of 8–12 thin, pointed bracts at the base. It begins to enlarge at the end of the day and is 3.2 to 5.7 cm long, completely surrounding the fruit. It is red or white (Shruthi., 2016).

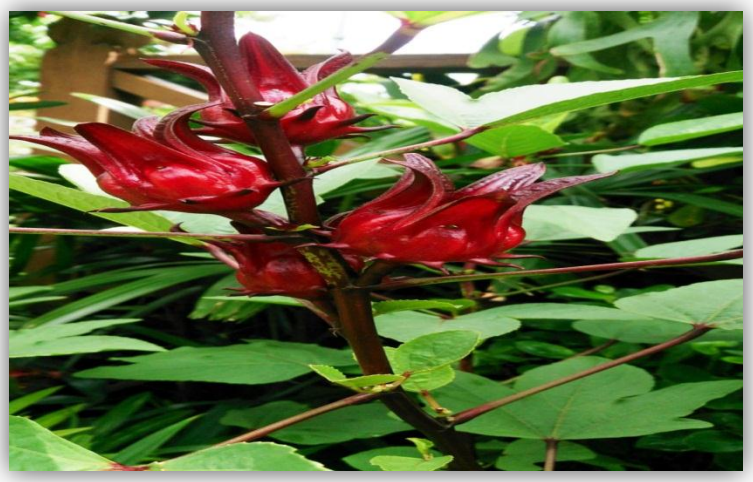


Figure (2): Stems and calyces of *H. sabdariffa.*

3.4. Flowers: The flower emerges on the branches at the leaf axils. They can reach 12.5 cm in diameter, with yellow petals and a pink or brown eye. They turn pink by the end of the day, open late in the morning, and close early in the afternoon (Shruthi., 2016).

Figure (3/4): Flower of *H. sabdariffa.*

3.5. Fruit: It is an ovoid capsule with 5 compartments, each consisting of 3 thin blades that are smooth inside and covered with fine, prickly hairs outside. The capsule contains the seeds (Paul, 1995).

Figure (5): Fruit of *H. sabdariffa.*

3.6. Seeds: The persistent red calyx of *H. sabdariffa* encloses a round fruit that contains numerous small, ovoid, brownish seeds. When mature, they are kidney-shaped, 3 to 5 mm long, and covered with tiny, thick, star-shaped hairs (Mahadevan et al., 2009).

Figure (6): Seeds of *H. sabdariffa*.

3.7. Roots: Roselle has a prolific root system with a long taproot and extended lateral roots, making it sensitive to soil moisture changes and capable of deep-water absorption (Lavanya et al., 2019).

Figure (7): Roots of *H. sabdariffa.*

4- Geographical distribution:

H. sabdariffa is an herbaceous plant of the Malvaceae family, widely widespread in the tropical and subtropical regions of both hemispheres (Cisse et al., 2008), also present in many regions of Central America and Asia. But it is originally from Africa, where the seeds were supposedly brought to America by slaves Africans. It was widely spread in Asia, where the species adapted. *H. sabdariffa* is also present in Thailand, Vietnam, Malaysia, China, Sudan, and Mexico. It is also present in other countries such as Egypt, Senegal, Ghana, Niger, Nigeria, Angola (Grubben, 2004), Tanzania, Mali, Chad, and Jamaica that produce in small quantities (Morton, 1987).

Figure (8): Geographical distribution of *H. sabdariffa.*

5- Phytochemistry:

Nutritional value:

The nutritional composition of fresh *H. sabdariffa* varies between studies, probably due to different varieties, genetic, environmental, ecology and harvest conditions of the plant. Early studies reported that *H. sabdariffa* contains protein (1.9 g/100 g), fat (0.1 g/100 g), carbohydrates (12.3 g/100 g) and fibre (2.3 g/100 g). They are rich in vitamin C (14 mg/100 g), β-carotene (300 μg/100 g), calcium (1.72 mg/100 g) and iron (57 mg/100 g) (Ismail, Ikram, & Nazri, 2008).

The leaves contain protein (3.3 g/100 g), fat (0.3 g/100 g), carbohydrate (9.2 g/100 g), minerals (phosphorus (214 mg/100 g), iron (4.8 mg/100 g) thiamine (0.45 mg/100 g), β-carotene (4135 μg/100 g), riboflavin (0.45 mg/100 g) and ascorbic acid (54 mg/100 g) (Ismail, Ikram, & Nazri, 2008).

The seeds contained crude fatty oil (21.85%), crude protein (27.78%), carbohydrate (21.25%), crude fibre (16.44%) and ash (6.2%). In terms of minerals, the most prevalent is potassium (1329 ± 1.47 mg/100 g), followed by sodium (659 ± 1.58 mg/100 g), calcium (647 ± 1.21 mg/100 g), phosphorus (510 ± 1.58 mg/100 g) and magnesium (442.8 ± 1.80 mg/100 g). The major saturated fatty acids identified in the seed oil are palmitic (20.84%) and stearic (5.88%) acids and the main unsaturated fatty acids are linoleic (39.31%) and oleic acid (32.06%) (Nzikou et al., 2011).

The main constituents of *H. sabdariffa* relevant in the context of its pharmacological are organic acids, anthocyanins, polysaccharides and flavonoids (Eggensperger and Wilker, 1996, Müller and Regensburg, 1990).

6- Bioactive constituents:

6-1. Organic acids:

H. sabdariffa extracts contain a high percentage of organic acids, including citric acid, hydroxycitric acid, hibiscus acid, malic and tartaric acids as major compounds, and oxalic and ascorbic acid as minor compounds. Based on previous studies, the percentage of organic acids in "hibisci flos" varies; hibiscus acid accounts for 13–24%, citric acid 12–20%, malic acid 2–

9%, tartaric acid 8% and 0.02–0.05% of ascorbic acid (vitamin C) (Eggensperger and Wilker, 1996, Schilcher, 1976).

In the late 1930s, citric and malic acids were first reported in aqueous extracts of the calyx (Buogo and Picchinenna, 1937, Indovina and Capotummino, 1938, Reaubourg and Monceaux, 1940) and also in five different strains (from Egypt, Senegal, India, Thailand and Central America) of *H. sabdariffa* var. sabdariffa (Khafaga, Koch, El Afry, & Prinz, 1980). Ascorbic acid is also present in *H. sabdariffa* but its content varies dramatically between fresh (6.7–14 mg/100 g (Ismail et al., 2008, Morton, 1987) and dried calyces (260–280 mg/100 g (Ismail, Ikram, & Nazri, 2008). The amount of ascorbic acid in the latter report being much higher than the ones previously reported in the literature. The differences observed might be due to different varieties, genetics, environment, ecology and harvest conditions.

6-2. Hydroxycitric acid:

(Fig 9) has an additional hydroxyl group at the second carbon of citric acid. This acid has four stereoisomers, (2S, 3S), (2R, 3R), (2S, 3R) and (2R, 3S), and their lactone forms. The principal organic acid found in the *H. sabdariffa* is the (2S, 3R)-hydroxycitric acid (Hida, Yamada, & Yamada, 2007). It is the principal organic acid found in the calyces of *H. sabdariffa*. It is worth noting that, (2S, 3R)-hydroxycitric acid from Hibiscus is different from the more commonly known (2S,3S)-hydroxycitric acid (HCA) extracted from, e.g., Garcinia sp., thus raising the question as to whether both diastereomers have identical or partially different pharmacological profiles.

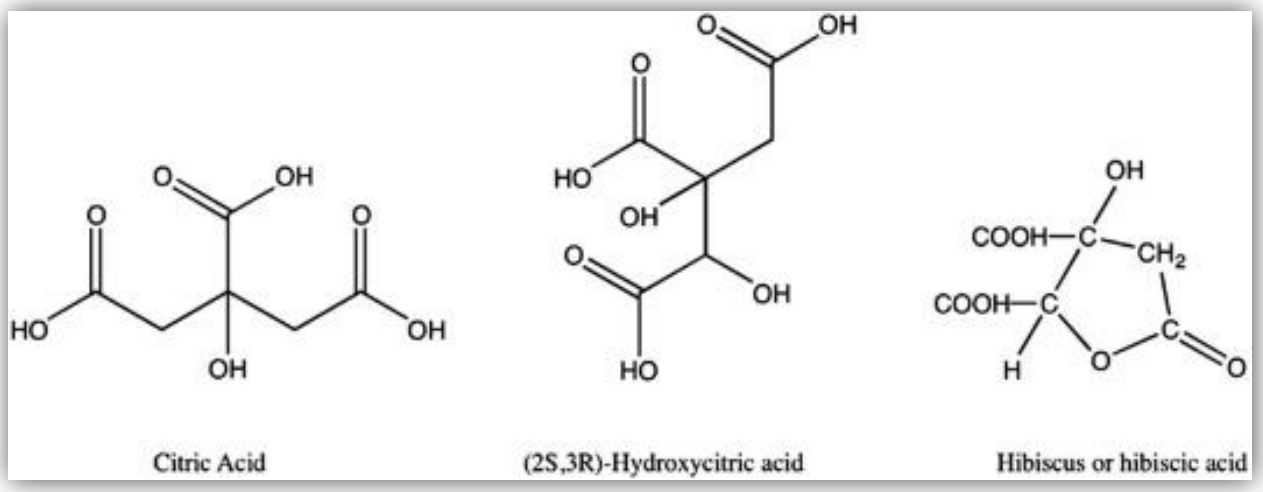


Fig. (9). Citric acid and its derivatives.

6-3. Hibiscus acid

(Fig. 1) is the lactone form of (+)-allo-hydroxycitric acid. It compromises a citric acid moiety with an additional hydroxyl group at the second carbon and has two diastereomers due to the existence of two chiral centers in the molecule (Boll et al., 1969, Eggensperger and Wilker, 1996, Griebel and Lebensm, 1939, Griebel and Lebensm, 1942).

Hydroxycitric acid, hibiscus acid and it derivatives as the major organic acids in the leaves and calyces extracts of *H. sabdariffa* (Beltran-Debon et al., 2010, Herranz-Lopez et al., 2012, Peng et al., 2011, Ramirez-Rodrigues et al., 2011a, Ramirez-Rodrigues et al., 2011b, Rodriguez-Medina et al., 2009)

6-4. Flavonoids:

H. sabdariffa contain polyphenols of the flavonol and flavanol type in simple or polymerised form. The following flavonoids have been described in *H. sabdariffa* extracts: hibiscitrin (hibiscetin-3-glucoside), sabdaritrin, gossypitrin, gossytrin and other gossypetin glucosides, quercetin and luteolin (McKay, 2009, Williamson et al., 2013); as well as chlorogenic acid, protocatechuic acid, pelargonidic acid, eugenol, quercetin, luteolin and the sterols β-sitosterol and ergosterol (McKay, 2009, Williamson et al., 2013).

Earlier the flowers of *H. sabdariffa* were recorded to contain 3-monoglucoside of hibiscetin (hibiscitrin) (Rao and Seshadri, 1942, Rao and Seshadri, 1942, Rao and Seshadri, 1948), 7-glucoside of gossypetin (gossypitrin) and sabdaritrin, which on acid hydrolysis yielded an hydroxyflavone named sabdaretin (Rao and Seshadri, 1942, Rao and Seshadri, 1942). The presence of these flavonol glycosides was low, with hibiscitrin being the major compound followed by gossypitrin and sabdaritrin (Rao and Seshadri, 1942, Rao and Seshadri, 1942). In 1961, gossypetin-3-glucoside (gossytrin) was isolated (Seshadri & Thakur, 1961). The petals of *H. sabdariffa* var. altissima also contain gossypetin-8-glucoside (0.4%) and gossypetin-7-glucoside (Subramanian & Nair, 1972).

From the leaves of *H. sabdariffa*, β-sitosteryl-β-d-galactoside (Osman, El-Garby-Younes, & Mokhtar, 1975) and from the seeds ergosterol (Salama & Ibrahim, 1979) were reported. β-sitosterol and ergosterol were also reported in *H. sabdariffa* extracts (McKay, 2009, Williamson et al., 2013).

The methanolic extract of the flowers also contains quercetin, luteolin and its glycoside (Salah, Gathumbi, & Vierling, 2002). Quercetin had already been identified in H. sabdariffa (Takeda & Yasui, 1985). One study reported that the amount of quercetin present in *H. sabdariffa* WE were 3.2 mg/g while rutin was 2.1 mg/g (Alarcon-Alonso et al., 2012). Quercetin and its conjugated glycosides (quercetin-3-glucoside), as well as, rutin (quercetin-3-rutinoside; Fig. 3) were frequently identified in *H. sabdariffa* WE, alongside with kaempferol (Beltran-Debon et al., 2010, Herranz-Lopez et al., 2012, Peng et al., 2011, Ramirez-Rodrigues et al., 2011a, Ramirez-Rodrigues et al., 2011b).

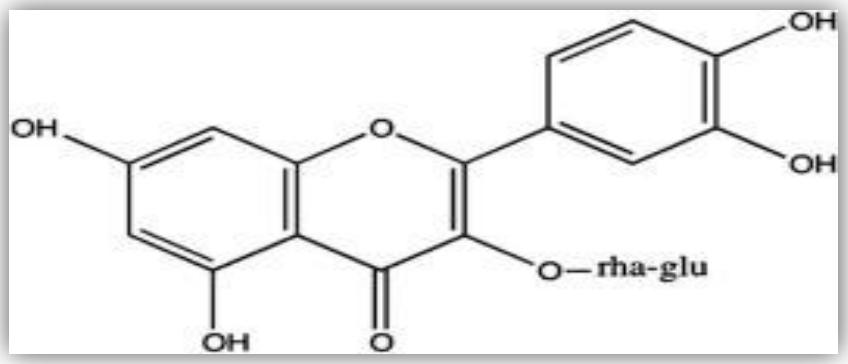


Fig (10) Quercetin-3-rutinoside.

The water extract of the dried leaves showed the presence of catechin (4.25%) and ellagic acid (28.20%) (Lin et al., 2012), while *H. sabdariffa* WE showed the presence of protocatechuic acid (24.24%), catechin (2.67%), gallocatechin (2.44%), caffeic acid (19.85%), gallocatechin gallate (27.98%) (Yang et al., 2010). Similar results were reported by Huang and co-workers (Huang et al., 2009).

6-5. Anthocyanins

The anthocyanins are a group of flavonoid derivatives and natural pigments present in the dried flowers of *H. sabdariffa* and their colour varies with pH.

Delphinidin and cyanidin-based anthocynins, include delphinidin-3-sambubioside (hibiscin), cyanidin-3-sambubioside (gossypicyanin), cyanidin-3,5-diglucoside, delphinidin (anthocyanidin) and others (Williamson et al., 2009).

The first anthocyanin from the calyx of *H. sabdariffa* to be isolated was “hiviscin”, also known as “hibiscin”, later named delphinidin-3-sambubioside and assigned the structure

of cyanidin-3-glucoside (Yamamoto & Osima, 1932), which was later renamed as delphinidin-pentoside-glucoside (Yamamoto & Osima, 1936). From the pigments of *H. sabdariffa*, three different anthocyanins were isolated: delphinidin-3-sambubioside (hibiscin), delphinidin-3-glucoside and cyanidin-3-glucoside (chrysanthenin) using material from Taiwan and Trinidad (Du and Francis, 1973, Shibata et al., 1969). The last study also identified cyanidin-3-sambubioside (gossypicyanin) . Later, the presence of cyanidin-3,5-diglucoside and cyanidin-3-(2G-glucosylrutinoside) in the flower pigments of *H. sabdariffa* var. altissima (Subramanian & Nair, 1972) was reported. A study conducted with 5 different strains of *H. sabdariffa* var. sabdariffa reported cyanidin-3-sambubioside and cyanidin-3-glucoside as the major compounds present in this plant (Khafaga et al., 1980). In one of the strains (Senegalese strain), delphinidin glycosides were absent. In this study, the anthocyanin content reached 1.7% to 2.5% of the dry weight in all strains. A similar anthocyanin content was observed in another study where their amount was about 1.5 g per 100 g of dry weight of *H. sabdariffa*, in terms of delphinidin-3-sambubioside (Du & Francis, 1973).

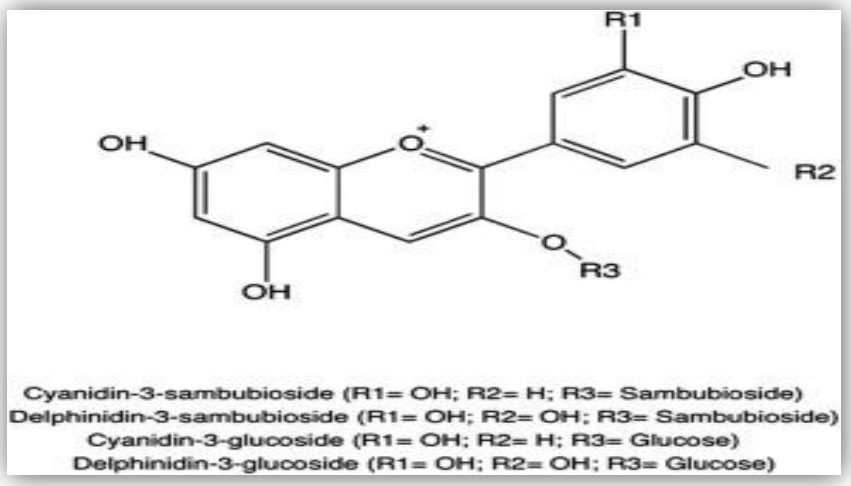
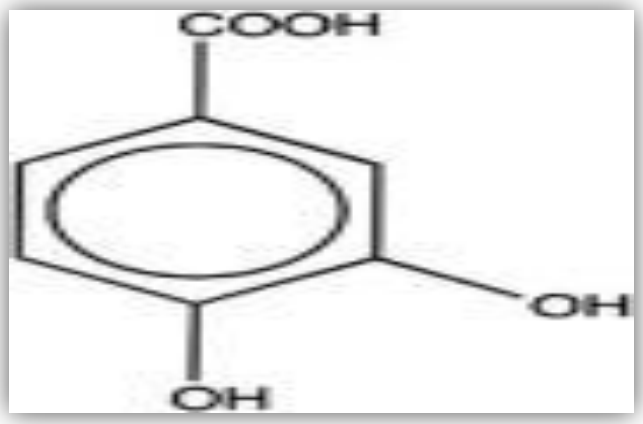


Fig (11). Chemical structures of main anthocyanins.

Several studies have identified delphinidin-3-sambubioside (delphinidin-3-O-(2-O-β-d-xylopyranosyl)-β-d-glucopyranoise) and cyanidin-3-sambubioside (cyanidin-3-O-(2-O-β-d-xylopyranosyl)-β-d-glucopyranoside) as the major anthocyanins present in extracts from *H. sabdariffa* (Alarcon-Aguilar et al., 2007, Alarcon-Alonso et al., 2012, Beltran-Debon et al., 2010, Degenhardt et al., 2000, Herranz-Lopez et al., 2012, Peng et al., 2011) and leaves (Rodriguez-Medina et al., 2009).

6-6. Phenolic acid:

Protocatechuic acid (PCA) is an important phenolic acid present in H. sabdariffa extract (Lee et al., 2002, Lin et al., 2003, McKay, 2009, Williamson et al., 2013). It was isolated from the dried flowers of H. sabdariffa and assigned the structure of 3,4-dihydrobenzoic acid) (Osman et al., 1975, Tseng et al., 1996).



Fig(12): Protocatechuic acid

Chlorogenic acid is another phenolic acid present in both leaf and *H. sabdariffa* extracts and belongs to a family of esters formed between certain trans-cinnamic acids (caffeic acid, ferulic acid and p-coumaric acid) and quinic acid (Clifford, Johnston, Knight, & Kuhnert, 2003). Several studies reported the presence of this acid and its derivatives in extracts of *H. sabdariffa* (Beltran-Debon et al., 2010, Herranz-Lopez et al., 2012, Peng et al., 2011, Ramirez-Rodrigues et al., 2011a, Ramirez-Rodrigues et al., 2011b, Salah et al., 2002) and leaves (Rodriguez-Medina et al., 2009). In one study, the amount of chlorogenic acid in the extract was reported to be 2.7 mg/g (Alarcon-Alonso et al., 2012).

6-7. Mucilage, pectin and carbohydrates (polysaccharides)

Polysaccharides are another key group of compounds present in large quantities in the *H. sabdariffa* WE. In one study, the ethanol-precipitated water extract yielded 10% of reddish polysaccharides. The following compounds were identified in two different fractions, arabinose, galactose, glucose, rhamnose and smaller amounts of galacturonic acid, glucuronic

acid, manose and xylose (Müller, Kraus, & Franz, 1989). Similar results were obtained in two other studies (Brunold et al., 2004; Müller & Franz, 1992).

The mucilage content was determined in the calyces of five strains of *H. sabdariffa* var. sabdariffa, reaching 24–28% in strains from Central America and Egypt but only 15% in an Indian strain. This amount was only reached at a later stage of development in the strains from Senegal and Thailand. The pectin content only accounted for 2–4% while the sugars reached a maximum of 3–5% in these five strains. Mucilage and pectin consisted of 60–80% anhydrouronic acid (Khafaga et al., 1980).

The petals of *H. sabdariffa* yielded 65% of dry weight of mucilage, which on hydrolysis produced galactose, galacturonic acid and rhamnose, while the leaves only yield 10% (El-Hamidi et al., 1967, Sengupta and Banik, 2011).

6-8. Volatile compounds

Volatile compounds are responsible for the aroma of *H. sabdariffa*. In a study conducted in 1992, more then twenty-five volatile compounds (accounting for less than 8% of total *H. sabdariffa* seeds composition) were reported in seed oil of *H. sabdariffa*. They were mainly unsaturated hydrocarbons, alcohols and aldehydes from C8 to C13. (Jirovetz et al., 1992) Subsequently, thirty-seven volatile compounds from five different groups from the *H. sabdariffa* WE were characterised. These compounds included fatty acid derivatives (such as 2-ethylfuran and hexanal), sugar derivatives (furfural and 5-methyl-2-furaldehyde), phenolic derivatives (eugenol), terpenes (such as 1,4-cineole, limonene) and miscellaneous compounds (e.g. acetic acid) (Chen et al., 1998). In another study, the volatile profile was examined in four aqueous extracts from fresh and dried calyx using two different, time–temperature extraction conditions by GC–MS. A total of thirty-two compounds were identified and could be divided into five chemical groups: aldehydes (fourteen compounds), alcohols (ten compounds), ketones (five compounds), terpenes (two compounds) and acids (one compound) (Ramirez-Rodrigues et al., 2011a, Ramirez-Rodrigues et al., 2011b). A total of seven aromatic volatiles were common to all four samples tested (hexanal, 3-octanone, octanal, 1-octen-3-one, nonanal, 2,4-nonadienal (E,E), and geranylacetone), (Da-Costa-Rocha et al., 2014).

7- Medical and nutritional uses:

7-1. Nutritional uses:

The source of a red drink known as Jamaica in Mexico (said to contain citric acid and salts, and is considered a diuretic). The calyx, called karkadeh in Switzerland, is a name not very different from the Arabic. *Hibiscus* is used in jams, jellies, and sauces. In the West Indies and other tropical regions, it is used to make jellies, syrups, gelatins, refreshing drinks, sweets, and cakes. Dried hibiscus is used in tea, jellies, marmalade, ice cream, sherbet, butter, pies, sauces, tarts, and other desserts. In the West Indies, calyxes are used to color and flavor rum. The tender leaves and stems are eaten as salad herbs and used to season curries.

The seeds have been used as an aphrodisiac substitute for coffee. The fruits of hibiscus are edible (Watt & Brier-Brandwick, 1962). Perry cites a study demonstrating the benefits of hibiscus in treating atherosclerosis and as an intestinal cleanser (Perry, 1980). Hibiscus is cultivated primarily for the bark fibers from its stems. The fiber strands, up to one and a half meters long, are used for rope making and as a jute substitute in burlap.

7-2. Folk Medicine:

H. Sabdariffa is said to be antiseptic, aphrodisiac, astringent, choleretic, demulcent, digestive, diuretic, laxative, cooling, dissolving, sedative, and stomach tonic. It is a folk remedy for abscesses, bilious conditions, cancer, cough, weakness, indigestion, dysuria, fever, headache, heart disease, high blood pressure, neurosis, scurvy, and urinary obstruction. A tea prepared by steeping a cup of it in water is said to be a folk remedy for cancer. Medicinally, its leaves are refreshing and widely used in Guinea as a diuretic, coolant, and sedative. Its fruits are used as an anti-scurvy. Its leaves, seeds, and ripe calyx are diuretic and anti-scurvy.

The succulent calyx, boiled in water, is used as a drink in cases of bilious attacks. The flowers contain gossyptin, anthocyanins, and the glucoside hispicin, which may have diuretic and choleretic effects, reducing blood viscosity, lowering blood pressure, and stimulating bowel movements. In Burma, the seeds are used to treat weakness, while the leaves are used as a laxative. The Taiwanese consider the seeds diuretic, laxative, and tonic. The Filipinos use the bitter root as an aphrodisiac and tonic (Perry, 1980).

The Angolans use the mucous leaves as an emollient and cough suppressant. People in Central Africa use the leaves as a poultice on abscesses. Alcoholics might consider one thing: taking the plant extract artificially reduces the rate of alcohol absorption, thus reducing the severity of alcohol's effects on chickens (Watt and Prior-Brandwick), (Duke, J. A, 1983).

7.3- Therapeutic use:

The species *H. sabdariffa* is said to have numerous therapeutic properties, which are attributed to the high concentrations of organic acids, including malic acid, ascorbic acid, and citric acid (Kohen and Downing, 1992). Other biological activities are linked to anthocyanin compounds, which have significant antioxidant activities (Sarni Manchad and Cheymier, 2006). Studies have shown that daily consumption of *H. sabdariffa* extract significantly reduces blood pressure in hypertensive subjects, and also shows great effectiveness against colds, cardiac pain (Ojeda et al., 2010), upper respiratory tract pain, nervous diseases (Panizza, 1997), loss of appetite, swelling (inflammation), stomach irritation, water retention, circulatory disorders, and to dissolve mucus. Other studies in diabetics show that a glass of *Hibiscus sabdariffa* L. infusion once a day helps combat insulin resistance. Indeed, this infusion can help maintain healthy blood sugar levels, and *H. sabdariffa* extract after a meal can reduce the absorption of starch and sucrose (Endrias, 2006).Also, the infusion of this plant plays a role of natural antidepressant by acting against the signs of fatigue, tone, lack of motivation thanks to certain bioflavonoids which are found in the flower of *H. sabdariffa* (Endrias, 2006).The seeds are used as a dietary supplement against anemia and the leaf powder is used as a nutrient-enriched flour (El-Sherif and Sarwat, 2007).

7.4-Industrial use:

H. sabdariffa has very varied applications in the industrial field, particularly in the areas of flavors, food colorants, and the generation of new beverages. It is a candidate, due to its seeds, to be a new source of vegetable oil (Endrias, 2006).

7.5-Cosmetic use:

Thanks to its antioxidant and coloring properties, hibiscus powder (*H. sabdariffa*) can be included in the composition of cosmetic products such as makeup, creams, shampoos, anti-aging treatments, softeners, and moisturizers, etc., as well as the oil used to produce scrubs and soaps (Ismail et al., 2008).

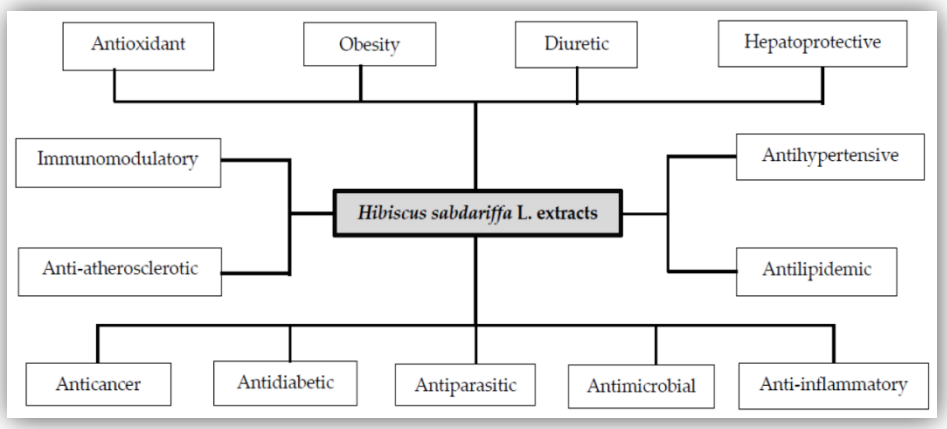


Figure (13): Schematic diagram of the therapeutic and pharmacological functions of hibiscus extracts (Morales et al ;2020).

8- Ideal conditions for its growth:

8-1. Ecology:

Suitable for tropical climates with well-distributed rainfall of 1500–2000 mm yearly, from sea-level to about 600 m altitude. Tolerates a warmer and more humid climate than kenaf, but is more susceptible to damage from frost and fog. Plant exhibits marked photoperiodism, not flowering at shortening days of 13.5 hours, but flowering at 11 hours. In United States plants do not flower until short days of late fall or early winter. Since flowering is not necessary for fiber production, long light days for 3–4 months is the critical factor. Roselle requires a permeable soil, a friable sandy loam with humus being preferable; however, it will adapt to a variety of soils. It is not shade tolerant and must be kept weed-free. It will tolerate floods, heavy winds or stagnant water. Ranging from Warm Temperate Moist through Tropical Wet to Very Dry Forest Life Zones, roselle is reported to tolerate annual precipitation of 6.4 to 42.9 dm (mean of 213 cases = 17.14) annual temperature of 12.5 to 27.5°C (mean of 213 cases = 23.11) and pH of 4.5 to 8.0 (mean of 119 cases = 6.1). (Duke, 1978, 1979).

8-2. Cultivation:

Soil preparation should be deep, about 20 cm, and thorough. Seed, 11–22 kg/ha depending upon the soil, is drilled about 15 cm by 15 cm at beginning of rainy season, mid-

April in India, planting to a depth of about 0.5 cm. Broadcasting is not recommended because of uneven stand, land, and hence lack of uniformity in fiber. When grown for its fiber, it is planted closely to produce long stems with little foilage. Weeding for first month is important. Fertilization practices vary widely. Roselle responds favorably to applications of nitrogen, and 45 kg/ha is a safe level in India, applied in the form of compost or mineral fertilizer in conjunction with a small quantity of phosphate. In Java green manure (*Mimosa invisa*) is p;owed under before it starts to 5 mature seeds. Also, in Java the following fertilizer rates are recommended for roselle: 80 kg N/ha, 36–54 kg P_2O_5/ha and 75–100 kg K_2O/ha. Rotations are sometimes used, the roselle, requiring several months to grow, making the land unavailable for other crops. The practice is recommended since the root-knot nematode, *Heterodera radicicola*, is a pest.

A sequence of a legume green-manure crop, then roselle and then corn is suggested. For home gardens of roselle, seeds are sown directly in rows about May 15. After germination, seedlings are thinned to stand 1 m apart. For larger plantings, seeds are sown in protected seedbeds and the seedlings transplanted to 1.3–2.6 m apart in rows 2–3.3 m apart. Applications of stable manure or commercial fertilizers are beneficial. Plants are subject to injury by root-knot nematodes and should not be planted on land infested with these pests.

8-3. Growth and development:

The vegetative growth period lasts between 4 and 6 months, the size of the plant 30 days after emergence is about 30 cm. The leaf harvest can begin 6 weeks after the sowing, it stimulates branching and consequently increases leaf production. Flowering begins when the length of the day decreases, at the earliest 2 months after sowing, and at the later than 7 months. The flower is generally self-pollinated. The fruits begin to ripen two or three months after fertilization (Grubben, 2004).

8-4. Harvesting:

For the calyces of fruits, about 3 weeks after tile onset of flowering, the first fruits are ready for picking. The fruit consists of the large reddish calyces surrounding the small seed pods. Capsules are easily separated, but need not be removed before cooking. For fiber, from planting to harvest is about 3–4 months, 10 months in Indonesia. Fiber quality is best if harvested just at flowering time. Stems are cut off at ground level, tied in bundles and retted until the fiber is freed from the wood. Then it is washed and dried in the sun. A skilled worker

can strip 36–45 kg dry clean fiber daily in this practice. Retting is by-passed if a decorticating machine is used (Duke, J. A., 1983).

9- Previous phytochemical studies

9-1. Antioxidant activity:

Studies on the *H. sabdariffa* plant have shown its strong antioxidant activity due to its high content of phenolic compounds, flavonoids, and anthocyanins. This was demonstrated by Tseng et al. (1997), who stated that extracts of the *H. sabdariffa* plant have high activity in inhibiting free radicals. There is also a study by Da-Costa-Rocha et al. (2014) which provided a general review of the antioxidant activity and its properties, attributed to the polyphenolic compounds present in it.

9-2. Anti-inflammatory activity:

Ali et al. (2005) demonstrated that the plant has anti-inflammatory activity, which is attributed to the inhibition of cyclooxygenase and lipoxygenase enzymes. Ajay et al. (2007) demonstrated that the plant extract of *H. sabdariffa* inhibits the production of nitric oxide in macrophages, which is one of the most important compounds involved in the inflammatory process.

9-3. Plant Chemical Composition:

Numerous studies have shown that *H. sabdariffa* contains high levels of anthocyanins and flavonoids, as well as organic acids like hibiscus acid. This was demonstrated by McKay and Blumberg (2007), who found that the plant is very rich in anthocyanins and polyphenols, especially anthocyanin compounds such as delphinidin and cyanidin, flavonoids, and organic acids like hydroxycitric acid and hibiscus acid. This was also clarified by Riaz & Chopra (2018).

10- The therapeutic effects of the *Hibiscus sabdariffa* L. plant:

10-1. The effect on cardiovascular health:

A series of systematic reviews and meta-analyses have shown that *H, sabdariffa* possesses effective properties in improving cardiovascular health, particularly in lowering blood pressure and cholesterol. For example, a review by Wang et al. (2025) showed that this

plant reduces blood pressure, improves total cholesterol, and lowers blood sugar. 4.2. Regulation of blood lipid levels: Zhang, Wang, & Lu (2019) showed that this plant works to balance fat levels in the body and also reduce LDL cholesterol, attributing this effect to its richness in polyphenolic compounds. 4.3. Its effect on obesity: A study by Nguyen & Tran (2024) indicated that hibiscus extract does not show any significant effect on weight loss or body mass index.

CHAPTER II

Material and Methodes

This chapter focuses on the experimental study of *H. sabdariffa* extracts using various solvents (hexane, ethanol, butanol, and water), with the aim of evaluating their biological activities, including antioxidant activity, anti-inflammatory activity, total reducing power, and sun protection factor (SPF).

A rigorous scientific approach was adopted for the extraction of active compounds from the plant material, followed by a series of laboratory analyses to determine the chemical and biological properties of the obtained extracts.

1. Material and Methodes:

1.1. Materials:

1.1.1. Plant Material:

Our work discusses the plant *H. Sabdariffa*, commonly known as "Karkadeh." It was purchased in dried form from an herbal shop, and we confirmed that it was cultivated in the region of Tamanrasset, specifically in Amachon (22°47′20″N 5°31′32″E), on October 25, 2024. We kept it under suitable conditions.

Fig (14): Dry flowers of *Hibiscus sabdariffa* L.

1-1-2. Chemicals:

Soduiom carbounate (Na2 Co3), Trichloroactic acid (TCA), Folin-ciacalte, Phosphate solution system, Ammonium molybdate, Hydrogen peroxcide (H2O2), Potassium ferricyanide

(K3Fe (CN)6), Ferric chloride (Fecl3), Soduim phosphate, Hexane, Butanol, Hydrochloric acid (Hcl), Sulphuric acid, Ethannol, Water

2-1. Methodes:

1-2-1. Preparation of plant sample :

The plant sample used in this study consisted of dried flowers of the *H. sabdariffa* plant. It was ground using an electric grinder to obtain a fine powder. Then, this powder was stored in clean, dry, airtight glass containers and placed in a cool, dark place.

Fig (15): Flowers of *H. sabdariffa*, completely dried and ground

1-2-2. Phytochemical screening

In this study, a qualitative chemical detection of secondary compounds in *H. sabdariffa* extracts was conducted using traditional reactions, including alkaloids, flavonoids, saponins, tannins, and steroids. Where 5 grams were previously soaked in 100 ml of distilled water for 24 hours, then filtered and used in this detection were.

Alkaloids:

Both Mayer's reagent and Wagner's reagent were used 2 mg of the soaked extract were placed with 2 ml of water, then we separated each 1 ml into a separate tube and added Mayer and Wiener's reagent to each of them , resulting in the appearance of yellow and reddish-brown precipitates, respectively, indicating the presence of alkaloids (Trease & Evans, 2002; Okereke et al., 2014).

Flavonoids:

They were detected using the Shinoda test, where 0.5 grams of the plant part were placed in 10 ml of ethanol and boiled. Afterward, they were filtered, and then a quantity of magnesium and drops of concentrated HCl were added to the mixture. After some time, a distinctive pink color appeared (Harborne, 1998; Fitrotunnisa et al., 2019).

Saponins:

They were detected through the foam test, where we placed 5 ml in a test tube and shook it well for 15 minutes, and the stable foam lasted for more than two minutes (Hamrita et al., 2022; Harborne, 1998).

Tannins:

It was detected by adding 0.5 ml of a 1% iron chloride solution to 1 ml of the sample, resulting in a dark blue color indicating its presence (Trease & Evans, 2002; Okereke et al., 2014).

Steroids:

They were detected using the Liebermann-Burchard test, where 1 ml of the extract was mixed with 1 ml of chloroform, then 1 ml of concentrated sulfuric acid was added to the wall of the tube, resulting in the formation of a brown precipitate, which is clear evidence of the presence of steroids (Harborne, 1998; Sofowora, 1993).

1-2-3. Extraction by Soxhlet:

The extraction of *H. sabdariffa* compounds was carried out using a Soxhlet apparatus, which is considered one of the most effective techniques for extracting active plant compounds from dry samples. The sample flowers were dried and partially ground, then 30 grams of the sample were weighed and placed in an extraction capsule made of filter paper. The extraction process was sequentially performed using four solvents arranged according to their polarity: hexane, ethanol, butanol, and water.

The extraction process was carried out sequentially using four solvents arranged according to their polarity: hexane, ethanol, butanol, and water. Each extraction was performed separately, using 300 ml of each solvent, and heated to the boiling point of each solvent under a closed and cooled system.

The extraction process took 3 hours for each solvent to ensure the extraction of most of the extracted compounds. After each cycle, the raw extract was filtered and then concentrated under low pressure using a rotary evaporator at appropriate temperatures for each solvent. It was then dried using an oven at a temperature not exceeding 45 degrees Celsius to maintain the stability of the biologically active compounds. After drying, they were collected, and their states were as follows: cohesive for ethanol and butanol, powder for the aqueous extract, and oily for the hexane extract. The extracts were stored in airtight and opaque bottles at low temperatures.

Yield (%) = (Weight of dry extract / Weight of dry plant material) × 100

2- Quantitative phytochemical analysis:

2.1. Determination of total phenolic contents:

A series of gallic acid concentrations with standard concentrations (1000-500-250 .... µg/ml) were prepared to draw the calibration curve. 1 mL of the Folin-Ciocalteu solution, diluted 10 times, was added to the three replicates of the four extracts and also to the series of gallic acid concentrations. Then incubate the tubes at room temperature for 5 minutes, then add 0.8 ml of sodium carbonate (7.5%). The mixture is incubated in the dark at room temperature for 40 minutes, then the absorbance is measured at 760 nanometers using a UV-Vis spectrophotometer (Ali et al., 2005).

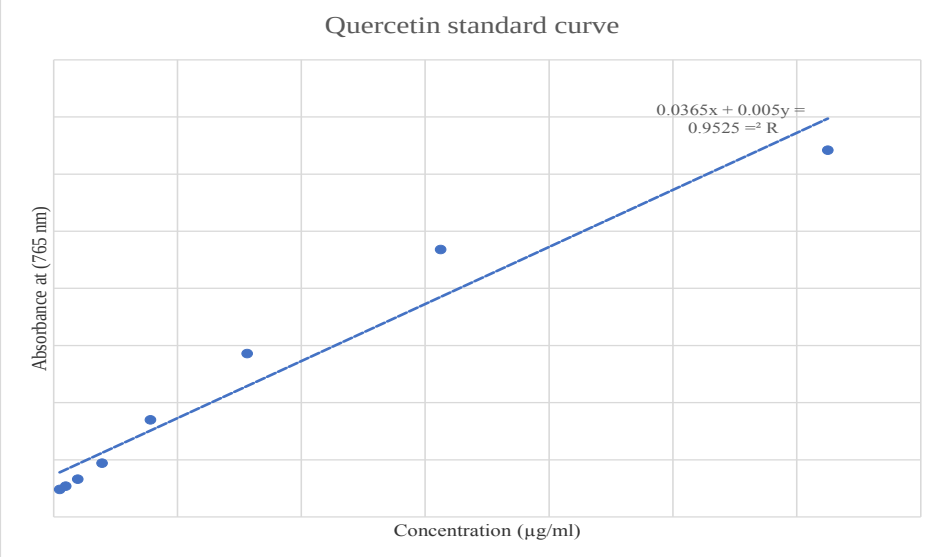


Figure (16): Standard curve of gallic acid for determination of total phenolic contents.

2-2. Determination of flavonoids contents:

The quantitative estimation of total flavonoids in extracts of the *H. sabdariffa* plant was conducted using a chemical reaction with aluminum chloride nitrate, as mentioned in (Chouikh et al. 2018). The study included four different extracts: (ethanolic, butanolic, hexanic, and aqueous). The standard solution was quercetin at a concentration of 1 mg/ml, with 2 mg dissolved in 2 ml of distilled water. Then, 1 ml of 2% aluminum nitrate ($AlCl_3$) was added to each of the three replicates of the extracts and the series of standard solution concentrations. The mixture was then placed in the dark for 30 minutes at room temperature. Then, the absorbance is read at a wavelength of 420 nanometers using a spectrophotometer. (Chouikh et al., 2018).

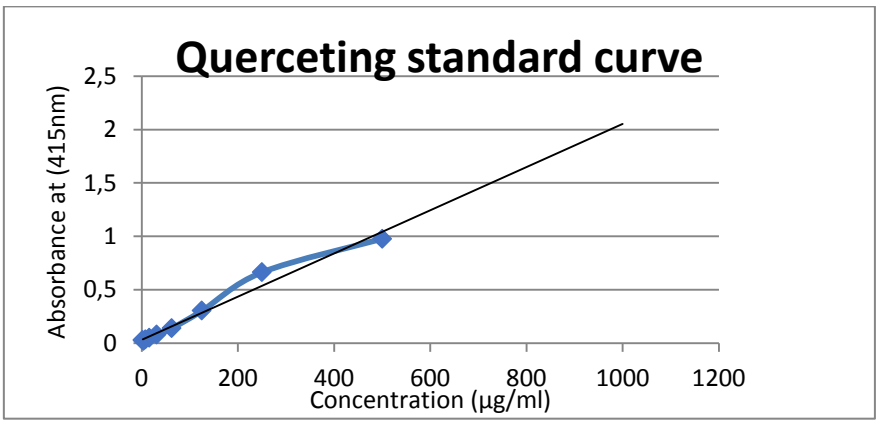


Figure (17): Standard curve of quercetin for estimating flavonoid content.

2-3. Determination of total tannins content:

We mixed 0.4 mL of the four extracts (1 mg/mL) (ethanolic, butanolic, aqueous, hexane) in a series of 3 replicates, and a series of different concentrations of catechin compound (where 1 mg was dissolved in 2 mL of distilled water, resulting in a concentration series of 1000, 500, 250... mg/mL). We added 1.5 mL of HCl and 3 mL of 4% vanillin, incubated for 15 minutes in the dark, and then measured the absorbance at a wavelength of 500 nanometers using a spectrophotometer (Olatunji et al., 2019).

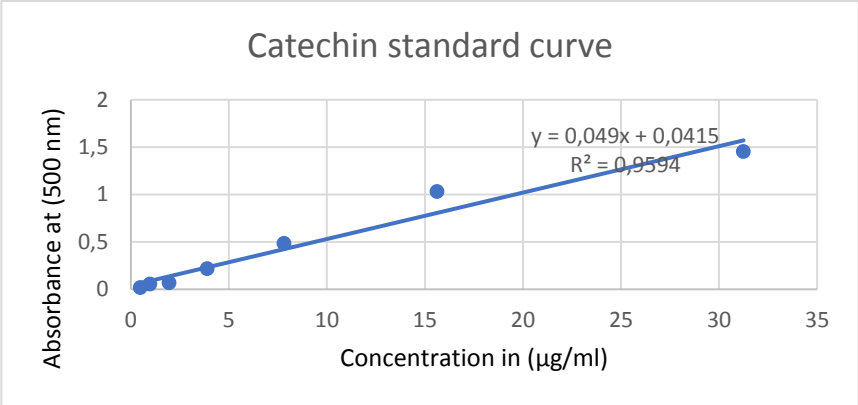


Figure (18): Standard curve of catechin for determination of condensed tannins

3. Biological activity tests:

3-1. Antioxidant activity:

3-1-1 Dpph assay:

To analyze the antioxidant activity of *H. sabdariffa* extracts, the DPPH radical scavenging assay was adopted. This is a simple colorimetric test that determines the ability of antioxidants in the extracts to reduce the stable DPPH radical, which is indicated by a color change from purple to light yellow (Brand-Williams et al., 1995).

Ascorbic acid was used as a reference solution, prepared by dissolving 2 mg in 2 ml of distilled water, while the four plant extracts were prepared at a concentration of 1 mg/ml. A series of ten serial dilutions were prepared for each sample.

To each concentration of the extract or standard acid, 0.5 mL of DPPH solution was added (2 mg of DPPH root was dissolved in 50 mL of methanol), and then the tubes were incubated in the dark for 30 minutes at room temperature (Molyneux, 2004). After incubation, the absorbance was measured at a wavelength of 517 nanometers using a UV-Vis spectrophotometer.

$$\text{Percentage of DPPH}^{\bullet}\text{ radical inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Antioxidant efficacy was assessed by the free causative inhibition ratio, and IC_{50} values (Concentration of extract necessary to inhibited of DPPH$^{\bullet}$ radical 50%) were calculated. They were determined by exploiting radical inhibition percentage curves in of sample concentration change

3-1-2. Ruducing power assay:

Potassium ferricyanide reacts with Fe^{+3} ions to form a complex with a very high extinction coefficient at 700 nm. Through this assay, the reduction of potassium ferricyanide is monitored by the antioxidants present in the studied extracts (Pisoschi *et al*., 2016). The reducing power of the extract was performed using the potassium ferricyanide method (Muthukrishnan *et al*., 2018). A volume of 0.5 mL of the different concentrations (mg/mL) of the extract was added to 1.25 mL of a phosphate buffer (0.2 M, pH 6.6) mixed with 1.25 mL of potassium ferricyanide (1%). The mixture was incubated in a hot bath at 50°C for 20 minutes, there action is stopped by adding 1.25 mL of trichloroacetic acid (10%), the tubes were then centrifuged at 3000 rpm/10 minutes. 1.25 mL of the supernatant, was mixed with 0.25 mL of ferric chloride ($FeCl_3$) (0.1%) and 1.25 mL of distilled water, and the optical density was read at 700 nm.

The results were compared with the value of EC_{50}, is the value of the effective concentration, which gives an absorption of 0.5 at a wavelength of 700 nm. They were determined by exploiting the absorption curves at a wavelength of 700 nm in of sample concentration change

3-1-3. Anti- hemolysis assay:

The objective of this test is to identify the extent to which plant extracts protect red blood cells from oxidants and free radicals that cause their degeneration, by determining the percentage of red blood cells (RBCs) lysis in the presence of the studied extracts. The efficacy of the extracts has been evaluated, following the protocol described by Chouikh *et al*. (2020). A volume of 40 µL of human erythrocytes (10%) was mixed with 2 mL of different concentrations (500, 250, and 125 µg/mL) of the extract and incubated for five minutes at 37°C. Then, we added 40 µL of hydrogen peroxide (H_2O_2) (30×10^{-3} M), 40 µL of ferric chloride ($FeCl_3$) (80×10^{-3} M) and 40 µL of ascorbic acid solution (50×10^{-3} M) respectively. After one hour of incubation at 37°C, the mixture was centrifuged with 700 rpm for 10 minutes. The absorbance of the supernatant was read at λ= 540 nm. The percentage of hemolysis was determined using the following formula*:*

$$\text{Prcentage Hemolysis \%} = (\text{Abs control} / \text{Abs sample}) \times 100$$

Hly_{50} is a value that represents the concentration at which 50% RBCs were lysed.

3-1-4. Phosphomolybdnum reducing power assay:

The total reducing power of the four extracts of the *H. sabdariffa* plant was analyzed using the spectrophotometric method, which relies on the antioxidants' ability to reduce a compound to a lower form, measurable at a wavelength of 695 nanometers. This method is considered the best standard for determining the reducing power of antioxidants, as the higher the absorbance, the greater the reducing power of the extract (Oyaizu, 1986). The extracts were prepared at a concentration of 1 mg/mL, with 3 replicates for each extract at the same concentration to ensure consistency. Gallic acid was adopted as a standard solution with a concentration of 0.5 mg/ml.

1 mL of the phosphomolybdenum reagent was added to each tube, which was prepared from a mixture of reagents (ammonium molybdate, sodium phosphate, sulfuric acid). The tubes were placed in a water bath at a temperature of 95 degrees Celsius for a full hour to ensure the reduction reaction (Prieto et al., 1999). The absorbance was measured at a wavelength of 695 nanometers using a UV-Vis spectrophotometer.

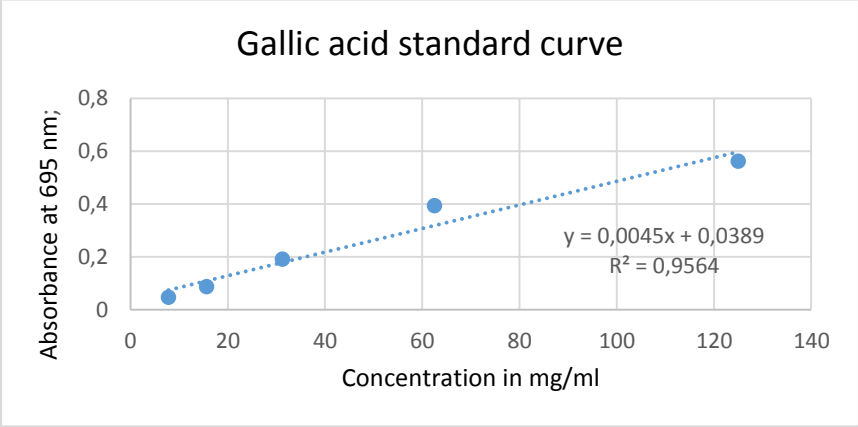


Figure (19): Standard curve of gallic acid for determination of the total antioxidant capacity of the plant extracts.

3-2. Determination of sun protection factor (SPF):

The sun protection factor for the studied plant extracts from the *H. sabdariffa* plant was estimated using the spectroscopic method, which relies on the principle of measuring the absorbance of ultraviolet rays in the wavelength range of 290 to 320 nanometers, as suggested by Sayre et al. (1979). This method has since been adopted in several botanical studies to determine the efficacy of natural plant extracts in protecting against sunlight.

The four extracts (hexane, ethanolic, butanolic, and aqueous) were prepared at a concentration of 1 mg/ml, where each extract was dissolved in ethanol because it is the

appropriate solvent that does not absorb in the spectral range to be studied. Then, the sample was read at wavelengths from 290 to 320 nanometers. After obtaining the values, SPF is calculated according to the following equation:

$$SPF = CF \times \Sigma (EE(\lambda) \times I(\lambda) \times Abs(\lambda))$$

Where:

CF is the correction factor (usually = 10). EE(λ) is the relative effect for each wavelength. I(λ) is the intensity of solar radiation at each wavelength. Abs(λ) is the absorbance measured at each wavelength (Sayre et al., 1979; Mansur et al., 1986).

3-3. Anti – inflammatory activity:

The anti-inflammatory activity was analyzed on extracts of the *H. sabdariffa* plant (hexane, ethanolic, butanolic, and aqueous) using the bovine serum albumin denaturation inhibition test, which is an effective and approved method for protein stabilization and prevention of denaturation, thereby allowing the determination of anti-inflammatory activity (Sakat et al., 2010).

The reaction mixture was prepared by adding 0.2 mL of 1% Bovine Serum Albumin (BSA) solution to 2.8 mL of the buffered PBS solution (pH 6.4). Then, 2 mL of normal saline (negative control) was added, along with a series of different concentrations of plant extracts at 1 mg/mL, and also sodium diclofenac as a reference solution with an additional 2 mL. The tubes were incubated at 37 degrees Celsius for 20 minutes, then heated at 51 degrees Celsius for another 20 minutes. Then, the tubes were placed at room temperature until they cooled down, and the absorbance was measured at a wavelength of 660 nanometers using a UV-Vis spectrophotometer.

The principle of this test is based on the fact that the reaction medium, when exposed to heat, causes a change in the structure of the BSA protein, leading to its degradation. On the other hand, compounds with anti-inflammatory activity stabilize the protein structure and reduce its degradation, resulting in a decrease in absorbance. (Oyedapo et al., 2010; Sakat et al., 2010).

$$\text{Percentage of protection from denaturation} = (1 - \frac{\text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}}) \times 100$$

2. Statistical Analysis

Results are expressed as mean ± standard deviation. Data were analyzed using one-way analysis of variance (ANOVA) using XLSTAT (version 2021.2.2) and Excel (version 2021).

CHAPTER III

Results

1. Phytochemical screening

Table 2: Phytochemical screening results

Phytochemical compounds		**Observation**
Alkaloids	Wagner	+
	Mayer	++
Steroids		+
Flavonoids		++
Saponins		±
Tanins		++
(+) Presence (++) Strong presence (±) Weak presence		

The phytochemical analysis revealed a strong presence of flavonoids and tannins. Alkaloids and steroids. Saponins were detected in low amounts.

3. Extraction yield

Table 3: Extraction process yield results

Extract Type	**Weight (g)**	**Yield (%)**
Fraction Butanol	5.21	17.37
Fraction Hexane	0.1	0.33
Fraction Ethannol	3.38	11.27

Fraction Aqueous	3.25	10.83

The highest extraction yield was obtained with butanol (17.37%), followed by ethanol (11.27%) and water (10.83%). The hexane extract showed a very low yield (0.33%), indicating poor solubility of the extractable compounds in non-polar solvents.

3- Quantitative phytochemical analysis

3-1. Determination of total phenolic contents

The total phenolic content varied markedly across the different polarity extracts of *H. sabdariffa* flowers, with statistically significant differences ($p<0.0001$) observed among them. The ethanol extract exhibited the highest phenolic concentration (186.3±30.20 mg GAE/g), closely followed by the butanol extract (167.3±15.26 mg GAE/g), indicating that moderately polar solvents are more efficient in extracting phenolic compounds. In contrast, the aqueous (44.57±4.13 mg GAE/g) and hexane (42.23±1.75 mg GAE/g) fractions demonstrated significantly lower phenolic contents, suggesting limited solubility of phenolics in highly polar or non-polar solvents.

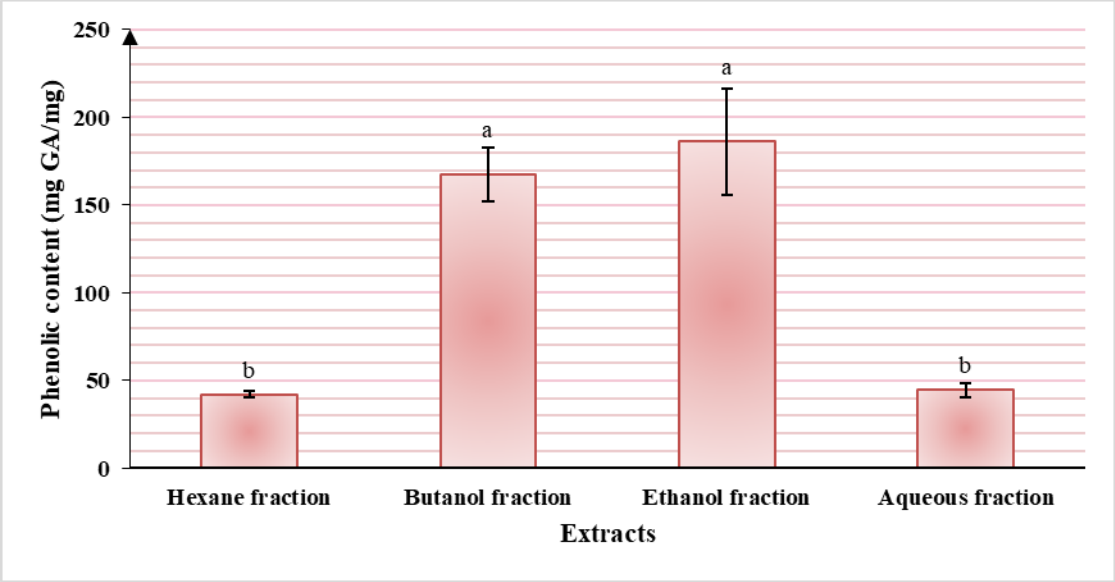


Figure (20): Total Phenolic Content of *H. sabdariffa* Extracts. (a-b, Duncan's test evidences).

3-2. Determination of flavonoids contents

The flavonoid content among the different polarity extracts of *H. sabdariffa* flowers revealed statistically significant differences ($p<0.001$), underscoring the strong influence of

solvent polarity on compound extraction. The butanol extract exhibited the highest flavonoid concentration (63.3±0.87 µg QE/mg), followed closely by the ethanol extract (60.63±2.12 µg QE/mg). In stark contrast, both the hexane (5.18±0.25 µg QE/mg) and aqueous (5.4±0.13 µg QE/mg) extracts demonstrated markedly lower flavonoid levels.

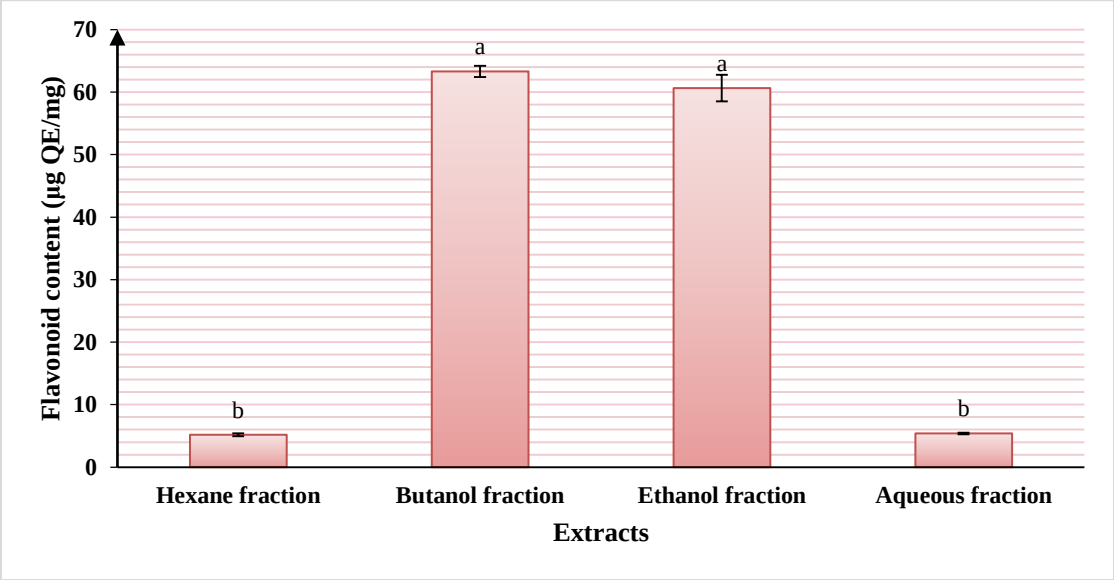


Figure (21): Flavonoid Content of *H. sabdariffa* Extracts. (a-b, Duncan's test evidences).

3-3. Determination of condensed tannin contents

The analysis of tannin content across *H. sabdariffa* extracts of varying polarity reveals statistically significant differences ($p<0.001$), highlighting the selective efficiency of solvents in extracting these compounds. The hexane fraction recorded the highest tannin concentration (19.74±0.19 µg CE/mg), which was significantly greater than those of the other extracts. In contrast, the butanol (13.94±1.17 µg CE/mg), ethanol (13.99±1.81 µg CE/mg), and aqueous (13.65±1.67 µg CE/mg) fractions displayed similar and lower tannin levels.

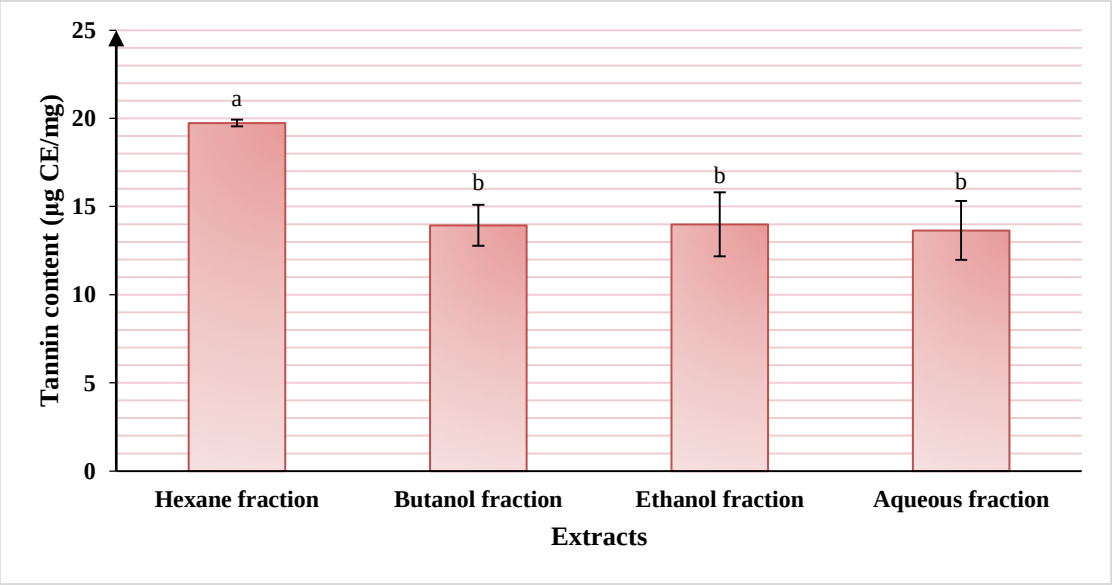


Figure (22): Tannin Content of *H. sabdariffa* Extracts. (a-b, Duncan's test evidences).

4- Biology activity

4-1. Antioxidant activity

4-1-1. Free radical-scavenging activity (DPPH• assay)

The IC_{50} values obtained for the DPPH• assay reveal notable differences (p<0.001) in antioxidant potential among the extracts of *H. sabdariffa*. Ascorbic acid exhibited the lowest value 6.72±0.93 µg/mL, confirming its role as a potent standard antioxidant. Among the extracts, the ethanol fraction showed the strongest radical scavenging activity (58.02±0.35 µg/mL), indicating the presence of highly active antioxidant constituents. In contrast, the hexane and aqueous fractions displayed considerably weaker activity, with IC_{50} values of 166.66±1.12 µg/mL and 884.02±6 µg/mL, respectively.

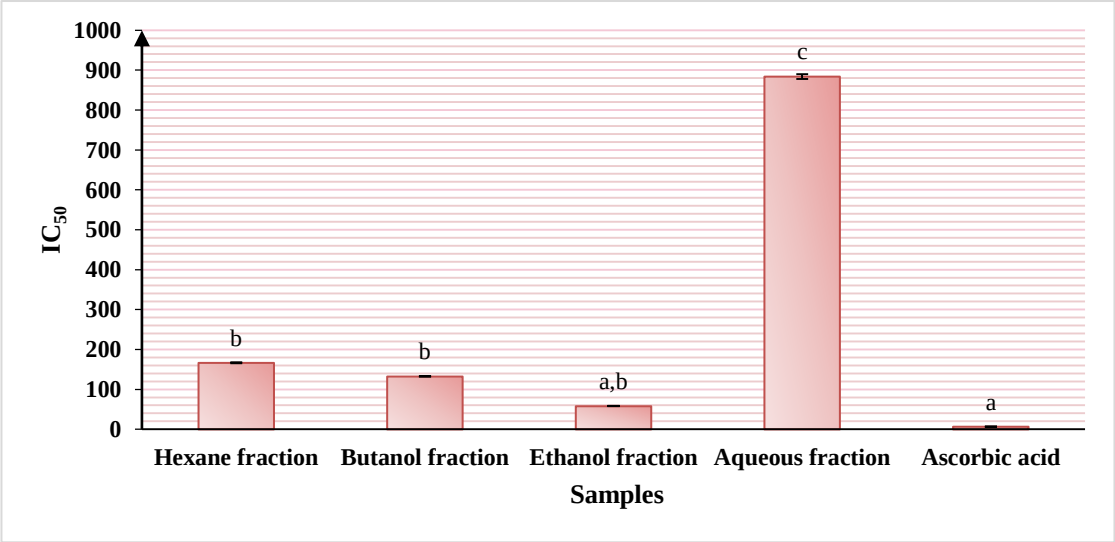
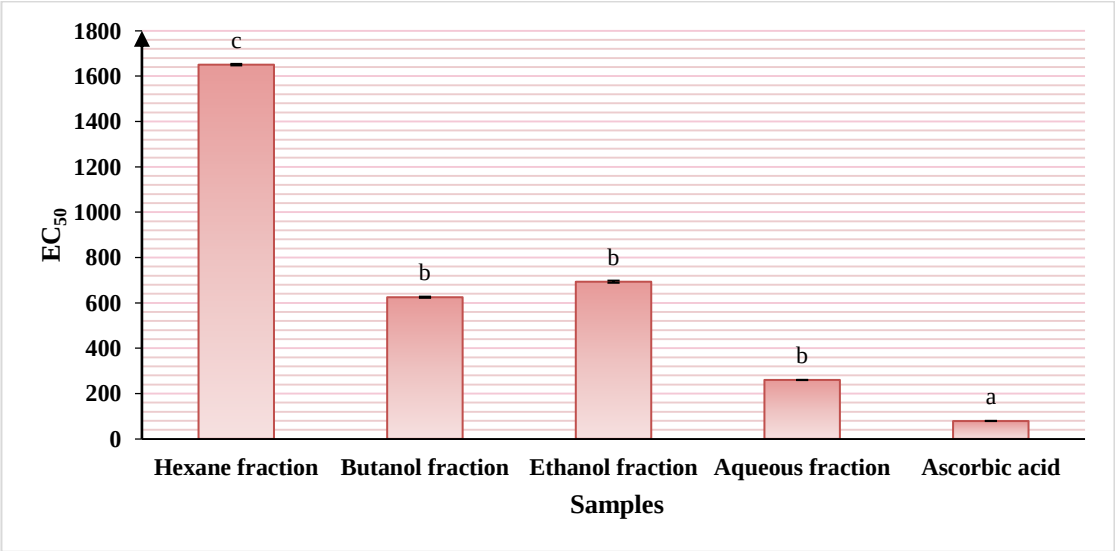


Figure (23): DPPH$^{\bullet}$ Radical Scavenging Activity (IC_{50}) of *H. sabdariffa* Flower Extracts and Ascorbic Acid. (a-b, Duncan's test evidences).

4-2. Reducing power

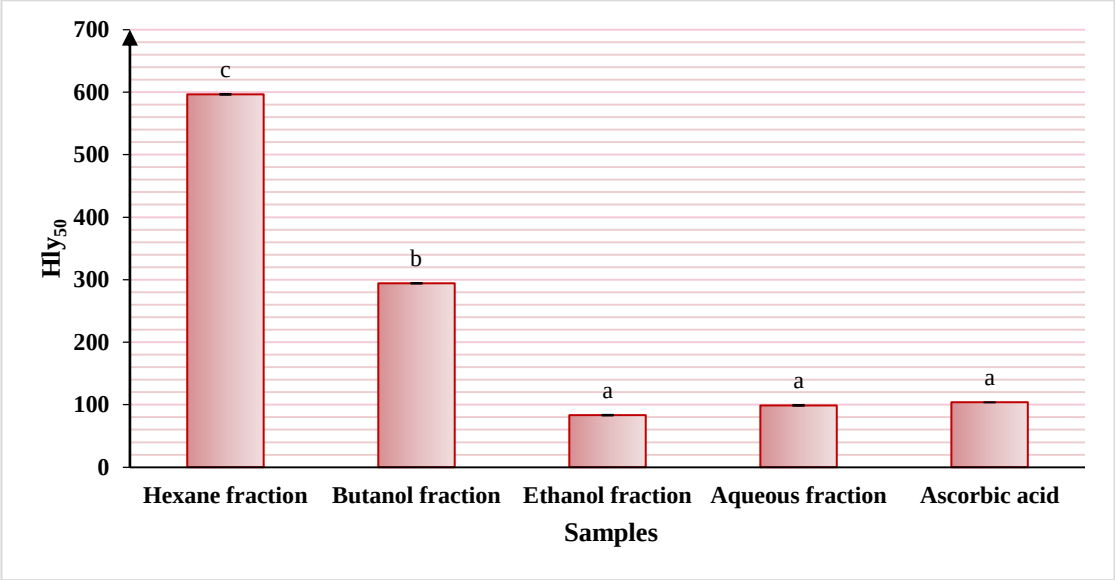
The bar chart illustrates the EC_{50} values of *H. sabdariffa* flower extracts of varying polarities and ascorbic acid, used as a reference antioxidant. In this context, lower EC_{50} values reflect stronger antioxidant activity. Ascorbic acid exhibited the strongest antioxidant activity (lowest EC_{50}), followed closely by the aqueous fraction. The butanol and ethanol fractions showed moderate antioxidant potential with significantly higher EC_{50} values, whereas the hexane fraction had the highest EC_{50} value and thus the weakest antioxidant effect.



Figure(24): Reducing Power (EC_{50}) of *H. sabdariffa* Flower Extracts and Ascorbic Acid. (a-b, Duncan's test evidences).

4-3. Anti-hemolysis activity

The bar chart presents the Hly_{50} values (µg/mL) of different polarity extracts of *H. sabdariffa* flowers and ascorbic acid, reflecting their anti-hemolytic (membrane-protective) capacity. In this context, lower Hly_{50} values indicate stronger anti-hemolytic activity, as less extract is required to protect 50% of red blood cells from lysis. The ethanol and aqueous fractions, along with ascorbic acid, showed the lowest Hly_{50} values (under \~120 µg/mL) and belonged to the same statistical group, indicating the highest protective activity. The butanol extract had a moderately higher Hly_{50} (\~350 µg/mL), suggesting reduced protective potential, while the hexane fraction exhibited the highest Hly_{50} value (\~600 µg/mL) and was significantly different, reflecting the weakest anti-hemolytic effect. These results demonstrate that polar extracts (ethanol and aqueous) are more effective at stabilizing red blood cell membranes, likely due to their content of hydrophilic bioactive compounds.



Figure(25): Comparative Anti-hemolytic Activity (Hly_{50}) of Different Extracts of *H. sabdariffa* Flowers. (a-c, Duncan's test evidences).

Phosphomolybdenum reducing power

Table(4): Evaluation of Total antioxidant capacity of Different Polarity Extracts of *H. sabdariffa* Flowers. (a-c, Duncan's test evidences).

Extracts	Total antioxidant capacity (µg EGA/mg Ex)
Hexane fraction	1.58 ± 0.44^{c}
Butanol fraction	1.911 ± 0.33^{c}
Ethanol fraction	47.21 ± 9.61^{a}
Aqueous fraction	17.80 ± 6.05^{b}

The antioxidant capacity of *H. sabdariffa* flower extracts varied markedly according to solvent polarity, with ethanol and aqueous fractions exhibiting notably higher activity compared to their less polar counterparts. The ethanol fraction demonstrated the strongest antioxidant potential, likely due to its high efficiency in extracting phenolic and flavonoid compounds known for their radical-scavenging properties. The aqueous extract followed with a moderate capacity, suggesting that some antioxidant constituents are also water-soluble. In contrast, the butanol and hexane fractions displayed minimal antioxidant activity, indicating a lower presence or poor solubility of antioxidant compounds in these solvents.

4-4. Sun protection factory

The evaluation of Sun Protection Factor (SPF) values across various polarity-based extracts of *H. sabdariffa* flowers, in comparison to quercetin as a reference compound, reveals a clear influence of solvent polarity on photoprotective capacity. The ethanol extract exhibited the highest SPF among the plant fractions (15.94±1.22), closely followed by the butanol extract (14.11±0.88). In contrast, the aqueous (6.87±1.09) and hexane (4.77±0.70) fractions demonstrated substantially lower SPF values, indicating a limited ability of highly polar or non-polar solvents to recover significant photoprotective constituents.

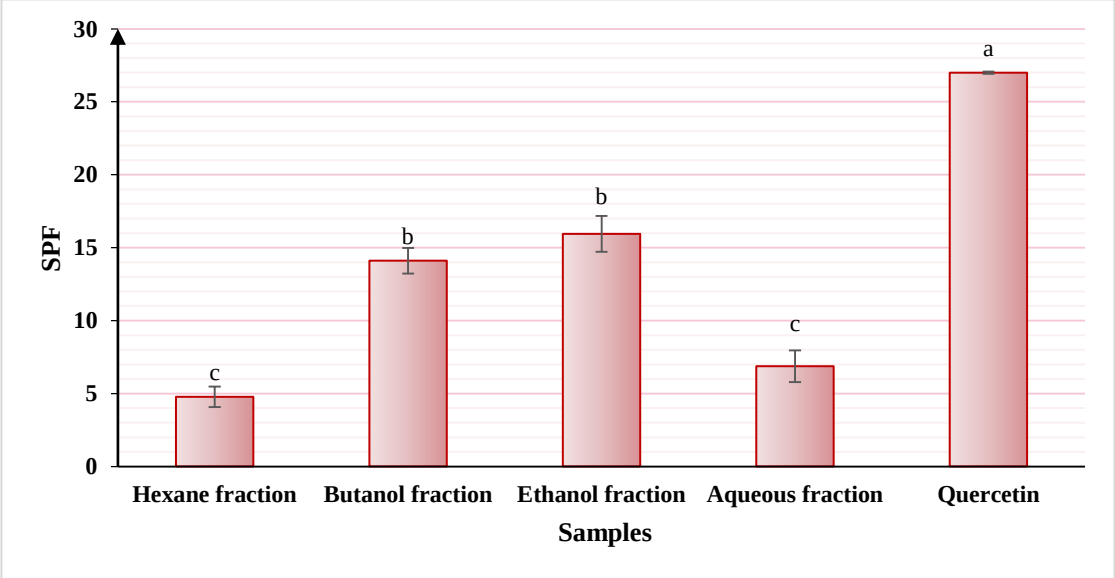


Figure (26): Sun Protection Factor (SPF) Values of Different Extracts of *H. sabdariffa* Flowers Compared to Quercetin. (a-c, Duncan's test evidences).

4-5. Anti-inflammatory activity

Table (5): Anti-Inflammatory Activity (%) of Different Extracts of *H. sabdariffa* Flowers at Varying Concentrations Compared to Diclofenac.

Concentration (µg/mg)	Hexane fraction	Butanol fraction	Ethanol fraction	Aqueous fraction	Diclofenac 50 mg
125	73.79	94.32	95.36	59.03	50.76
250	84.93	97.21	96.60	83.29	52.53
500	88.44	98.66	98.85	87.10	96.70

The anti-inflammatory activity of various polar extracts of *H. sabdariffa* flowers demonstrates a concentration-dependent effect, with all extracts exhibiting enhanced inhibition at increasing doses. Among the tested fractions, the ethanol and butanol extracts consistently showed the highest anti-inflammatory potential across all concentrations, with maximal inhibition at 500 µg/mg reaching 98.85% and 98.66%, respectively—surpassing the reference drug diclofenac, which achieved 96.70% at the same concentration. The hexane fraction displayed moderate activity, particularly at the highest dose (88.44%), while the aqueous fraction showed a marked increase from 59.03% at 125 µg/mg to 87.10% at 500 µg/mg, indicating a substantial dose-response effect. These findings suggest that ethanol and butanol are the most effective solvents for extracting anti-inflammatory compounds from *H. sabdariffa* flowers.

5- Pearson Correlation Analysis of Bioactive Compounds and Functional Activities in *H. sabdariffa* Extracts:

The Pearson correlation matrix reveals significant interrelations among the bioactive compound contents (phenolics, flavonoids, tannins) and the functional parameters (DPPH$^{\bullet}$ radical scavenging activity (IC50), Reducing power (EC50), Anti-hemolytic activity (Hly50), total antioxidant capacity, and SPF). A strong positive correlation is observed between phenolic and flavonoid contents (r = 0.97), indicating their co-extraction and shared contribution to biological activity. Both phenolics and flavonoids exhibit a strong negative correlation with DPPH$^{\bullet}$ radical scavenging activity (r = -0.63 and -0.64, respectively), reflecting that higher levels of these compounds are associated with stronger antioxidant activity (lower IC50 values). Additionally, SPF values are strongly correlated with phenolic (r

= 0.94) and flavonoid (r = 0.96) contents, suggesting their prominent role in photoprotection. Conversely, tannin content shows a positive correlation with reducing power (r = 0.88) and anti-hemolytic activity (r = 0.84). Notably, total antioxidant capacity correlates positively with phenolics (r = 0.47) and SPF (r = 0.54), but negatively with tannins (r = -0.50) and anti-hemolytic activity (r = -0.71). Overall, these results demonstrate that phenolics and flavonoids are major contributors to antioxidant and photoprotective activities, while tannins exhibit a distinct functional pattern, especially in relation to anti-hemolytic activity and reducing power.

Table (6): Pearson Correlation Matrix Between Phytochemical Contents and Biological Activities of *H. sabdariffa* Extracts

	Phenolic content	**Flavonoids content**	**Tannin content**	**IC_{50}**	**EC_{50}**	**Hly_{50}**	**Total capacity**	**SPF**
Phenolic content	1	**0.971744**	-0.48931	-0.62577	-0.2871	-0.41941	0.471998	**0.939947**
Flavonoids content	**0.971744**	1	-0.49759	-0.63664	-0.29199	-0.3737	0.367101	**0.958302**
Tannin content	-0.48931	-0.49759	1	-0.27145	**0.881292**	**0.843419**	-0.49757	-0.58885
IC_{50}	-0.62577	-0.63664	-0.27145	1	-0.54275	-0.36897	-0.08158	-0.50435
EC_{50}	-0.2871	-0.29199	**0.881292**	-0.54275	1	**0.906811**	-0.35885	-0.42034
Hly_{50}	-0.41941	-0.3737	**0.843419**	-0.36897	**0.906811**	1	-0.7053	-0.55078
Total capacity	0.471998	0.367101	-0.49757	-0.08158	-0.35885	-0.7053	1	0.536035
SPF	**0.939947**	**0.958302**	-0.58885	-0.50435	-0.42034	-0.55078	0.536035	1

CHAPTER IV

Discussions & Conclusions

1- Quantitative phytochemical analysis

1-1. Determination of total phenolic contents:

The studied results showed a varying difference in the total phenolic content among the four extracts with different polarities from the plant Hibiscus sabdariffa L. The ethanolic extract had the highest total phenolic content (186.3±30.20 mg/g), followed by the butanolic extract (167.3±15.26 mg/g). The aqueous (44.57±4.13 mg) and hexane (42.23±1.75 mg) extracts had the lowest content, indicating that polar solvents are effective in extracting phenolic compounds. And this was demonstrated by the study of Mohamed et al. (2013), which showed that the ethanolic extract of *H. sabdariffa* is rich in phenolics, unlike the aqueous and hexane extracts. This is due to the ability of ethanol to dissolve phenolic compounds because they are moderately polar (Mohamed et al., 2013). Some have indicated that the high level of phenolics is due to the presence of anthocyanins and flavonoids. A study by Da-Costa-Rocha et al. (2014) showed that the total phenolic compounds in *H. sabdariffa* are concentrated in the flowering part of the plant, and that using ethanol or moderately polar solvents yields the highest extraction due to the polar solvent's ability to break hydrogen bonds, resulting in strong extraction efficiency. And in the study by Baba et al. (2020) indicated that the aqueous and hexane extracts showed a low content of total phenolics in *H. sabdariffa*, which is attributed to the inability of water and hexane to extract phenolic compounds due to the difference in polarity. The study proved that phenolics can be moderately polar or associated with complex proteins and sugars, requiring organic solvents for their separation**.**

1-2. Determination of flavonoids contents:

The results showed that the total flavonoid content in *H. sabdariffa* flower extracts varied significantly depending on the polarity of the solvent used. The butanol extract recorded the highest concentration (63.3±0.87 micrograms quercetin equivalent/mg), followed by the ethanol extract (60.63±2.12 micrograms/mg), while the aqueous (5.4±0.13) and hexane (5.18±0.25) extracts showed very low levels of flavonoids. This variation indicates that the flavonoids in *H. sabdariffa* have a high solubility in moderately polar organic solvents. These results are consistent with the findings of Ali et al. (2021), which indicated that flavonoids, particularly quercetin and anthocyanins, are highly concentrated in the alcoholic extracts of the *H. sabdariffa*

plant, while they are less concentrated in aqueous or non-polar extracts. The study attributed this to the chemical structure of flavonoids, which includes hydroxyl groups that make them soluble in polar organic solvents, such as ethanol and butanol (Ali et al., 2021). A study by Mohamed et al. (2013) showed similar results, where the butanol extract recorded the highest flavonoid content compared to the other solvents. The study indicated that butanol, due to its moderate polarity, is ideal for extracting flavonoids that may be bound to sugar molecules (glycosides) or exist as complex compounds that are difficult to extract with water or non-polar solvents (Mohamed et al., 2013). The study by Da-Costa-Rocha et al. (2014) confirmed that the flavonoid compounds in *H. sabdariffa*, including anthocyanins such as delphinidin and cyanidin, constitute a significant portion of its biologically active components and are more readily extracted using moderately polar organic solvents. These compounds are responsible for the distinctive red color of the flowers, as well as the strong antioxidant activity exhibited by the plant. On the other hand, the study by Salem et al. (2020) recorded a significant decrease in the flavonoid content in the aqueous and hexane extracts of the same plant. It indicated that flavonoids with moderate to high polarity are not efficiently extracted with water due to their binding to complex cellular components or the insufficiency of water in breaking the bonds within plant cells. Hexane, being a non-polar solvent, is unable to extract flavonoid compounds with a polar structure.

1-3. Determination of condensed tannin contents:

The results showed that the content of condensed tannins varied significantly depending on the polarity of the solvent used. The hexane extract recorded the highest concentration (19.74±0.19 micrograms catechin equivalent/mg), statistically surpassing the other extracts. In contrast, the other extracts (butanolic, ethanolic, and aqueous) recorded similar and lower concentrations (around 13.65–13.99 µg/mg). These results indicate that the tannins in *H. sabdariffa* have chemical properties that allow them to be extracted in non-polar solvents. The study by Ali et al. (2021) confirmed this analysis and indicated that *H. sabdariffa* contains a variety of tannins in different forms, which can be polar or associated with fatty or waxy substances in the plant tissue. This allowed hexane to extract them efficiently. This study also mentioned that the tannins in this plant are not limited to those soluble in water or alcohol only (Ali et al., 2021). In another study, Mohamed et al. (2013) showed that the tannin content was low in the ethanolic and aqueous extracts, which proved that these compounds are not strongly polar as commonly believed, or a type of them may exist in the form of complex compounds with a non-polar nature, which can be extracted by hexane. On the other hand, the study by Da-Costa-Rocha et al. (2014) showed that the

tannins in *H. sabdariffa* are distributed between hydrolyzable tannins and condensed tannins, with the latter possibly being associated with cell walls or waxy substances on the surface of plant tissues, which supports the ability of non-polar solvents like hexane to extract them efficiently. As shown by the study by Baba et al. (2020), the quality of the solvent used significantly affects the extraction of tannins, and non-polar extracts may contain tannin compounds bound to fats or proteins, which polar solvents cannot easily extract. The study recommended using a variety of solvents to obtain a comprehensive picture of the tannin content in plants.

2- Biology activity:

2-1. Antioxidant activity:

2-1-1. Free radical-scavenging activity (DPPH• assay):

The results of the DPPH• test showed a significant difference in antioxidant capacity among the four extracts, where the effectiveness was measured by calculating the IC_{50} value. Ascorbic acid (6.72±0.93 µg/mL) had the highest efficacy as a reference antioxidant. As for the extracts, the ethanolic extract showed the strongest activity (58.02±0.35 µg/mL), followed by the hexane extract (166.66±1.12 µg/mL), while the aqueous extract had the lowest value (884.02±6 µg/mL). These results indicate that effective antioxidant compounds, such as phenols and flavonoids, dissolve more efficiently in ethanol than in other solvents, which is reflected in the lower IC_{50} value, indicating higher efficacy. These results are consistent with the study by Mohamed et al. (2013), which showed that the ethanolic extract has strong free radical scavenging activity compared to the aqueous extract. This was demonstrated by the high content of flavonoids and phenolics soluble in ethanol, which are compounds capable of donating hydrogen and also inhibiting free radicals (Mohamed et al., 2013). The study by Da-Costa-Rocha et al. (2014) confirmed these results, indicating that anthocyanins and flavonoids have antioxidant activity when extracted with polar solvents such as ethanol. It was mentioned that the inhibition efficacy depends on the compounds' ability to donate electrons or hydrogen atoms to free radicals. In the study by Ali et al. (2021), the antioxidant activity of *H. sabdariffa* extracts was compared using several different solvents, with the best results recorded for the ethanolic extract, followed by extracts with low polarity, while the aqueous extract was the weakest. The study indicated that the aqueous extract often contains sugars and compounds that are ineffective as antioxidants, and that water cannot efficiently extract complex phenolic compounds. As for the study by Salem et al. (2020), it demonstrated the relationship between high phenolic and

flavonoid content and antioxidant activity, confirming that ethanol and butanol are the best solvents for extracting antioxidants from *H. sabdariffa*. It indicated that the low effectiveness in the aqueous and hexane extracts was due to the low amount of active compounds in those extracts, or their insolubility in those solvents.

2-1-2. Reducing power:

The results show that there is a clear variation in EC_{50} among the extracts. Ascorbic acid (as a reference antioxidant) recorded the highest reducing capacity (low EC_{50}), followed by the aqueous extract. As for the ethanolic and butanolic extracts, they showed moderate activity with a slight increase in EC_{50}, while the hexane extract recorded the lowest activity (highest EC_{50}).

These results indicated that the aqueous extract contains compounds that are soluble in water and have strong reductive properties (water-soluble vitamins such as vitamin C), or polyphenolic compounds with the ability to donate electrons. Abubakar et al. (2020) agreed, demonstrating that the aqueous extract exhibited a remarkable reducing capacity compared to alcoholic extracts, attributing this to its content of flavonoid compounds and polysaccharides.

The study by Salem et al. (2020) also confirmed these results, showing that the reducing activity is not only related to the total phenolic content but also to the quality of the compounds.

On the other hand, the study by Da-Costa-Rocha et al. (2014) indicated that *H. sabdariffa* extracts contain water-soluble anthocyanin compounds, which have a high capacity to donate electrons in reduction systems, reflecting the high efficacy of the aqueous extract in this type of tests.

In the study by Mohamed et al. (2013), they observed that the alcoholic extracts showed good efficacy but were not the highest in percentage. They also indicated that there are antioxidant compounds in the plant that may change their structure or lose their efficacy when extracted with alcohol, unlike water.

2-1-3. Anti-hemolysis activity:

The results showed that the ethanolic and aqueous extracts recorded the lowest Hly_{50} value (<120 µg/mL), indicating a strong protective effect against hemolysis. They were equally

effective as ascorbic acid, confirming that they contain compounds capable of stabilizing the cell membrane and oxidation. In contrast, the butanol extract showed moderate activity (Hly_{50} ~350 µg/mL), while the hexane extract exhibited high activity (Hly_{50} ~600 µg/mL), which had the weakest anti-hemolytic activity.

The study by Ali et al. (2011) agreed with the examined results, which demonstrated that the ethanolic extract recorded a clear protective effect against hemolysis caused by hydrogen peroxide, due to its high content of anthocyanins and flavonoids. A study by Odigie et al. (2003) confirmed that the aqueous extract has a protective effect on cells, indicating that water-soluble phenolic compounds may enhance the stability of cell membranes and make them resistant to oxidation.

The study by Tseng et al. (2017) showed that the polar extracts of *H. sabdariffa* positively affect the fluidity and stability of the cell membrane under oxidative stress conditions, and this study agreed with our results on the aqueous and ethanolic extracts. As for the study by Ajiboye et al. (2011), it showed that *H. sabdariffa* extracts rich in polyphenolic antioxidants provide high protection for red blood cells against hemolysis by inhibiting lipid peroxides, supporting the hypothesis that polar compounds are the most effective in protecting the cell membrane.

2-1-4. Phosphomolybdenum reducing power:

The results of this analysis revealed a clear variation in the total antioxidant capacity among the extracts, with the ethanolic extract showing the highest value (47.21±9.61 µg EGA/mg), followed by the aqueous extract (17.80±6.05 µg EGA/mg), while the butanolic and hexanic extracts recorded low values (1.911±0.33 and 1.58±0.44, respectively). These differences reflect the efficiency of polar solvents in extracting antioxidant compounds capable of reducing molybdenum ions, which is an important indicator of overall reductive activity. These results are consistent with the findings of Tseng et al. (2017), which showed that the ethanolic extract of *H. sabdariffa* contains a high percentage of phenolic compounds and anthocyanins that are associated with a high reducing capacity in the phosphomolybdic acid test, due to their ability to donate electrons.

A study by Bako et al. (2020) also showed that the ethanolic and aqueous extracts of the *H. sabdariffa* plant contain polyphenolic compounds and effective antioxidants such as quercetin and

gallic acid, which are responsible for the ability to reduce phosphomolybdic acid and form green complexes representing total antioxidant activity.

The study by Mahadevan et al. (2015) confirmed that the phosphomolybdic test reflects the total antioxidant content capable of reducing Mo(VI) to Mo(V), which is achieved more effectively in the presence of phenolic compounds soluble in ethanol and water, compared to non-polar organic solvents like hexane. The study by Ali et al. (2011) demonstrated that anthocyanins extracted with ethanol play a crucial role in the overall antioxidant activity.

2-2. Sun protection factor:

The SPF evaluation results indicate significant differences between extracts obtained with solvents of varying polarities. The ethanolic extract showed the highest UV protection value (15.94±1.22), followed by the butanolic extract (14.11±0.88), while the values were noticeably lower in the aqueous extract (6.87±1.09) and the hexane extract (4.77±0.70). These results reflect the crucial role of solvent polarity in extracting photochemically active compounds such as phenolics and flavonoids, which are known for their ability to absorb or block ultraviolet rays.

These results are consistent with the findings of Kaur & Saraf (2010), which indicated that polyphenolic plant compounds, particularly flavonoids like quercetin and rutin, possess strong absorption properties in the ultraviolet range (UVB and UVA), making them effective as ingredients in sunscreen formulations. It has been shown that *H. sabdariffa* extracts rich in these compounds, especially those extracted with ethanol, achieve high SPF values. A study by Mahran et al. (2020) also showed that the ethanolic extract of *H. sabdariffa* contains high concentrations of anthocyanins, which are plant pigments with strong absorption properties in the ultraviolet spectrum and play a pivotal role in protecting cells from solar radiation damage.

The study by Yazdi et al. (2018) also confirmed that the polyphenols extracted from *H. sabdariffa* using ethanol or butanol provide effective protective activity against ultraviolet rays by directly absorbing the rays, in addition to their antioxidant properties that reduce DNA and protein damage caused by radiation.

2-3. Anti-inflammatory activity:

The results show that *H. sabdariffa* extracts exhibit a concentration-dependent anti-inflammatory activity, as the inhibition rate increases with higher concentrations in all

extracts. It is observed that the ethanolic and butanolic extracts exhibited the highest activity across the three concentrations (125, 250, 500 µg/mg), with their effectiveness at the highest concentration (98.85% and 98.66% respectively) surpassing that of the reference drug diclofenac (96.70%). In contrast, the hexane extract recorded moderate activity (88.44% at 500 µg/mg), while the aqueous extract showed significant improvement with increasing concentration, from 59.03% to 87.10%.

These results reflect the efficiency of medium-polarity solvents (ethanol and butanol) in extracting anti-inflammatory active compounds, such as phenols and flavonoids, which work through several mechanisms, including the inhibition of COX and LOX enzymes, and the reduction of inflammatory mediators like prostaglandins and cytokines.
The study by Ali et al. (2011) confirms that flavonoid-rich extracts from *H. sabdariffa*, especially those extracted with ethanol, show a clear inhibition of the COX-2 enzyme and reduce inflammation more effectively than many traditional compounds. The study by Tseng et al. (2020) also supports these findings, showing that anthocyanins extracted from the plant's flower calyces reduce inflammation by inhibiting NF-κB pathways associated with the inflammatory response.

A study by Abubakar et al. (2019) showed that the butanol extracts from *H. sabdariffa* exhibited a significant effect in chronic and acute inflammation models in mice, attributing this effect to their high content of polyphenols and proanthocyanidin compounds. The study by Mohamed et al. (2021) confirmed that using moderate polar solvents like ethanol produces extracts with higher biological activity due to their ability to extract multiple compounds with overlapping therapeutic functions, especially in combating

3- Pearson Correlation Analysis of Bioactive Compounds and Functional Activities in *H. sabdariffa* Extracts:

The Pearson correlation reveals significant relationships between the content of active compounds (phenols, flavonoids, tannins) and various biological activities:
A strong positive correlation between phenols and flavonoids ($r = 0.97$), indicating that they are often extracted together from the same fractions, which explains the close effectiveness of ethanol and butanol.

A strong inverse correlation between phenols/flavonoids and DPPH activity (IC_{50}) (r = -0.63 and -0.64, respectively), indicating that an increase in these compounds is associated with a decrease in IC_{50} value, meaning greater antioxidant activity.

A strong positive correlation between SPF and the content of phenols (r = 0.94) and flavonoids (r = 0.96), indicating their effective contribution to photoprotection. In contrast, tannins showed a strong positive correlation with reducing power (EC_{50}, r = 0.88) and anti-hemolytic activity (r = 0.84), indicating that they mainly contribute to these functions.

CONCLUSION

Through this study, we were able to highlight the biological and chemical importance of the extracts of the *Hibiscus sabdariffa* L. plant. A theoretical section was completed, covering the general aspects of the plant in terms of its classification, traditional uses, and active components. The experimental part focused on comparing four extracts (ethanolic, butanolic, hexanic, and aqueous) in terms of their content of phenolic, flavonoid, and tannin compounds, along with evaluating their biological activity through several tests, including DPPH, FRAP, SPF, phospholipid peroxidation inhibition, hemolytic inhibition, and anti-inflammatory activity .

The results showed that the ethanolic extract was the richest in phenolic compounds and the most active as an antioxidant and also in the sun protection test, while the butanolic extract was distinguished by its effectiveness in resisting hemolysis and inflammation. As for the aqueous extract, it showed its effectiveness in the FRAP test, while the hexane extract recorded weak and low results compared to the other extracts .

These results have demonstrated the great potential of this plant as a natural source of biologically active compounds, which opens up future prospects for developing therapeutic or preventive preparations based on Hibiscus sabdariffa L. extracts.

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Annexes

1.Some used materials and devices:

Figure (27) : Soxhlet apparatus

Figure (28) : Libra apparatus.

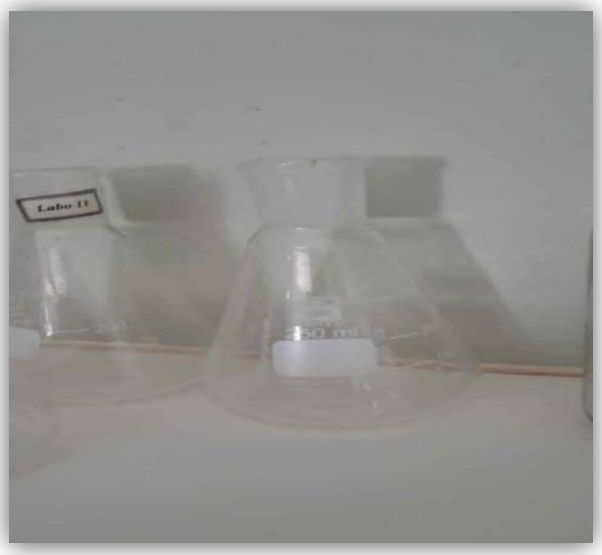


Figure (29): Laboratory glassware (flask)

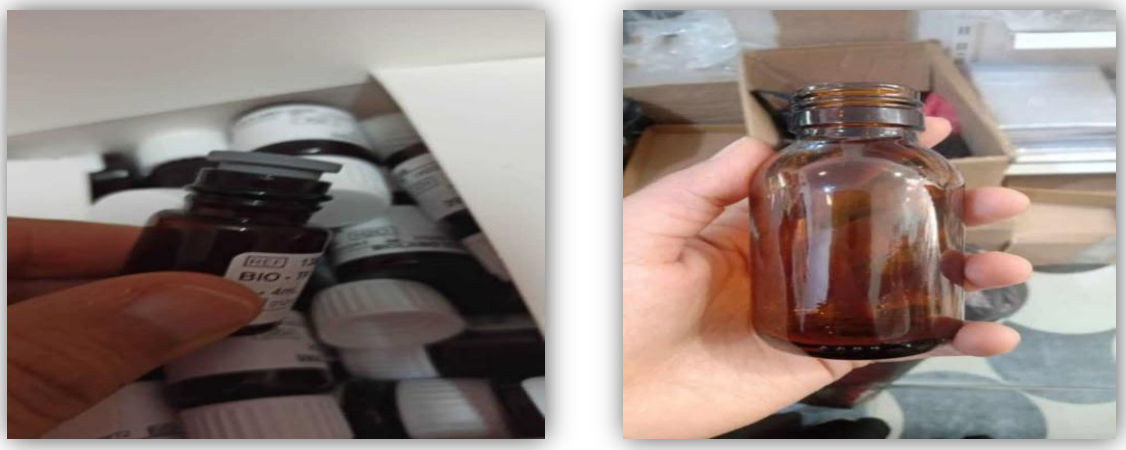
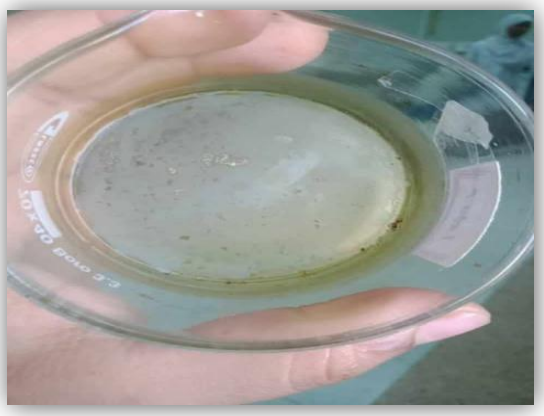
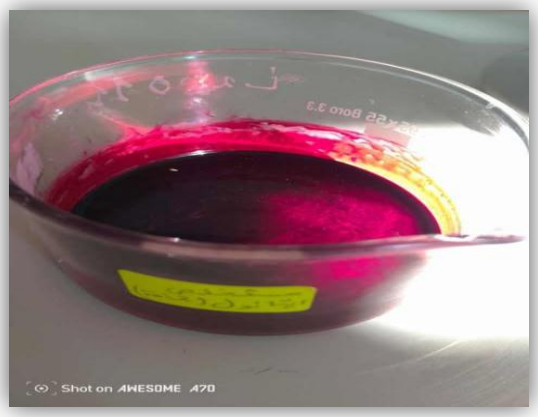


Figure (30): Opaque storage glassware

2.Plant extracts (hexane, butanolic, ethanolic and aqueous) :

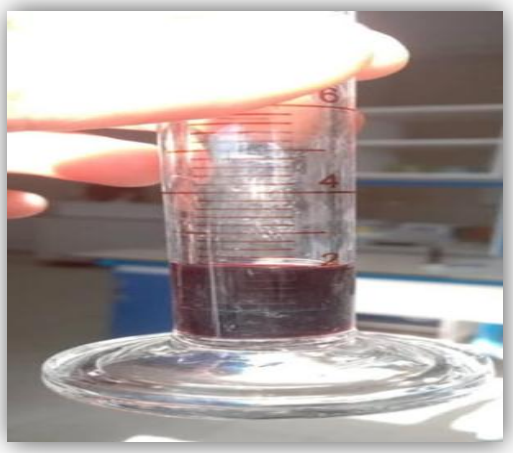
Figure(31): Aqueous extract **Figure(32):** Butanolic extract





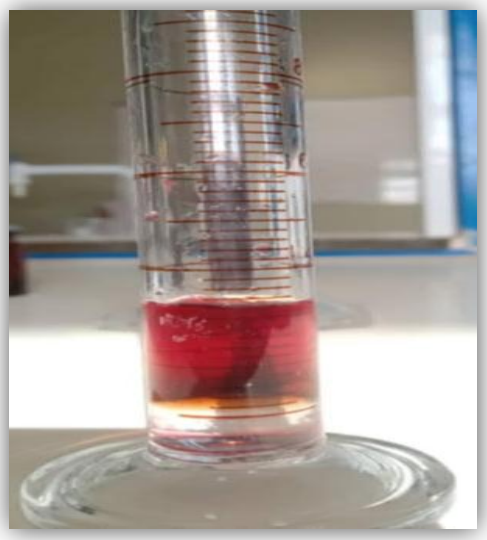
Figure(33): Hexanic extract **Figure(34):** Ethalonic extract

3 The results of phytochemicals screening :



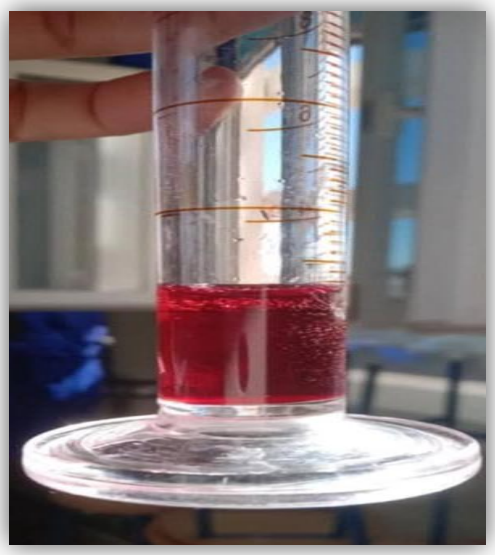
Figure(35): Chemical detection of tanins

Figure(36): Chemical detection of alkaloids



Figure(37): Chemical detection of steroids

Figure(38): Chemical detection of flavonoids



Figure(39): Chemical detection of saponins