

University Ferhat Abbas
Setif 1
Faculty of Nature and Life
Sciences

الجمهورية الجزائرية الديمقراطية الشعبية
وزارة التعليم العالي والبحث العلمي

جامعة فرحات عباس
سطيف 1
كلية علوم الطبيعة والحياة



Department of Biochemistry

Master thesis

Presented by

Rahma Guellati

Meriem Chellali

For the fulfillment of the diploma of

Master

Field: Biological Sciences

Specialty: Pharmacotoxicology

Topic

***In Vitro Antioxidant Activity and Gastrointestinal
Effect of the Decocted Extract of *Hibiscus sabdariffa****

Jury

President

Fatima Benchikh

Pr. UFA Setif 1

Supervisor

Walid Mamache

SLA. UFASetif 1

Examinator

Hassiba Benabdallah

Pr.UFA.Setif 1

Laboratory of Phytotherapy Applied to Chronic Diseases

2025/2026

الملخص

أجريت هذه الدراسة للتحقيق في المحتوى الكيميائي النباتي، النشاط المضاد للأكسدة والتأثيرات على التقلصات المعوية المعوية للمستخلص المائي لنبات الكركدية (*Hibiscus sabdariffa* L (HSAq). أظهرت النتائج أن مردود الاستخلاص بلغ 33.7%. أظهر التحليل الكمي احتواء المستخلص على مستويات متوسطة من المركبات الحيوية الفعالة، شملت عديدات الفينول الكلية (9.83 ± 73.49 ميكروغرام مكافئ لحمض الغاليك / لكل ملغ من المستخلص)، والفلافونويدات (0.77 ± 8.44 ميكروغرام مكافئ للكركستين / لكل ملغ من المستخلص)، والدباغ (2.05 ± 51.83 ميكروغرام مكافئ حمض التانيك / لكل ملغ من المستخلص).

أظهر المستخلص المائي للكركدية (HSAq) نشاطاً مضاداً للأكسدة ملحوظاً من خلال عدة اختبارات، تمثلت في: اختبار التقاط الجذور الحرة DPPH (149.17 ± 0.12 ميكروغرام/مل)، اختبار ($26.38 \pm 239.79 = IC_{50}$) ABTS (ميكروغرام/مل)، اختبار القدرة الاختزالية الفينانثرولين ($6.77 \pm 23.08 = EC_{50}$ ميكروغرام/مل)، بالإضافة إلى اختبار السعة الكلية المضادة للأكسدة ($7.53 \pm 216.8 = EC_{50}$ ميكروغرام/مل).

أظهرت تقييمات إفراغ المعدة (GE) أن المستخلص المائي للكركدية (HSAq) بجرعة 400 ملغ / كغ لم يُحدث تغييراً ملحوظاً في إفراغ المعدة ($79.62 \pm 2.40\%$). ومع ذلك، قام HSAq بتنشيط العبور المعوي (IT) بشكل يعتمد على الجرعة، حيث أظهرت جرعة 400 ملغ / كغ أعلى نسبة تثبيط ($42.11 \pm 1.59\%$). أظهر تثبيط وتنشيط إنزيم تصنيع أكسيد النيتريك (NO) باستخدام L-NNA أو L-arginine أن مسارات أكسيد النيتريك (NO) لا تشارك في التأثير الناتج عن HSAq. في المقابل، أدى تثبيط إنزيم COX بواسطة الإندوميثاسين إلى انخفاض العبور المعوي ($44.25 \pm 3.92\%$). استمر هذا التثبيط في وجود HSAq ($51.73 \pm 4.34\%$). مما يدل على أن تأثير HSAq مرتبط بشكل كبير بتنشيط تخليق البروستاغلاندينات (PG).

تشير هذه النتائج إلى الإمكانات العلاجية المحتملة للمستخلص المائي للكركدية (HSAq) كعامل مضاد للأكسدة يمتلك تأثيرات تثبيطية انتقائية على حركية الأمعاء، دون التأثير على الإفراغ المعوي الطبيعي.

الكلمات المفتاحية: *Hibiscus sabdariffa*، المستخلص المائي، المحتوى الكيميائي النباتي، النشاط المضاد للأكسدة، العبور المعوي، الإفراغ المعوي.

Abstract

The present study investigated the phytochemical content, antioxidant activity, and gastrointestinal motility effects of the aqueous extract of *Hibiscus sabdariffa* L (HSAq). The results showed that the extraction yield was 33.7%. Quantitative analysis revealed moderate amounts of bioactive compounds, including total polyphenols (73.49 ± 9.83 μg GAE/mg extract), flavonoids (8.44 ± 0.77 μg QE/mg extract), and tannins (51.83 ± 2.05 μg TAE/mg extract).

HSAq exhibited notable antioxidant properties across multiple assays: DPPH radical scavenging ($\text{IC}_{50} = 149.17 \pm 0.12$ $\mu\text{g/ml}$), ABTS assay ($\text{IC}_{50} = 239.79 \pm 26.38$ $\mu\text{g/ml}$), phenanthroline-based reducing power ($\text{EC}_{50} = 23.08 \pm 6.77$ $\mu\text{g/ml}$), and total antioxidant capacity ($\text{EC}_{50} = 216.8 \pm 7.53$ $\mu\text{g/ml}$).

Gastric emptying (GE) evaluations showed that HSAq at a dose of 400 mg/kg did not significantly modify gastric emptying ($79.62 \pm 2.40\%$). Therefore, HSAq significantly inhibited intestinal transit (IT) in a dose-dependent manner, with the dose of 400 mg/kg showing the highest inhibition ($42.11 \pm 1.59\%$). The inhibition and activation of nitric oxide (NO) synthase with L-NNA or L-arginine showed that the NO pathways are not involved in the effect induced by HSAq. Meanwhile, regarding the cyclooxygenase (COX) pathway, inhibition of COX by Indometacin decreased IT ($44.25 \pm 3.92\%$), and this reduction persisted in the presence of HSAq ($51.73 \pm 4.34\%$). This explains that the HSAq effect is mediated by the synthesis inhibition of PG.

These findings support the potential therapeutic use of HSAq as an antioxidant agent with selective inhibitory effects on intestinal motility, without affecting normal gastric emptying.

Keywords: *Hibiscus sabdariffa*, aqueous extract, phytochemical content, antioxidant activity, intestinal transit, gastric emptying.

Table of content

المُلخَص

Abstract

List of abbreviations

List of figures

List of tables

I. Introduction.....	1
II. Materials and Methods.....	4
1. Materials and Methods.....	4
1.1. Plant Material	4
1.2. Preparation of HSAq	4
1.3. Laboratory animals	4
1.4 Chemicals and Reagents	4
2. Phytochemical assessment.....	5
2.1. Total polyphenol content Assay	5
2.2. Total flavonoid content assay.....	6
2.3. Total tannin content assay	7
3. <i>In vitro</i> biological Activities.....	8
3.1 The DPPH Radical Scavenging Assay	8
3.2. ABTS Radical Scavenging Assay.....	8
3.3 Assessment of Reducing Power (Phenanthroline Method)	9
3.4. Total Antioxidant Capacity (TAC) Assay	9
4. <i>In vivo</i> intestinal transit and gastric emptying rate tests.....	9
4.1 Assessment of intestinal transit (IT).....	10
4.2 Assessment of Gastric Emptying (GE)	10
5. Statistical analysis	10
III. RESULTS	11
1. Extraction yield.....	11
2. Phytochemicals screening.....	11
3. Antioxidant activities	12
4. Effects of HSAq on gastric emptying and intestinal transit rate	12
4.1 Effects of HSAq on IT.....	12
4.2 Effect of HSAq on GE.....	14
4.3 Evaluation of the Possible Mechanisms of HSAq on Intestinal Transit.....	15
IV. Discussion.....	16

V. Conclusion and Perspectives.....	20
VI. Bibliographic references	21

I. Introduction

Introduction

Oxidative stress is a critical biological phenomenon in which antioxidant defences fail to neutralize excess oxidant molecules. While it is well-established that reactive oxygen species (ROS) serve vital roles in signaling pathways, external pressures, specifically heavy metals, UV radiation, and various environmental pollutants, can trigger their excessive accumulation. This surge essentially outpaces the biological system detoxification mechanisms, resulting in a cascade of biomolecular damage that compromises tissue integrity (Pizzino et al., 2017). An overwhelming oxidant challenge leads to the degradation of essential biomolecules; maintaining a controlled level of oxidation, termed oxidative eustress, is indispensable for the regulation of life processes via redox signaling. Recent research emphasizes the sophisticated mechanisms through which cells integrate these opposing effects. This redox equilibrium is sustained through a multi-layered defense of prevention, interception (Sies et al., 2017).

The protective role of antioxidants is mediated through a diverse array of biochemical mechanisms; they scavenge free radicals and inhibit Fenton-like reactions by chelating redox-active metals. Also, they contribute to the repair of biomolecules that have been damaged by oxidation and help to regulate the balance of the antioxidant enzymatic system within the cell (Galano, 2025). The human body relies on an interplay between intrinsic cellular defenses and exogenous antioxidants derived from the diet. Together, these systems play a critical role in regulating the cellular oxidation-reduction equilibrium (Aguilar et al., 2016). Antioxidants have gained significant scientific attention, primarily due to their documented capacity to neutralize the potentially deleterious effects of oxidative stress caused by free radicals (Rahaman et al., 2023). Diverse array of plant-based matrices, ranging from common fruits and vegetables to various seeds and spices, serves as a primary reservoir for naturally occurring antioxidant compounds (Lourenço et al., 2019). Polyphenols represent a vital class of secondary metabolites whose bioactivity is fundamental to maintaining human health and improving systemic wellness (Coşkun et al., 2025). Traditional medicine remains a cornerstone of drug discovery, underpinning modern efforts to identify and refine new therapeutic pathways (Belhouala & Benarba, 2021).

Among these botanical sources, *H.sabdariffa* L. (Roselle), is a member of the Malvaceae family cultivated extensively across Africa, South East Asia, Central America, Mexico, and Egypt. This medicinal plant is highly valued in the food industry. Its calyces are frequently utilized as natural acidulants and flavorings for preserves and beverages, as well as potent organic colorants (Shruthi et al., 2016). The *H.sabdariffa* is a seasonal, upright plant

Introduction

that reaches a height of about 2.4 meters. It is characterized by smooth, reddish cylindrical stems and green leaves with red veins (Da-Costa-Rocha et al., 2014).

Chromatographic analysis of *H.sabdariffa* revealed major anthocyanins (delphinidin-3-sambubioside and cyanidin-3-sambubioside), organic acids, and phenolic acids, which are responsible for its red color and sour taste (Li et al., 2024). Additionally, delphinidin-3-sambubioside and 3-caffeoylquinic acid account for approximately 60% of total phenolics in *H.sabdariffa* calyces (Piovesana et al., 2019b).

Analysis of *H.sabdariffa* revealed glucose as the main sugar, malic acid as the primary organic acid, α -tocopherol as the major tocopherol, and linoleic acid as the principal fatty acid. Among non-anthocyanin compounds, 5-(hydroxymethyl) furfural was most abundant, while delphinidin-3-O-sambubioside was the predominant anthocyanin (Jabeur et al., 2017).

H.sabdariffa has been extensively studied for its therapeutic effects in humans. Consumption of its calyx extracts exerts multiple health benefits, including antihypertensive, hypoglycemic, lipid-lowering, antianemic, anti-inflammatory, antioxidant, anti-xerostomic, and diuretic properties, while also aiding weight management (Montalvo-González et al., 2022). Its anti-inflammatory properties have been particularly investigated in relation to degenerative diseases, sepsis, neoplasms, intestinal dysfunctions, and atherosclerosis (Nogueira da Cruz et al., 2025).

In North African traditional medicine, calyx preparations are used for sore throats, coughs, and reproductive issues, while the leaf pulp serves as an emollient for wounds and abscesses (Da-Costa-Rocha et al., 2014).

H.sabdariffa has long been used in folk medicine and cuisine as a meal, natural dye, and beverage. In China and Thailand, the flowers, leaves, and calyx are commonly consumed as a refreshing drink (Almajid et al., 2023). This perennial plant thrives in tropical and subtropical regions, including China, Egypt, Indonesia, Mexico, Nigeria, Thailand, and Saudi Arabia. Known as "Jamaica" in Spanish, it has a long history of culinary, botanical, cosmetic, and therapeutic applications (Izquierdo-Vega et al., 2020).

These diverse properties make *H. sabdariffa* a promising candidate for the treatment of gastrointestinal disorders. Despite the wealth of literature characterizing the phytochemical content of *H.sabdariffa*, such as flavonoids, tannins, and polyphenols (Da-Costa-Rocha et al., 2014), the direct correlation between these bioactive compounds and their mechanical effect on the gastrointestinal tract's smooth muscles remains insufficiently explored. There is a notable lack of functional data regarding how the aqueous extract specifically modulates gastric emptying and intestinal transit.

Introduction

In this context, the present study was conducted to evaluate the phytochemical profile and pharmacological properties of the aqueous extract of *H. sabdariffa* (HSAq).

The evaluation began with a phytochemical assessment and an analysis of the antioxidant activity using DPPH, ABTS, reducing power (phenanthroline method), and TAC assays.

Finally, the effects on gastric emptying and intestinal transit were investigated.