

## Analysis of the Pharmacological and Phytochemical constituents of the phenolic extracts of *Hibiscus sabdariffa* L. dried calyces plant by HPLC Techniques

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تحليل المركبات الكيميائية والعلاجية للمستخلص الفينولي لكئوس نبات الكرديّة المجفف

بأستخدام تقنيّة HPLC

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فرع الادوية والسموم، كلية الطب، جامعة واسط، واسط، العراق

### المستخلص:

التركيب الكيميائي لنواتج الايض الثانوية للنباتات مهم في اكتشاف مركبات ذات تاثيرات علاجية . نبات الكرديّة له استخدامات واسعة في علاج الطب الشعبي، لذلك تهدف هذه الدراسة الى الكشف النوعي والكمي للمركبات الفعالة للمستخلص الفينولي لكاس نبات الكرديّة المجفف بواسطة جهاز الكروماتوگرافي ذات الاداء العالي High Performance Liquid Chromatogram ( HPLC) . اوضحت النتائج ان النبات يمتلك المستخلص الفينولي (16 %), المستخلص التربيّني (5 %), المستخلص القلويدي (8 %). اشار تحليل HPLC اشار الى وجود (7) مركبات فينولية للمستخلص الفينولي هي: Vitamin C (443.39 مايكروغرام/مل), Gossypetrin (289.52 مايكروغرام/مل), Sabdaretin (222.61 مايكروغرام/مل), Gossypetin (7.12.24 مايكروغرام/مل), Chlorogenic (657.22 مايكروغرام/مل), Coumaric A (432.22 مايكروغرام/مل), و Hebiscetin (336.46 مايكروغرام/مل). وواضحت النتائج ان اعلى تركيز من المركبات الفينولية كان Gossypetin (712.24 مايكروغرام/مل), و اقل تركيز كان Sabdaretin (222.61 مايكروغرام/مل). اخيرا يمكن ان نستنتج من هذه الدراسة ان المستخلص الفينولي للنبات يمتلك مركبات صيدلانية .

**الكلمات المفتاحية:** الكشف الكيميائي الصيدلاني, نبات الكرديّة, تحليل hplc.

## Abstract

The chemical constituents of the secondary metabolic products of plants have important role in the discovery of therapeutically effective compounds. *Hibiscus sabdariffa* L. is widely utilized in herbal medicine for treatment and prevention of several diseases, the objective of this study is the Quantitative and Qualitative analysis of the phenolic extracts of the plants. High Performance Liquid Chromatography Techniques (HPLC) were used in the study, the result showed that the phenolic extracts of the plant composed of crud phenols (16%), crud alkaloid (8%), and crud terpens (5%). The HPLC identification of the extract indicates the presence of ( 7 ) compounds for phenolic extracts ; Vitamin C (443.39 µg/ml), Gossypetrin (289.52 µg/ml), Sabdaretin(222.61 µg/ml), Gossypetin (712.24 µg/ml ), Chlorogenic acid (657.22 µg/ml), Coumaric A (432.22 µg/ml ), and Hebiscetin (336.46 µg/ml). The highest phenolic compounds concentration was Gossypetin (712.24 µg/ml), while the lowest was Sabdaretin (222.61 µg/ml). The conclusion is that, the phenolic extract of the plant has many phytochemical and pharmacological components.

## Key words:

Phytochemical screening, *Hibiscus sabdariffa*, secondary metabolites, phenolic extracts, HPLC.

## Introduction

Human utilization for a variety of local herbs is accepted to contribute significantly to the improvement of human health, in terms of prevention, and or cure of sicknesses because plants have source of therapeutic compounds (1). *Hibiscus sabdariffa* L. (family Malvaceae), commonly known in English as roselle or red sorrel and in Arabic as karkadeh (2). *H. sabdariffa* is utilized in many parts of the world to make hot and cold drinks because the calyces of this plant are rich ascorbic

acid (vitamin C ). In folk medicine, the calyx extracts are utilized for the treatment of several grievances, including antioxidant activity, liver diseases, high blood pressure, and fever (3).

*H. sabdariffa* has secondary metabolites which have antioxidant and free radical scavenging properties such as phenolic acids, vitamins, tannins, terpenoids, flavonoids, and coumarins (4,5,6). Antioxidants are intimately included in prevention of cellular damage -the common pathway for cancer , aging, and

a variety of diseases by interact with free radicals and terminate the chain reaction before vital molecules are damaged (7, 8). This plants are rich in secondary metabolites which have antimicrobial properties (9). And which have antiseptic, aphrodisiac, digestive, purgative, demulcent, astringent, cholagogue, and resolvent, and it is used as a folk remedy in the treatment of cancer, cough, dyspepsia, fever, abscesses, debility, hangover, neurosis, heart ailments, and hypertension

(10,11,12,13). Qualitative and Quantitative determinate of secondary metabolites of *H. sabdariffa* by using HPLC (14,15). The aim of this study to determined yield of major compounds for secondary metabolites such as terpenoids, alkaloids and phenol from *H. sabdariffa* dried calyx and Identification, Quantitative and Qualitative of the active compounds for phenolic extracts of this plant by High Performance Liquid Chromatogram (HPLC)

## Materials and Methods

The calyces of *H. sabdariffa* (Figure 1), were collected from different area of Wassit Province during the year, cleaned,



Figure (1) *Hibiscus sabdariffa* plant

### Terpenoids extract

Crude terpenoids was extracted according to Harborne (16), 10 g of plant powder were blended with 200 ml of chloroform, and after that put in soxhlet

dried, powdered by using a grinder, and preparation for extracts.

apparatus for 8 hours of extraction, then evaporated in a rotary evaporator at 40°C for (1-2) hours, and kept until utilize.

### Alkaloids extract

Crude Alkaloids was extracted according to Harborne (16), 100 g of plant powder were blended with 350 ml ethanol : distill water mixture in a ratio at 1:4 in electrical blender for 5 minutes. The solution was filtered through muslin cloth

, then through Bukhnar funnel by using Whattman (No.1) filter papers, then evaporated at 45 °C in a rotary evaporator, drops of 2% sulphuric acid were added to adjust the pH(1-2), then transferred to a separation funnel and extracted with chloroform three times .

The solution was separated into two layers, the lower layer was chloroform, which was neglected . The upper layer was the aqueous layer to be used . "Concentrated ammonium hydroxide was added to this layer adjust the pH(9-10),

then the solution was again extracted in a separation funnel with chloroform : methanol mixture in ratio of 3:1 twice, and one time with chloroform alone".

The solution was separated into two layers, the lower layer, chloroform layer or chloroform: methanol layer, was evaporated in a rotary evaporator at 40°C for (1-2) hours . The upper layer, the aqueous layer, was evaporated in a rotary evaporator at 40°C for (1-2) hours, and kept in a refrigerator until utilize .

### Phenols extract

Crude phenols were extracted from the plant according to Ribereau-Gayon (17), and Harborne (16, 200 g of plant powder were separated into two equivalent amounts of, 300 ml of (1%) hydrochloric acid was added to one section, and 300 ml of distilled water was The two supernatants were blended. equivalent amounts of n-propanol was added to the blend and until the solution isolated into two layers sodium chloride was added. The lower layer removed with Ethyl acetic acid derivation by the separating funnel, and the dissolvable layer was dissipated in a revolving evaporator for (1-2) hours at 40°C. The upper layer was dissipated in a revolving

added to the next, the two amounts were transferred electrical blender for 5 minutes .

At that point the two blends were transferred for 30-40 minutes to bubbled water bath, the two blends were cooled and filter through muslin cloth, then transferred to a centrifuge( speed of 3000 rpm for 10 minutes) . evaporator for (1-2) hours at 40°C the dried material of both layers were blended and dissolved in 5ml of (96%) ethanol , then transferred to oven then was kept in cooler until utilize .

### Chemical composition analysis for phenolic extracts of *H. sabdariffa*

Analysis of chemical composition by injecting 20 µl of the phenolic extract of each sample in High Performance Liquid Chromatogram (HPLC Shimadzu-C-6A) for identification. The procedure that used outlined by Hartley and Buchan. The separate conditions were represented in

table (1). The peaks were detected by UV detector. The analysis was carried out in the laboratories of Ministry of Science and Technology. Compound concentration might determined by the following equation:

Area of the sample

Concentration of sample (µg/ml) = ----- × standard conc. × Dilution factor

Area of the standard

**Table(1) Conditions of High Performance Liquid Chromatograph used for analysis of phenolic compounds of the *H. sabdariffa* calyx extracts .**

Parameter	Characteristic
Column dimensions	3µm particle size (100×4.6 mm I.D)
Flow Rate	1.2 ml / min
Detector	UV spectrophotometer at 280 nm
Volume injection sample	20 µl
Type of Column	C-18
Mobile face	1%a <u>trifluoro</u> acetic acid (TFA) in deionized water: solvent B was 0.1 % TFA in gradient program from 0% B to 100% B for 12 minutes.
Temperature	30 °C

## Results and Discussion

The calyces of *H. sabdariffa* was extracted for discovery of terpens,

alkaloids and phenols. The yield of major compounds in extracts were found in table (2).

**Table (2) Yield of compounds of *H. sabdariffa* expressed as %**

Plants Name	Extracts type	Yield (%)
<i>Hibiscus sabdariffa</i>	Terpens	5
	Alkaloids	8
	Phenols	16

This indicated *H. sabdariffa* dried calyx which have terpens, alkaloids and phenols and this results agree with (18) were indicated phytochemical analysis for dried calyces of *H. sabdariffa* by utilizing alcoholic extraction method, the plant have some chemicals such as alkaloids, tannins, saponins, glycosides, phenols

and flavonoids. (19) were investigated found that phenols, alkaloids, tannins, flavanoids, Saponin were present in leaves, stem, and root of *H. sabdariffa*. TLC analysis also confirmed these results. Quantitative analysis of leaves, roots and stems shows best results in which phenolic have been found to be present more in leaf of the plant

Ethanollic extracts of *H. sabdariffa* calyces which have antibacterial activity(20).

Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal

significance, to make the best and judicious use of available natural wealth(21,22). Results of HPLC analysis in this study indicate the presence of 7 phenolic compounds in phenolic extracts as show in table (3) and figure(2),(3).

Table (3) Types and concentration of phenolic compounds in plant extracts .

Phenolic compounds ( $\mu\text{g/ml}$ )	<i>H. sabdariffa</i>
Vitamin C	443.39
Gossypetrin	289.52
Sabdaretin	222.61
Gossypetin	712.24
Chlorogenic acid	657.22
Coumaric A	432.22
Hebiscetin	336.46
<b>Total concentration (<math>\mu\text{g/ml}</math>)</b>	<b>3093.66</b>

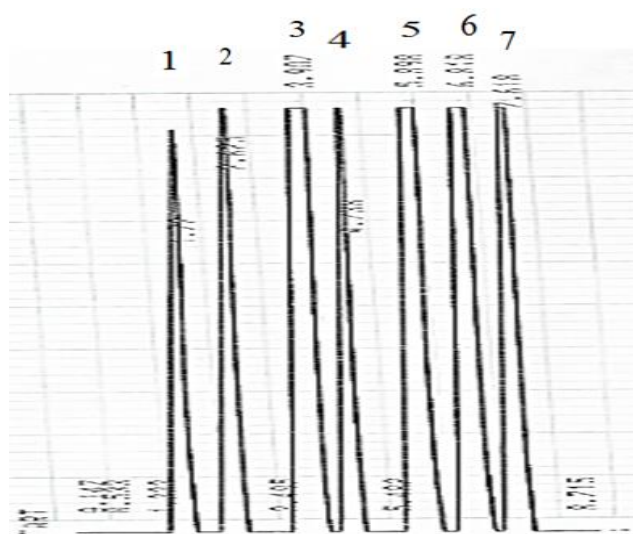


Figure (2) HPLC profile of Phenols Standard

(1) Vitamin C, (2) Gossypetrin, (3) Sabdaretin, (4) Gossypetin, (5) Chlorogenic acid,  
(6) Coumaric A, (7) Hebiscetin.

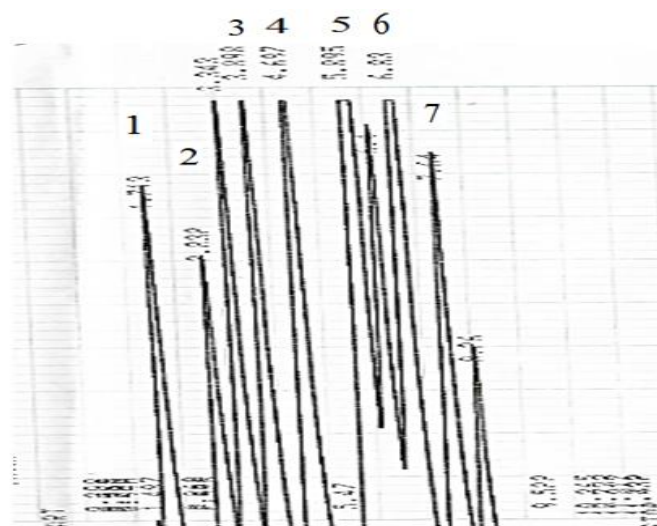


Figure (3) HPLC profile of *Hibiscus sabdariffa* calyx Phenols

(1) Vitamin C, (2) Gossypetrin, (3) Sabdaretin, (4) Gossypetin, (5) Chlorogenic acid, (6) Coumaric A, (7) Hebiscetin.

Results shows the highest phenolic compounds was Gossypetin (712.24 µg/ml), while Sabdaretin (222.61 µg/ml) was the lowest. Several experimental studies indicated phenolic compounds which have biological and

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