Republic of Iraq
Ministry of Higher Education
& Scientific Research

Al-Zahrawi University
College Pharmacy

A Phytochemical Investigation of Cyperus Rotundus L. family Cyperaceae Extracts

This research is prepared to get a bachelor's degree in pharmacy department

Prepared by
Alaa Ali Radhi

Rusul Hassan Yas

Zahraa Mohsen Ubaid

Zahraa Ihsan Mahmoud

Fatimah Al-Zahraa Abdul Hameed

Supervisor

Dr.Mohammed Jassim Hassan

summary

This study presents a phytochemical investigation of Cyperus rotundus L. (family Cyperaceae), a medicinal plant widely distributed in tropical and subtropical regions, including Iraq. The research focused on the roots of the plant, aiming to evaluate the chemical composition of its extracts using different extraction methods and to identify and quantify bioactive compounds via High-Performance Liquid Chromatography (HPLC).The plant material was collected, dried, and powdered. Two extraction methods were employed: cold maceration and hot reflux extraction, both using 90% methanol after defatting with petroleum ether. The resulting crude methanolic extracts were then fractionated successively with chloroform, ethyl acetate, and n-butanol based on polarity. The percentage yields of each fraction were calculated for both methods. For the cold method, the ethyl acetate fraction gave the highest yield (32.53%), followed by n-butanol (16.30%) and chloroform (7.19%). For the hot method, the ethyl acetate fraction again yielded the highest (35.82%), followed by n-butanol (21.77%) and chloroform (11.75%).HPLC analysis was performed on the ethyl acetate and n-butanol fractions from both extraction methods. The analysis identified four major bioactive compounds: chlorogenic acid, catechin, quercetin, and genistein. Their identities were confirmed by comparing retention times and UV spectra with authentic standards.Quantitative analysis revealed that the hot reflux method was more efficient than cold maceration, yielding higher concentrations of all detected compounds. Catechin was the most abundant compound, with the highest concentration found in the hot ethyl acetate fraction (509.22 µg/g of fraction), followed by the hot n-butanol fraction (369.99 µg/g). Chlorogenic acid was also highest in the hot ethyl acetate fraction (211.76 µg/g), while quercetin and genistein followed a similar pattern, with their highest concentrations also in the

hot ethyl acetate fraction (117.49 µg/g and 56.33 µg/g, respectively). Notably, genistein was absent in both n-butanol fractions, indicating its preferential solubility in less polar solvents such as ethyl acetate.The study concludes that hot reflux extraction combined with polarity-based fractionation using ethyl acetate is an efficient method for isolating bioactive compounds from Cyperus rotundus. The findings highlight the significant role of temperature and solvent polarity in optimizing extraction yields. The study recommends further bioactivity assessment of the isolated compounds, validation of the HPLC method for reproducibility, and consideration of scalable, environmentally sustainable extraction techniques for potential pharmaceutical application

Keywords: Cyperus rotundus, phytochemical screening, HPLC, ethyl acetate fraction, n-butanol

A Phytochemical Investigation of Cyperus Rotundus L. family Cyperaceae Extracts

Introduction

Medicinal plants have been used since ancient times and continue to represent an important source of therapeutic agents due to their relative safety, lower toxicity, affordability, and accessibility [1]. For centuries, traditional herbal medicine has been trusted worldwide for the management of various diseases [2].

Cyperus rotundus L., a perennial herb belonging to the family Cyperaceae, is one of the most widely distributed medicinal plants in tropical and subtropical regions. It is commonly known as nut grass or purple nut sedge. The plant is characterized by slender, rough, creeping rhizomes with small tubers measuring approximately 1–3 cm in length. These tubers are blackish on the outside and reddish-white internally with a characteristic odor. The stems may reach about 25 cm in height, and the leaves are linear, dark green, and grooved on the upper surface. The inflorescences are small with 2–4 bracts, consisting of tiny flowers with a reddish-brown husk. The fruit is three-angled, oblong-ovate, yellow in color and turns black when mature. Due to its adaptability, C. rotundus is widely dispersed in many regions, including Iraq, where it is considered a common and popular medicinal plant [3].

In folk medicine, it has also been used as a diuretic, aphrodisiac, sedative, carminative, and a treatment for dysentery and renal colic [4]. Numerous biological and pharmacological properties, including cytotoxic [5], antimicrobial [6], anti-inflammatory [7], anti-allergic [8], anti-diarrheal [9], and hepatoprotective [10], are also exhibited by *C. rotundus*, both in vitro and in vivo. Previous studies on the phytochemistry of *C. rotundus* have identified a variety of secondary metabolites,

including phenolic acids, alkaloids, flavonoids, iridoids, phenylpropanoids, furochromones, and saponins [11, 12, 13].

Medicinal plants, including C. rotundus, offer promising potential in this regard due to their rich phytochemical composition and broad spectrum of biological activities. Therefore, the present study aims to evaluate the phytochemical constituents of Cyperus rotundus L. extracts, particularly focusing on the roots. The study includes extraction using methanol, phytochemical screening, and chemical characterization of the bioactive compounds using HPLC analysis. As C. rotundus is a widely available and popular plant in Iraq, studying its extracts may contribute to the development of affordable and locally sourced therapeutic options. The study aims to investigate the phytochemical constituents and main characteristics of Cyperus rotundus, which is a commonly used medicinal plant in Iraq.

The study aims to:

Screen the phytochemical components and features of *Cyperus rotundus*, a popular plant in Iraq.

Methods

Plant materials

Cyperus rotundus roots will be collected, characterized and the identification of the plant sample will be confirmed. The plant materials will be cut into small pieces, dried in the shed, and then powdered using a plant grinding machine.

Extraction and Fractionation of plant material

1- Cold method: three hundred grams of shade-dried pulverized root of plant were defatted by maceration with petroleum ether for 24 hours then allowed to dry at room temperature. The defatted plant materials were extracted by maceration with 90% methanol (3 × 1 L) in a 2 L conical flask. Then it will be carefully closed and kept for 72 hours. The extract was filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator to get a dry extract. About three-quarters of dry methanolic extract was suspended in 300ml water and partitioned successively with chloroform, ethyl acetate, and n-butanol (3x300ml) for each fraction. The three fractions were evaporated to dryness. Each fraction was weighted and assigned for further analysis [14,15].

2-Hot method: three hundred grams of shade-dried pulverized root of plant were defatted by maceration with petroleum ether for 24 hours then allowed to dry at room temperature. The defatted plant materials were extracted by reflux with 90% methanol (3 × 300ml) for 3 hours. The extract was filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator to get a dry extract. About three-quarters of dry methanolic extract was suspended in 300ml water and partitioned successively with chloroform, ethyl acetate, and n-butanol (3x300ml) for each fraction. The three fractions were evaporated to dryness. Each fraction was weighted and assigned for further analysis [14,15].

High-performance liquid chromatography (HPLC) examination of ethyl acetate fraction of cold and hot method and n-butanol fraction of cold and hot method.

HPLC was carried out for examination of some chemical compounds present in ethyl acetate fraction of cold and hot method and n-butanol fraction of

cold and hot method, the retention times of analyzed samples was compared to retention times of standard materials under the same conditions. The following standards were used in examination: Quercetin, Kaempferol, Myricetin, Gallic acid, luteolin, caffiec acid, apigenin, genistein, chlorogenic acid, syringic acid, silybin, vanillic acid and catecheine.

HPLC conditions for analyzing the fractions

-System components shown in the table below.

Table (2-1): HPLC Instrument Components

Component	Model or version
Binary high pressure gradient pump	P6.1L
Diode array detector	DAD 2.1L
Sample loop (20 µl) and injector	D1357
Analyses and system control software	Claritychrom, V 7.4.2.107

-The separation was achieved on C18 (250X4.6) 5-µm particles size from water corporation, USA

-Gradient of mobile A (1% acetic acid in HPLC grad water) and mobile B (acetonitrile). The gradient as the below table

Table (2-2): HPLC Mobile Phase Gradient

Time	Mobile A concentration %	Mobile B concentration %	Flow rate ml/min
0	90	10	1
28	60	40	1
39	40	60	1
60	10	90	1

- The column was thermostatically controlled at 28^0C.

- The injection volume was kept at 20 µl.

-HPLC chromatograms were detected using a photo diode array UV detector at three different wavelengths (272, 280 and 310 nm).

-The detection of each compound was performed by matching retention time and absorbance spectrum of the standards [16].

Preparation of standards and samples for HPLC

Standard solutions for HPLC: Quercetin, Kaempferol, Myricetin, Gallic acid, luteolin, caffeic acid, apigenin, genistein, chlorogenic acid, syringic acid, silybin, vanillic acid and catecheine were prepared by dissolving a few milligrams in 1 ml of HPLC grade methanol. Dried samples were suspended in 1 mL HPLC grade methanol. The standard and sample solutions were filtered through 2.5 µm disposable filter. Injection volume was 20 µL of the sample injected into HPLC [17].

UV-diodarray

Few milligrams of each isolated compound were dissolved individually in 1 mL absolute methanol, and the UV absorbance was scanned from 200-400 nm, and then compared with UV absorbance of authentic standards.

3. Results

Extraction of plant material

Extraction is the most important step in the evaluation of the tested plant, selecting the proper method for extraction is necessary and depend on the type of the bioactive compounds that desired to be extracted from the plant for further separation and characterization.

Fractionation of extracts

The percentage yield (%w/w) of each fraction of plant extract was determined for cold method and for hot method and the results are shown in the table (3.1) and table (3.2) respectively.

Table (3.1): Percentage Yield (%w/w) of Fractions of 14.6 Grams Crude Extracts with Different Solvents (cold method).

Fraction	Weight of extract	Percentage of extract
Chloroform	1.05g	7.19 %
ethyl acetate	4.75g	32.53 %
n-butanol	2.38g	16.30 %

Table (3.2): Percentage Yield (%w/w) of Fractions of 10.47 Grams Crude Extracts with Different Solvents (hot method).

Fraction	Weight of extract	Percentage of extract
Chloroform	1.23g	11.75 %
ethyl acetate	3.75g	35.82 %
n-butanol	2.28g	21.77 %

High-performance liquid chromatography (HPLC) examination of ethyl acetate fraction of cold and hot method.

HPLC chromatogram of ethyl acetate fraction of cold method by matching with standards show the presence chlorogenic acid, catechin, quercetin and genistein and as in figure (3.1).

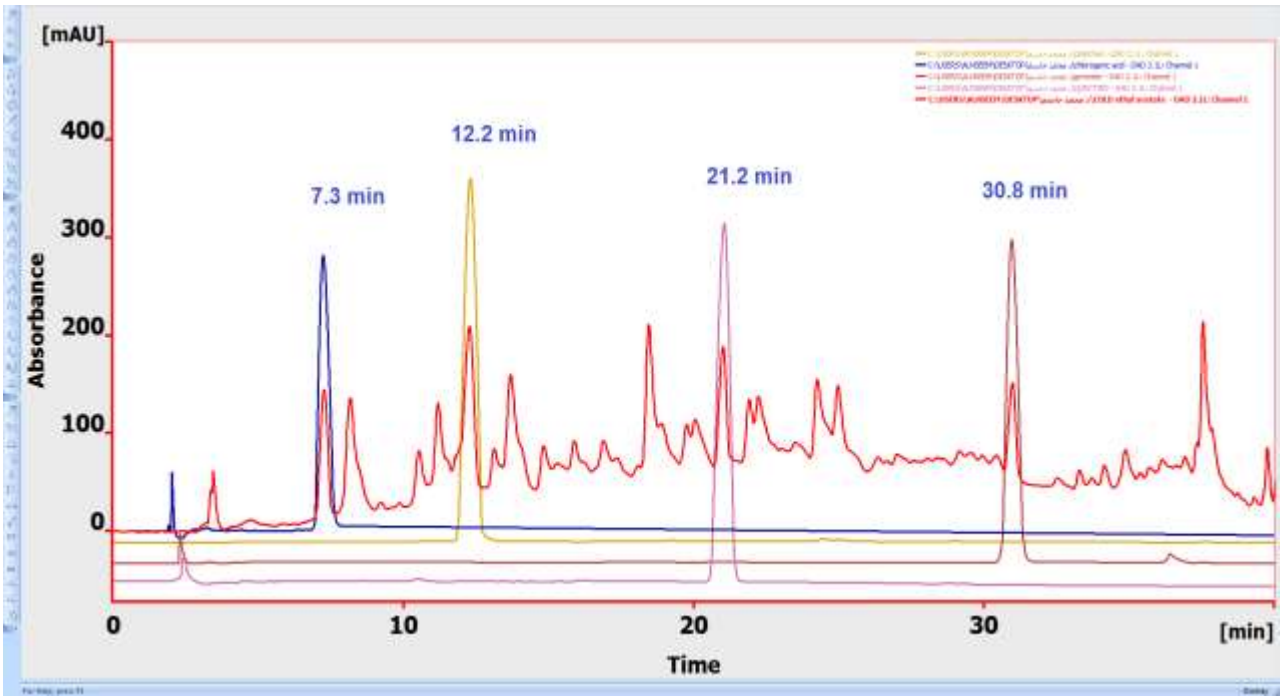

Figure (3.1): HPLC chromatogram of ethyl acetate fraction of cold method.

Table (3.3): Retention Times in Minutes of Flavonoids & Phenols in ethyl acetate fraction of cold method

Compound	Retention time of standard	Retention time in ethyl acetate fraction of cold method
Chlorogenic acid	**7.3**	**7.3**
Catechin	**12.2**	**12.2**
Quercetin	**21.2**	**21.2**
Genistein	**30.8**	**30.9**

HPLC chromatogram of ethyl acetate fraction of hot method by matching with standards show the presence chlorogenic acid, catechin, quercetin and genistein and as in figure (3.2).

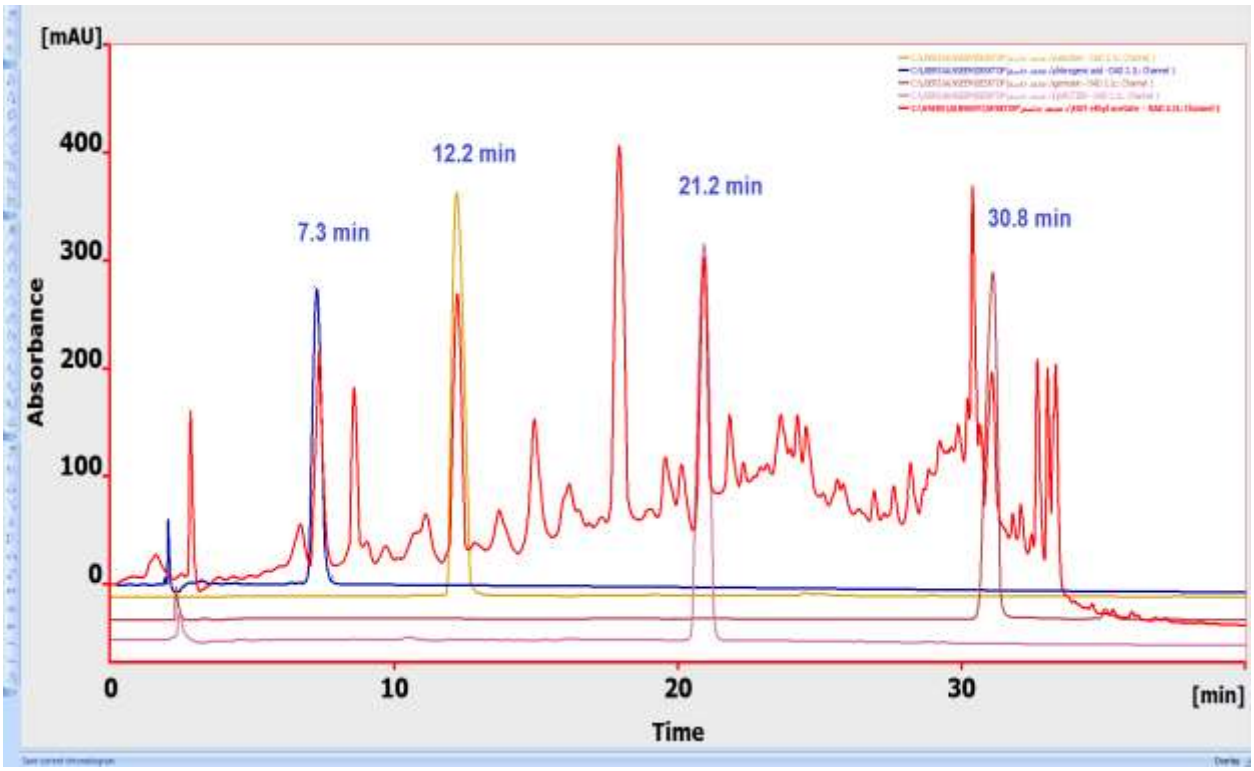

Figure (3.2): HPLC chromatogram of ethyl acetate fraction of hot method.

Table (3.4): Retention Times in Minutes of Flavonoids & Phenols in ethyl acetate fraction of hot method

Compound	Retention time of standard	Retention time in ethyl acetate fraction of hot method
Chlorogenic acid	7.3	7.4
Catechin	12.2	12.2
Quercetin	21.2	21.2
Genistein	30.8	30.8

High-performance liquid chromatography (HPLC) examination n-butanol fraction of cold and hot method.

HPLC chromatogram of n-butanol fraction of cold method by matching with standards show the presence chlorogenic acid, catechin and quercetin as in figure (3.3).

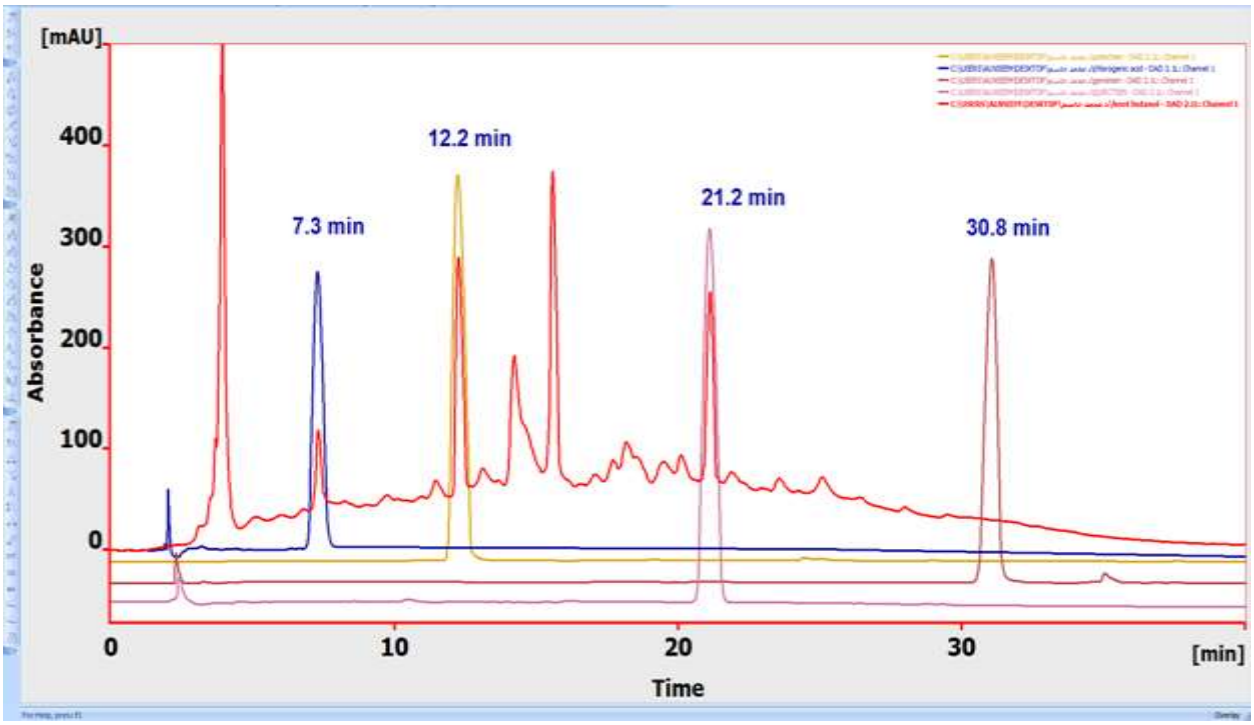

Figure (3.3): HPLC chromatogram of n-butanol fraction of cold method.

Table (3.5): Retention Times in Minutes of Flavonoids & Phenols in n-butanol fraction of cold method

Compound	Retention time of standard	Retention time in n-butanol fraction of cold method
Chlorogenic acid	7.3	7.4
Catechin	12.2	12.3
Quercetin	21.2	21.2

HPLC chromatogram of n-butanol fraction of hot method by matching with standards show the presence chlorogenic acid, catechein and quercetin as in figure (3.4).

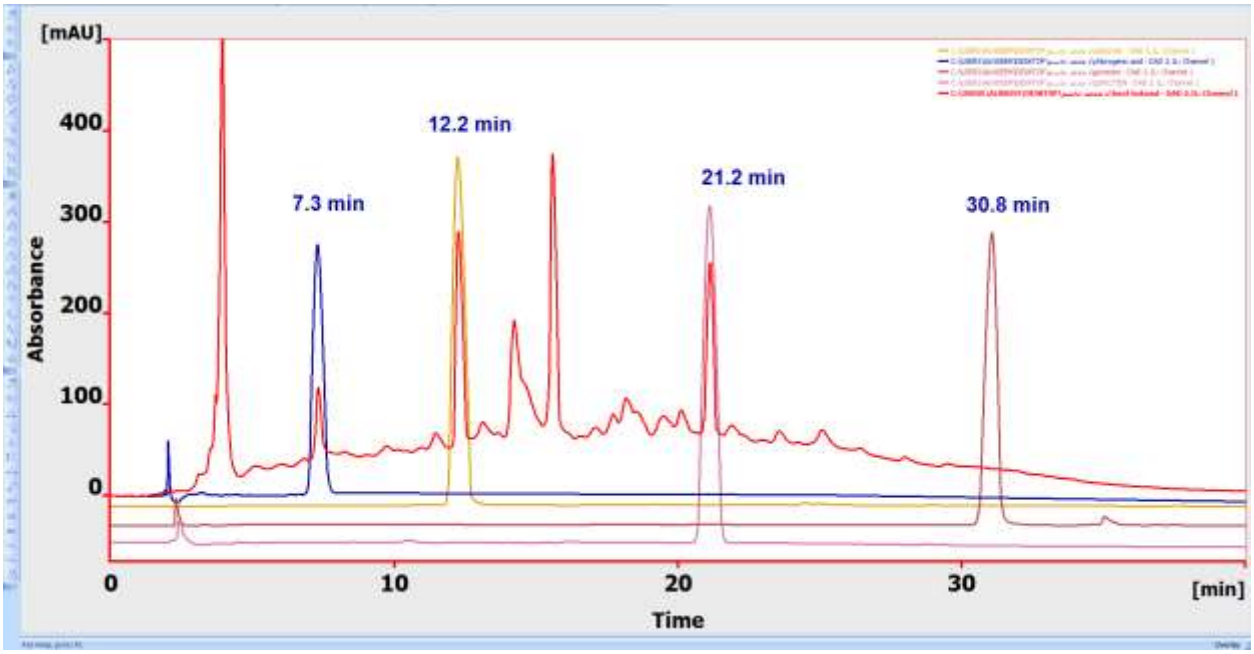

Figure (3.4): HPLC chromatogram of n-butanol fraction of hot method.

Table (3.6): Retention Times in Minutes of Flavonoids & Phenols in n-butanol fraction of hot method.

Compound	Retention time of standard	Retention time in n-butanol fraction of hot method
Chlorogenic acid	7.3	7.4
Catechin	12.2	12.2
Quercetin	21.2	21.3

UV-diodarray.

-UV spectrum of chlorogenic acid presented in cold ethyl acetate fraction, hot ethyl acetate fraction, cold n-butanol fraction and hot n-butanol fraction with chlorogenic acid standard shown in figure (3.5).

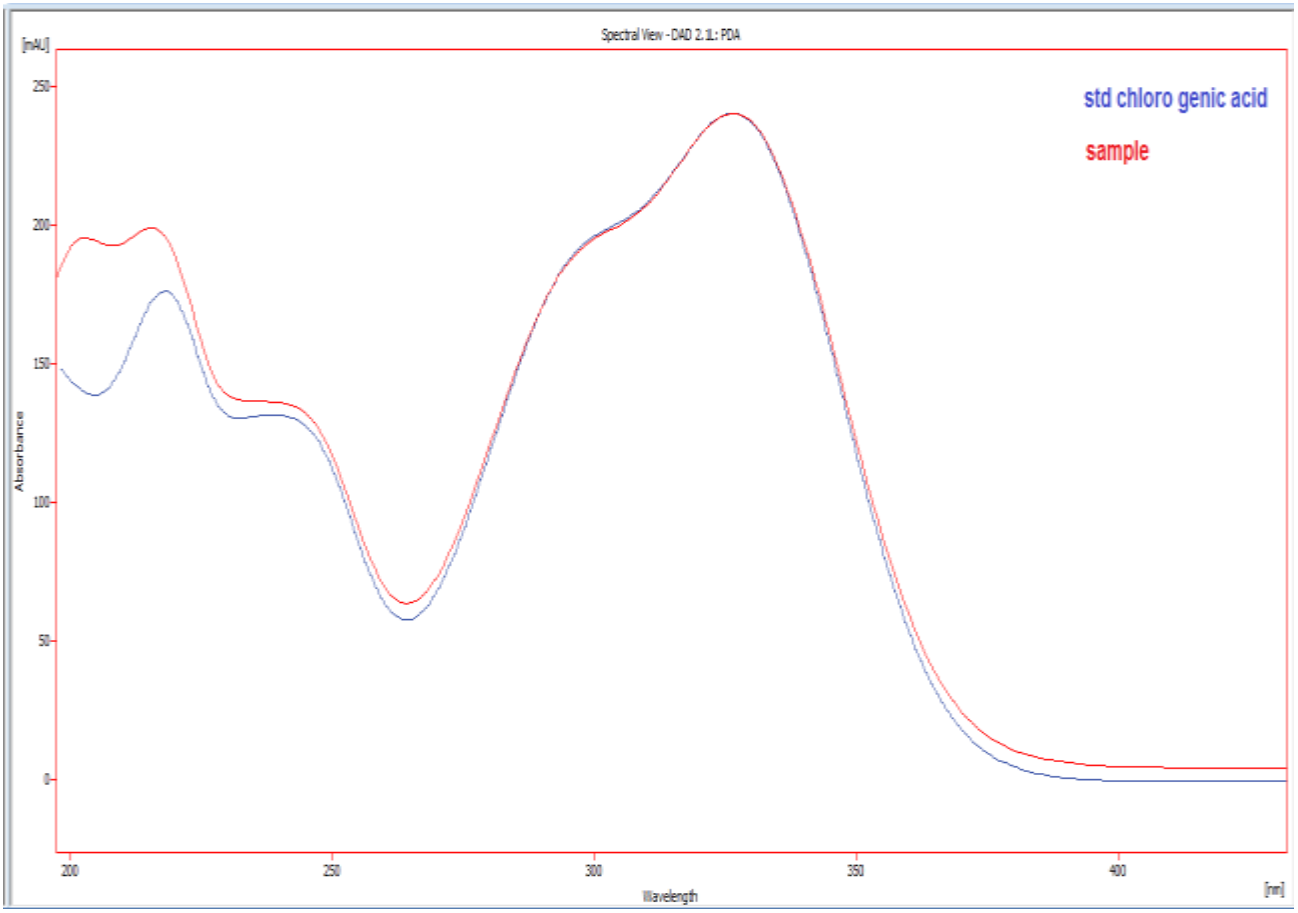

Figure (3.5): UV spectrum of the isolated chlorogenic acid & chlorogenic acid standard.

-UV spectrum of catechin presented in cold ethyl acetate fraction, hot ethyl acetate fraction, cold n-butanol fraction and hot n-butanol fraction with catechin standard shown in figure (3.6).

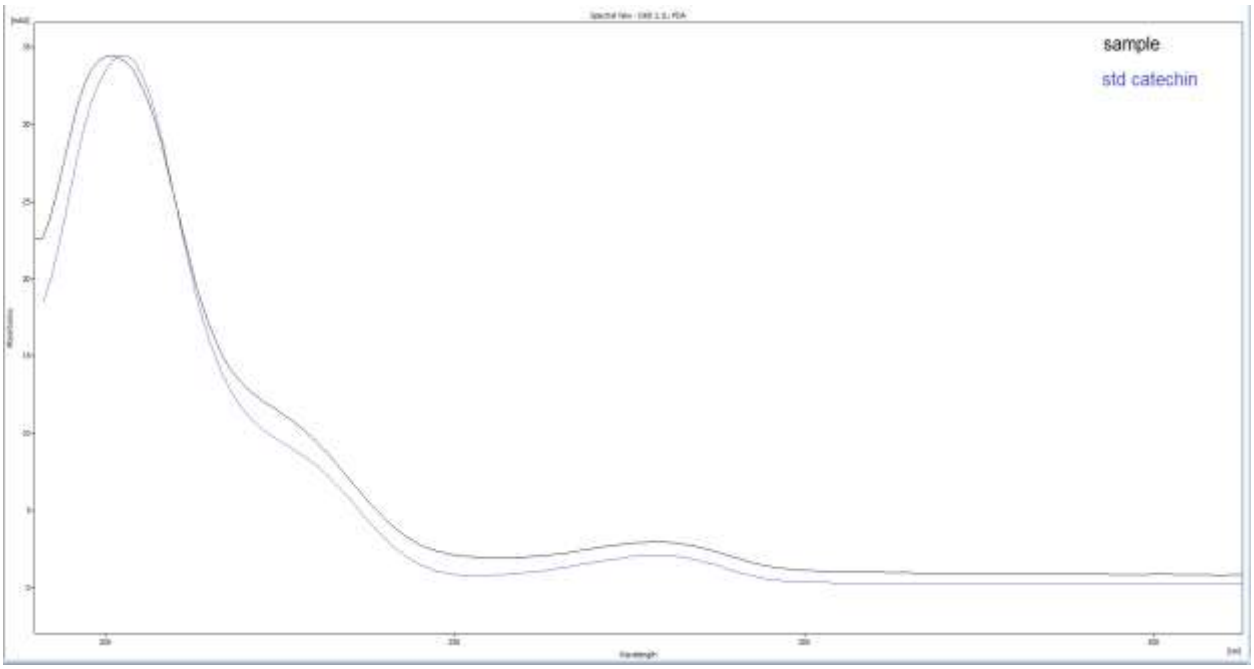

Figure (3.6): UV spectrum of the isolated catechin & catechin standard.

-UV spectrum of quercetin presented in cold ethyl acetate fraction, hot ethyl acetate fraction, cold n-butanol fraction and hot n-butanol fraction with quercetin standard shown in figure (3.7).

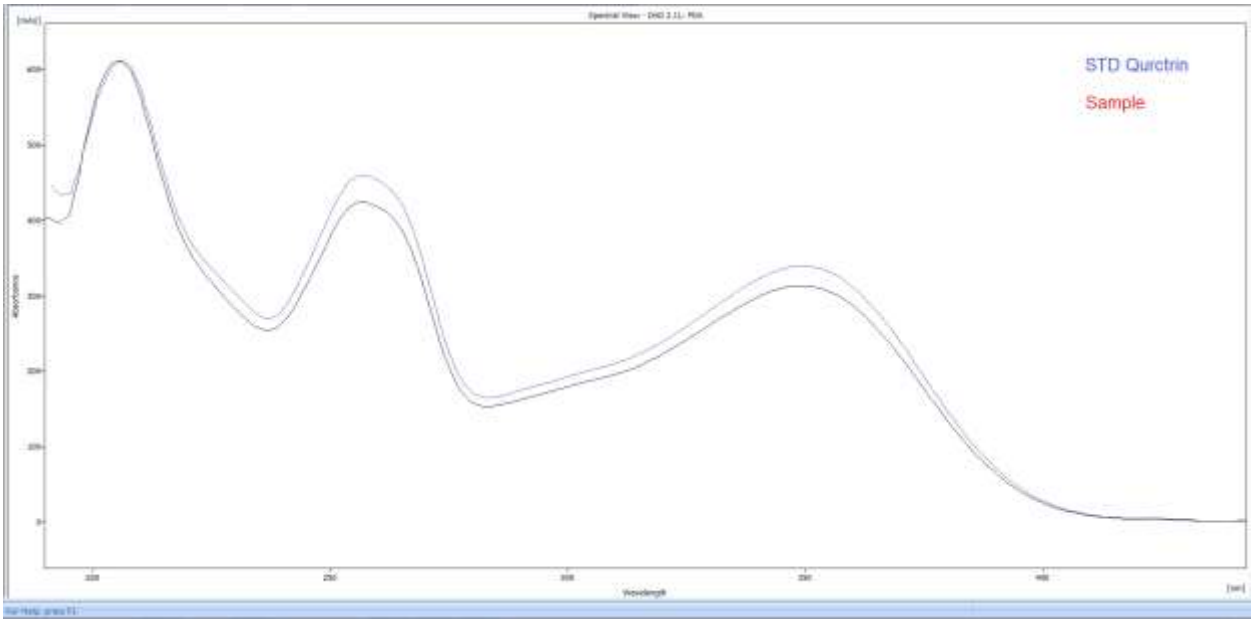

Figure (3.7): UV spectrum of the isolated quercetin & quercetin standard.

-UV spectrum of genistein presented in cold ethyl acetate fraction and hot ethyl acetate with genistein standard shown in figure (3.8).

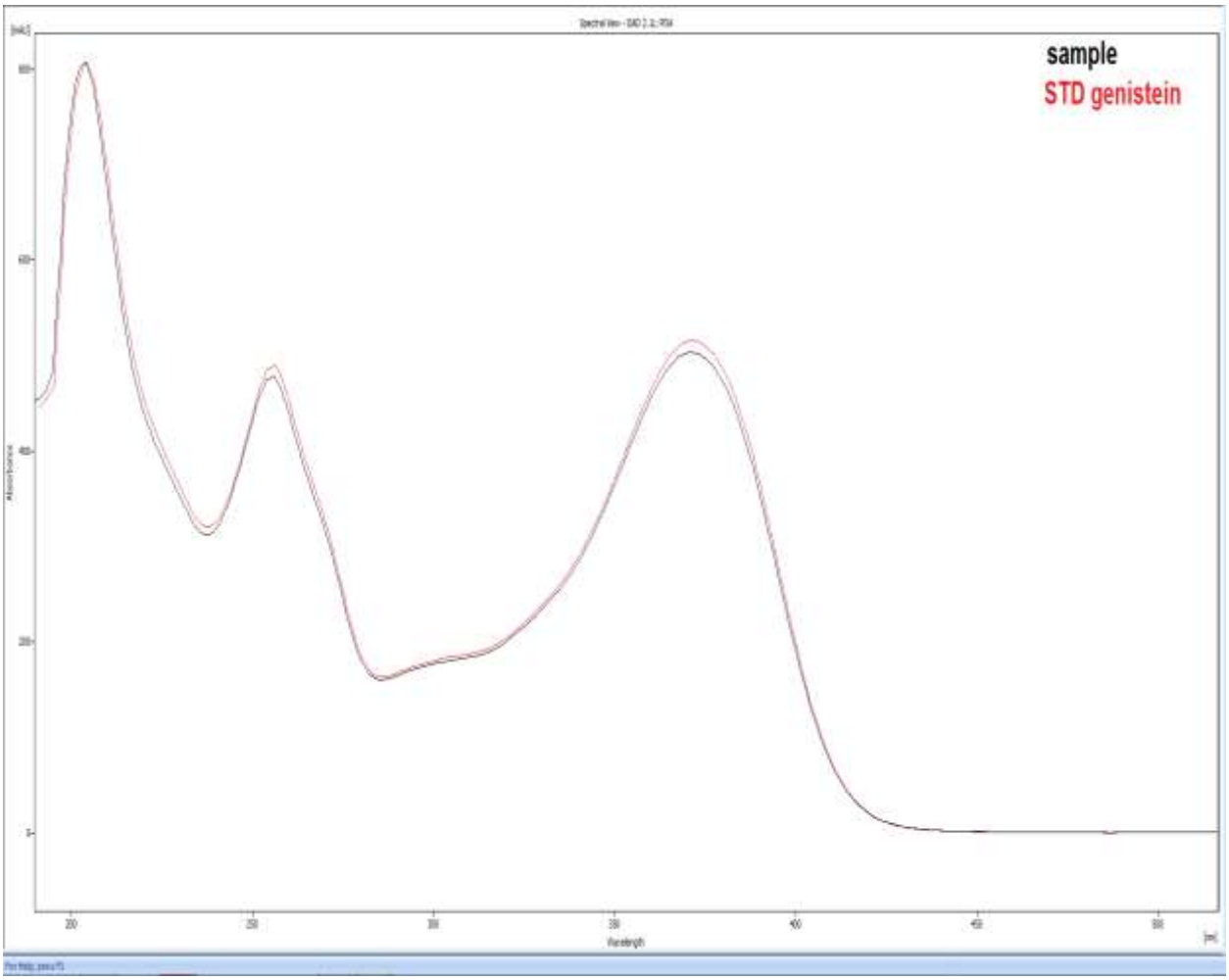

Figure (3.8): UV spectrum of the isolated genistein & genistein standard

Quantitative HPLC analysis of the isolated compounds.

The concentration of each isolated compound was calculated by serial concentrations of external standard materials to build calibration curve between concentration and its equivalent peak area. The following figures show the calibration curve of standards of each isolated compound.

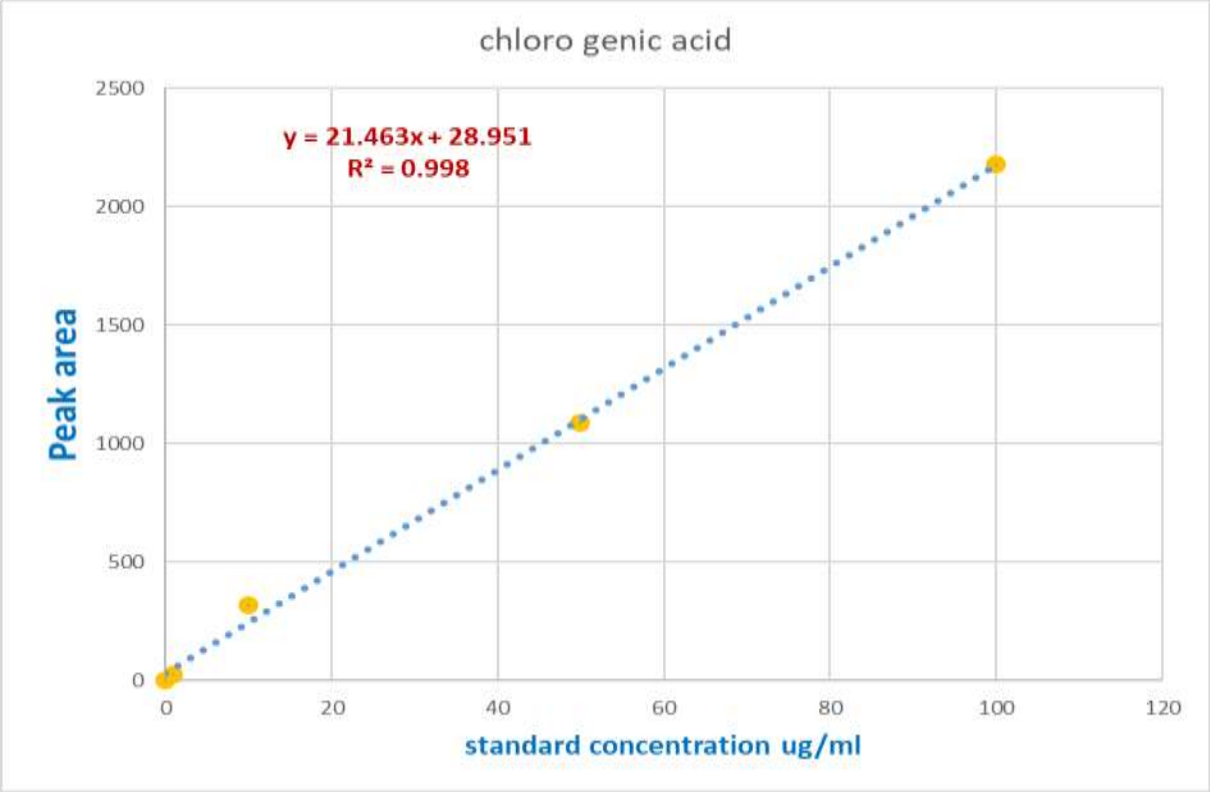

Figure (3.9): calibration curve of chlorogenic acid standard.

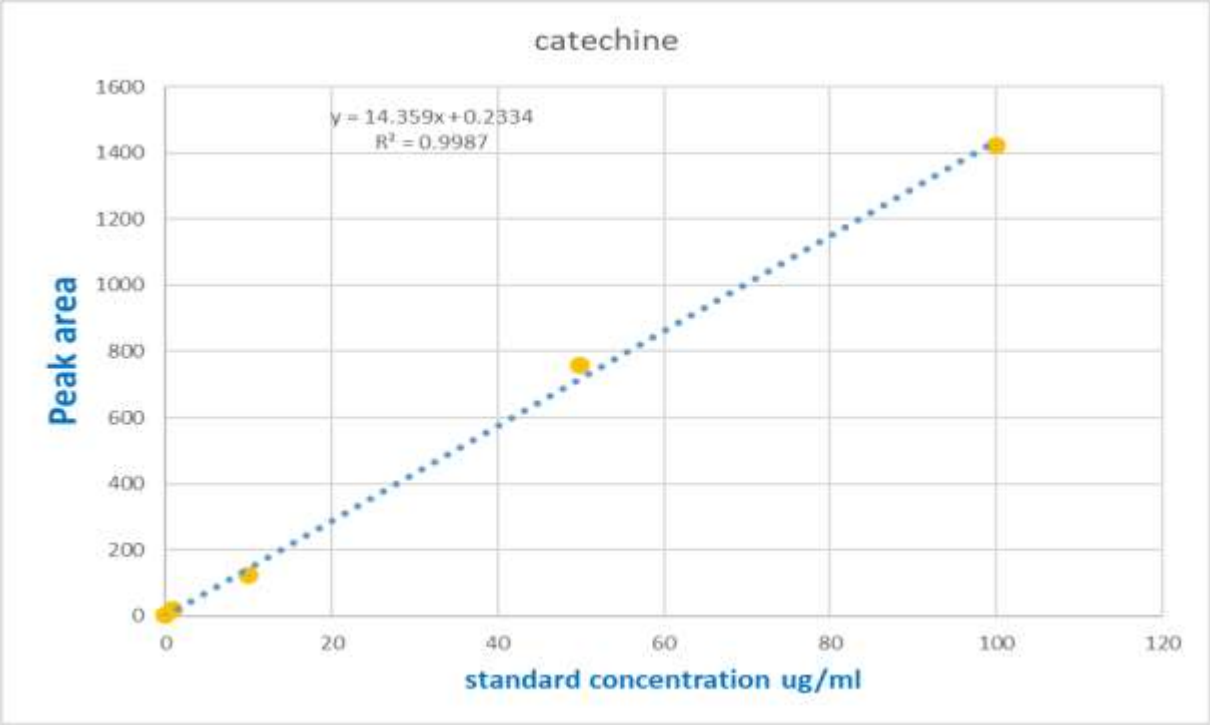

Figure (3.10): calibration curve of catecheine standard

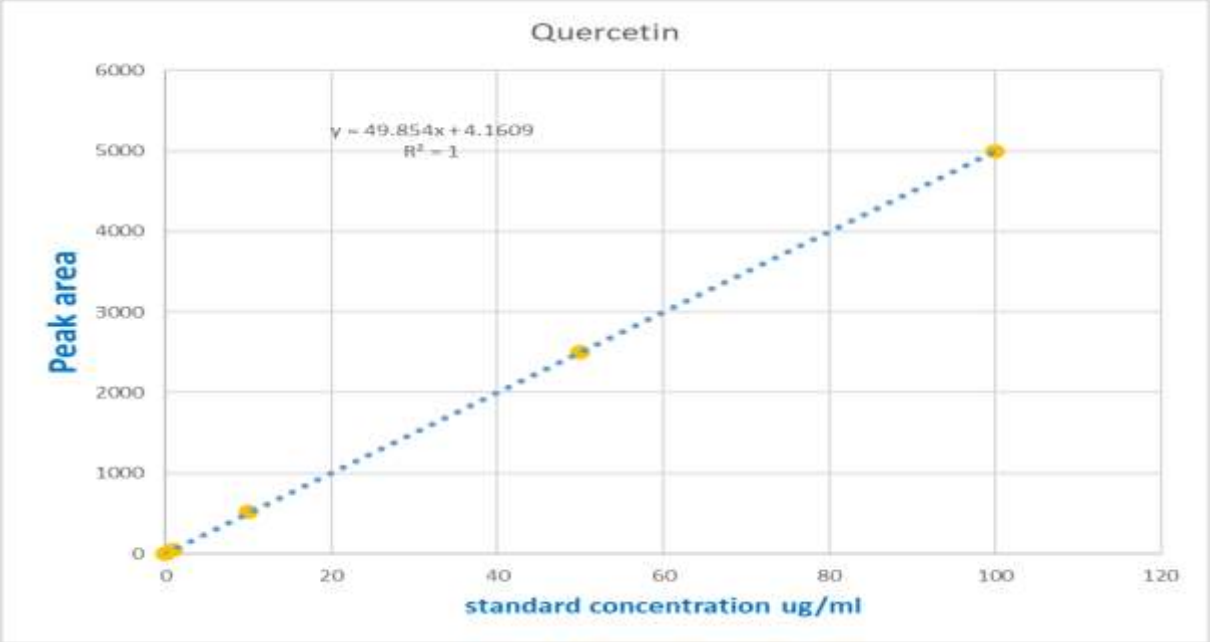

Figure (3.11): Calibration curve of quercetin standard.

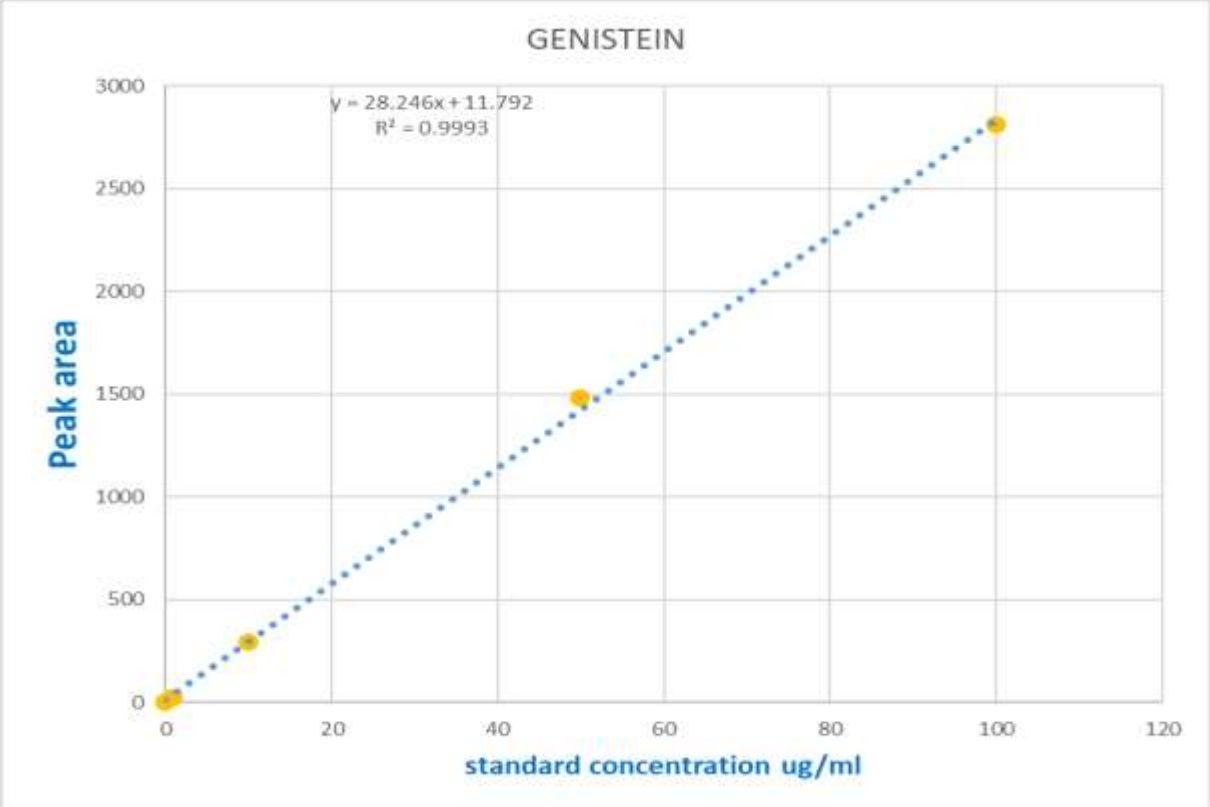

Figure (3.12): calibration curve of genistein standard.

Table (3.7): The Concentration and Weight of Isolated Chlorogenic acid in Each fraction

Fraction	Concentration of chlorogenic acid (µg/ml)	Weight of chlorogenic acid (µg/gram of fraction)
Cold ethyl acetate	7.51	163.37
Hot ethyl acetate	25.62	211.76
Cold n-butanol	6.92	124.75
Hot n-butanol	1.37	27.52

Table (3.8): The Concentration and Weight of Isolated Catechin in Each fraction

Fraction	Concentration of catechin (µg/ml)	Weight of catechin (µg/gram of fraction)
Cold ethyl acetate	9.68	210.47
Hot ethyl acetate	61.61	509.22
Cold n-butanol	15.96	287.65
Hot n-butanol	18.49	369.99

Table (3.9): The Concentration and Weight of Isolated Quercetin in Each fraction

Fraction	Concentration of Quercetin (µg/ml)	Weight of Quercetin (µg/gram of fraction)
Cold ethyl acetate	1.61	35.01
Hot ethyl acetate	6.35	117.49
Cold n-butanol	2.96	53.47
Hot n-butanol	3.80	76.06

Table (3.10): The Concentration and Weight of Isolated Genistein in Each fraction

Fraction	Concentration of Genistein (µg/ml)	Weight of Genistein (µg/gram of fraction)
Cold ethyl acetate	1.99	43.42
Hot ethyl acetate	6.81	56.33
Cold n-butanol	0	0
Hot n-butanol	0	0

4. Discussion

Extraction Fractionation of plant material

The results of this study indicate that the reflux extraction method is more efficient than maceration, as it yielded a greater diversity of compounds in higher quantities. This enhanced efficiency can be attributed to continuous heating and solvent recycling during reflux, which improve mass transfer and facilitate the extraction of phytochemicals. Furthermore, to obtain a comprehensive phytochemical profile of the whole plant, fractionation of the crude extract is recommended to enable the separation of major chemical classes. This separation is primarily based on differences in physicochemical properties, particularly polarity and solubility, which influence the distribution of compounds among various solvents [18].

High-performance liquid chromatography (HPLC) examination of ethyl acetate fraction of cold and hot method and n-butanol fraction of cold and hot method.

The quantitative HPLC analysis revealed notable variations in the concentration and distribution of the isolated compounds among different fractions. In general, the hot ethyl acetate fraction exhibited the highest extraction efficiency for most compounds, including chlorogenic acid, catechin, quercetin, and genistein, indicating that elevated temperature enhances the solubility and extraction of these phytochemicals. This observation is consistent with previous studies, which reported that reflux extraction improves mass transfer and increases extraction yield due to continuous heating and solvent recycling [19].

Catechin showed the highest concentration among the studied compounds, particularly in the hot ethyl acetate fraction, suggesting its higher affinity for

moderately polar solvents under heated conditions. In contrast, the n-butanol fractions demonstrated comparatively lower yields for most compounds, with the exception of catechin. Notably, genistein was absent in both cold and hot n-butanol fractions, indicating its preferential solubility in less polar solvents such as ethyl acetate. Furthermore, the higher concentrations observed in hot extraction compared to cold extraction confirm that temperature plays a significant role in improving extraction efficiency. Reflux extraction has been reported to be more efficient than maceration, providing higher yields in shorter time due to enhanced solvent–matrix interaction [18]. These findings support the principle that the distribution of phytochemicals depends largely on their polarity, solubility, and the extraction conditions applied.

5. Conclusion

In conclusion, this study demonstrated that Reflux extraction proved more efficient than maceration, yielding higher amounts and a greater diversity of phytochemicals. Hot ethyl acetate fractions contained the highest concentrations of chlorogenic acid, catechin, quercetin, and genistein, while fractionation allowed effective separation of compounds based on polarity and solubility. Catechin was the most abundant compound, and genistein was preferentially extracted in ethyl acetate. These findings demonstrate that combining reflux extraction with fractionation provides a reliable method for isolating bioactive compounds from plant materials. Future studies could explore the bioactivity of these isolated compounds and optimize extraction conditions for potential pharmaceutical and nutraceutical applications.

6. Acknowledgment.

The Authors would like to thank Assistant Lecturer Mohamed Jasim for his assistance in performing the experiments and in the interpretation of the results.

7. Recommendations

Based on the results of this study, the following recommendations are proposed:

- Optimization of Extraction Methods: Reflux extraction should be prioritized for isolating bioactive compounds, with careful adjustment of temperature, solvent type, and extraction time to maximize yield.
- Enhanced Fractionation Techniques: Further purification using polarity-based fractionation combined with methods such as column chromatography could improve the isolation of specific chemical classes.
- Bioactivity Assessment: Detailed studies on the pharmacological or nutraceutical activities of catechin, genistein, and other isolated compounds are recommended to evaluate their potential applications.
- Analytical Method Validation: HPLC methods should be fully validated for reproducibility, sensitivity, and accuracy to ensure consistent quantification of compounds across studies.
- Industrial Application and Sustainability: Scaling up the hot ethyl acetate reflux method may be considered for industrial use, and the use of greener solvents or energy-efficient extraction techniques should be explored to minimize environmental impact.

8. Conflict of Interest

The authors declare no conflict of interest.

9. Ethical Approval

No ethical approval was required for this study, as it did not involve human participants, animal subjects, or any sensitive biological materials

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