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A comparative phytochemical investigation and antioxidant activity of *Quisqualis indica* L.

Hadeel M. Habeeeb[1*]

[1]Department of Biology, College Science for Women, University Baghdad, Iraq.

*Corresponding Author: Hadeel.makki@csw.uobaghdad.edu.iq

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Abstract

Quisqualis indica L. is an herbaceous plant with many active ingredients that function as antioxidants and anticancer agents. Traditional medicine has long employed *Quisqualis indica* L. as an anti-inflammatory, anti-diarrheal, and anti-diabetic plant. The study aims to compare the antioxidant activity of leaves and flowers. By employing a particular reagent, a qualitative examination revealed the presence of alkaloids, terpenoids, tannins, flavonoids, and saponins. Flavonoids and phenolic compounds were isolated from leaves and flowers such as hydroxybenzoic acid, gallic acid, catechin, Rutin, kaempferol, and sinapic acid. According to the HPLC analysis, flowers contain a higher percentage of flavonoids and phenolic compounds than leaves. For the ascorbic acid, the antioxidant activity shows that the plant has significant inhibition activity against free radicals in both the leaves and the flowers, and the flowers are more active than the leaves. Also, the antioxidant activity increases with increasing concentration from 25 to 400 mg mL^{-1}; the IC_{50} values for the flowers were 100 µg mL^{-1} and 200 µg mL^{-1} for the leaves.

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Introduction

In ancient times, herbal medicines were the primary source of treatment for many diseases (Ssenku *et al*., 2022). Even today, in many places, they are still used for healthcare. Therefore, herbal medicine can be considered a traditional system of medicine that is used in medical practices since antiquity (Yuan *et al*., 2016). formerly known as Holarrhena floribunda, displays a wide range of pharmacological activities such as anti-inflammatory, antipyretic, immunomodulatory, anti-staphylococcal, anthelmintic, and antiseptic activities. Plant components have distinct energy primary that cause the activities, Quisqualis indica is a combretaceae perennial vine (Riaz *et al.*, 2023).

Quisqualis indica plants are distinguished by oval leaves with parallel veins (Sahu *et al*., 2012). The blooms range in color from pale pink to deep pink or crimson, according to Kulshreshtha *et al*. (2018). The plant's leaves and flowers include alkaloids, flavonoids, saponins, and tannins (Barik *et al*., 2020). Leaves have more terpenoids than flowers, which have more phenols. Thu *et al*. (2022) found that oils are in the flowers and triterpenes, sterols, and aromatic chemicals in the leaves. (Mohammed and Habeeb, 2022).

Abd El Rahman *et al.* (2008); Revathi and Radh (2016); and Nemade and Aher (2023) have proved that plants improve health, with many benefits like Acetylcholinesterase inhibition, cholesterol reduction, fever reduction, worm prevention, antioxidants, inflammation reduction, infection prevention, and cancer prevention. The presence of stress in cells can be triggered by many factors like smoking, pollution, pesticides, or internal reactive oxygen species (ROS) such as

superoxide anion, singlet oxygen and hydrogen peroxide. Stress can lead to damage in acids, proteins and enzymes altering their integrity within living organisms as studied by González Palma *et al.* (2016). The body's antioxidant defense mechanisms work to minimize and stabilize these factors. Current research is focusing on the use of tannins and flavonoids, in supplements to combat free radical damage effectively and alleviate ROS effects according to Saeed *et al*. (2012).

The main challenges are to extract and isolate specific active groups from medicinal plants without removing other active chemicals (Ghenabzia *et al*., 2023), and then assess the biochemical behavior and bioavailability, including toxicity (Sasidharan *et al*., 2011) and in vivo. This paper aims to extract and investigate active compounds, then isolate flavonoids and phenolic compounds, conduct qualitative flavanol and phenolic compounds analysis, and examine their antioxidant activity.

Materials and Methods

Plant collection and extraction

The plant leaves and flowers were collected in November from Baghdad nurseries, and then washed. The leaves were dried, but the flowers were used freshly, the dry leaf was powdered and the flowers were extracted with a (1:10) ratio (plant: solvent) in a soxhlet with petroleum ether for 4 hours at 60–80 °C separately, then the plant material was extracted with methanol at 60–80 °C for 6–8 hours, then filtered (Ali *et al.*, 2022).

Qualitative analysis of an active compound

The active components investigated were detected according to Ali *et al*. (2022). The 1g of crud extract (leaves and flowers) was dissolved in 10 ml of methanol. 1 ml of this extract was directed to the detection of active compound presence, alkaloids, terpenoids, tannins, flavonoids, and saponins using specific two different reagents for each compound, as follows:

1. Alkaloids: Mayer and Wagner reagents are used for the detection process.

2. Terpenoids: Two reagents, chloroform, and H_2SO_4, as well as an aldehyde reagent, were utilized to detect the terpenoids.

3. Tannins: by adding $FeCl_3$ reagent and lead acetate reagent.

4. Magnesium crystals with 1% HCl and saponins with H_2SO_4 reagent.

5. Saponins reagents for foam, $HgCl_2$, and other substances.

Phenolic compound and Flavonoids purification

The leaves and flowers methanolic extract were concentrated, then sequentially distilled water and ethyl acetate was added for each flower and leaf, and then shaken. After that, the organic layer was collected and dried. The presence of phenolic compounds and flavonoids was detected (Wagner and Bladt, 2009).

Quantitative analysis of phenolic compound flavonoids

The purified phenolic compound flavonoids were quantitatively determined by high-performance liquid chromatography (HPLC), which was done using the Sykamn Germany HPLC system, with C18-ODS column dimensions (250 mm * 4.6 mm, 5µm). 100 µm of samples were injected, and the sample was developed using acetonitrile and 0.01% trifluoroacetic acid at 1 mL min^{-1} in the mobile phase at a 1 mL min^{-1} flow rate. The developing program followed the sequence concentration (10% from 0–5 min; 25% from 5-7 min; 40% from 7–13 min) of acetonitrile, respectively, returning to the first concentration condition. Then a UV-visible detector at 278 nm was used

to detect the phenolic compounds, flavonoid concentrations calculated by the following equation (Ngamsuk *et al*., 2019).

The concentration of flavonoids = [area of specimen/area of standard ***** concentration of standard ***** dilution factor].

Antioxidant activity

Leaf and flower phenolic compound flavonoids antioxidant abilities were examined through scavenging 1,1'-diphenyl-2-picrylhydrazyl (DPPH) according to Marwah *et al*. (2007) 2 mL of stock solution 100 mM DPPH in methanol was mixed with 2 ml of leaf or flower flavonoids to make the reaction medium. Ascorbic acid was used as a standard. After mixing, the mixture was incubated at 35 °C in the dark for 20 min. at 512 nm. The absorbance was then recorded, and the treatment was triple-repeated. The IC_{50} (concentration of sample required to scavenge 50% of DPPH radicals) The DPPH scavenging ability is calculated based on the following equation:

% DPPH radical scavenging activity = [1-(A sample/A control) * 100]

A control = absorbencies of control

A sample = absorbencies of sample

Statistical Analysis

The SAS program was previously used to determine different factors in the study of parameters. LSD test and ANOVA were used to significantly compare between means in this study.

Results and Desiccation

Qualitative analysis of an active compound

The active compound test of *Quisqualis indica* leaf and flower crud extract shows the presence of alkaloids, terpenoids, tannins, flavonoids, and saponins in Table 1, this result goes with Barik *et al*. (2020) of presence of alkaloids, tannins, flavonoids, and saponins in leaf and flower extracts, while the terpenoids are found in leaves and absent in flowers, but Shah *et al*. (2019) shows the absence of alkaloids and tannins in *Quisqualis* leaves and flowers. The contrast in the presence or absence of some active compounds in the same genus may be related to the environment and where the plant is cultivated (Pant *et al*., 2021).

Table 1. Phytochemical screening of active ingredients in *Quisqualis indica* leaf and flower crud extract

Active compounds	leave		flower	
	Reagent A	Reagent B	Reagent A	Reagent B
Alkaloids	+ white precipitate	+ brown precipitate	+ white precipitate	+ brown precipitate
Terpenoids	+ Brown-redush	+ brown precipitate	+ Brown-redush	+ brown precipitate
Tannins	+ green- blue	+ yellow precipitate	+ green- blue	+ yellow precipitate
Flavonoids	+ red- orange	+ red	+ red- orange	+ red
Saponins	+ foam	+white precipitate	+ foam	+white precipitate

+ Presence of active compound

Quantitative analysis of phenolic compound and flavonoids

The results of phenolic compound and flavonoids quantitative analysis after flavonoids and phenolic compound isolation from crude extract, which is shown in (Table 2), indicate the presence of sex compounds in the purified extract, which show that the extract of leaves and flowers contains hydroxybenzoic acid, gallic acid, catechine, rutin, kaempferol, and

sinapic acid figure (1 and 2); Tables 3, 4 and 5, which refer to the retention time of each stander, leaves, and flowers. Table 3 shows that hydroxybenzoic acid, the same molecule, has a retention period of 2.51 for standard hydroxybenzoic acid and 2.58, and 2.55 for leaves and flowers, respectively. Stander gallic acid was 3.02, in addition, it was 3.05 and 3.01 for leaves and flowers, it was 3.05 and 3.01; in the stander category retention time, the leaves were 5.95, the flowers were 5.96, and the catechine stander was 5.90. The retention times for rutin, kaempferol, and sinapic acid are as follows: 6.52 for rutin, 7.85 and 7.81 for leaves and flowers, 9.35 for kaempferol, 9.32 and 9.35 for leaves and flowers, and 11.25 for sinapic acid stander and 11.26 and 11.27 for leaves and flowers, respectively. The approximate retention times of the flowers, leaves, and standers are identical, suggesting that the isolated compounds are similar to the standers. The result shows that all phenolic compounds and flavonoids were higher in the flowers than the leaves, while gallic acid has the highest compound concentration and sinapic acid has the lowest amount among the active compounds in each flower and leaf. This result differs from Rastogi *et al.* (2019), which elucidate that the quantity of leaf flavonoids is higher than flower flavonoids and agrees with them in the presence of some active compounds (gallic acid and rutin), also the result is compatible with Bairagi *et al.* (2012), and Chaudhary *et al*. (2021) and in the presentation of some types of flavonoids in Iraqi plants.

Table 2. Type and Quantity of phenolic compound and flavonoids isolated from *Quisqualis indica* by HPLC chromatography

NO	Name	Leaves (ppm)	Flowers (ppm)
1	Hydroxybenzoic acid	12.8	15.1
2	gallic acid	33.9	42.6
3	catechine	22.5	29.8
4	Rutin	21.6	30.6
5	kaempferol	24.9	29.7
6	Sinapic acid	9.6	12.9

Table 3. The retention time and peak area of HPLC Chromatography of standers phenolic compound and flavonoids

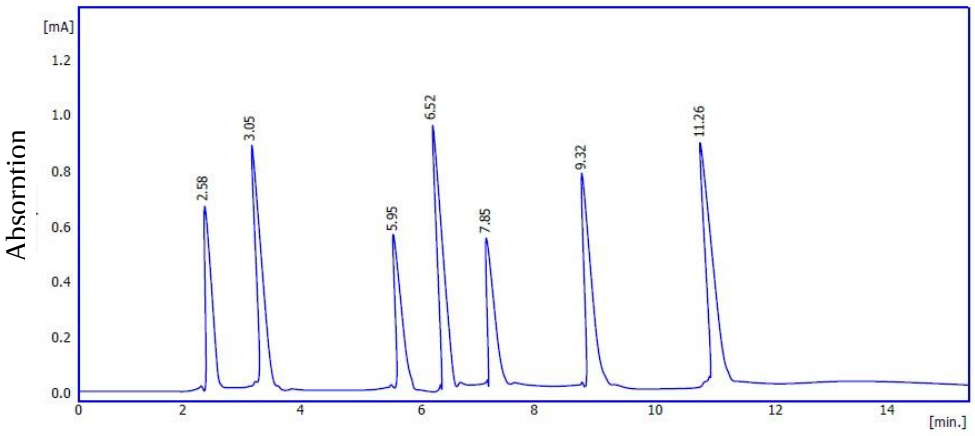
No	Compounds	Retention time	Peak area
1	hydroxybenzoic acid	2.51	1421.05
2	gallic acid	3.02	1521.44
3	catechine	5.90	1244.79
4	Rutin	6.52	952.11
5	kaempferol	9.35	1877.48
6	Sinapic acid	11.25	952.14

Table 4. The retention time and peak area of HPLC Chromatography of *Quisqualis indica* Leaves

No	Compounds	Retention time	Peak area
1	hydroxybenzoic acid	2.58	2564.25
2	gallic acid	3.05	3652.14
3	catechine	5.95	1569.89
4	Rutin	7.85	2698.59
5	kaempferol	9.32	3655.49
6	Sinapic acid	11.26	6521.47

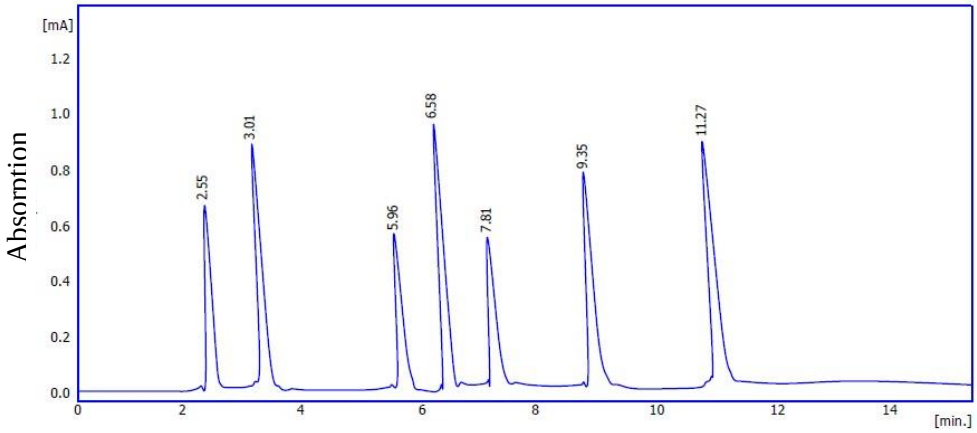
Table 5. The retention time and peak area of HPLC Chromatography of *Quisqualis indica* Flowers

No	Compounds	Retention time	Peak area
1	hydroxybenzoic acid	2.55	3652.89
2	gallic acid	3.01	5412.49
3	catechine	5.96	2145.88
4	Rutin	7.81	4875.80
5	kaempferol	9.35	5991.41
6	Sinapic acid	11.27	8562.08



Result chromatography (Uncal – F:\ leaves)

Figure 1. HPLC chromatography of leaves phenolic compound and flavonoids



Result chromatography (Uncal – F:\ flower)

Figure 2. HPLC chromatography of flowers phenolic compound and flavonoids

Antioxidant activity assay

The results in Table 6 elucidate the antioxidant activity of leaves and flowers phenolic compound and flavonoids isolated from *Quisqualis indica* using the DPPH method and compares them with ascorbic acid as a moisturizer, which indicates the *in vitro* ability of *Quisqualis indica* phenolic compound and flavonoids to scavenge the free radical factors released in solution. The results showed that scavenger activity increased with phenolic compound and flavonoids concentration increased for leaves and flowers and exceeded flower flavonoids on leaf flavonoids, and the

higher inhibition rate was 76.62 and 73.18 in concentration 400 µg/mL, while the lowest inhibition rate was 36.95 and 28.70 in 25 µg/mL for flowers and leaves, respectively, while the IC_{50} was 100µg/mL for flowers (57.25) and 200µg/mL for leaves and ascorbic acid (66.08 and 54.80) respectively. The results presented significantly differ ($p<0.001$) between all concentrations used in the experiment. The antioxidant activity of the *Quisqualis indica* plant agrees with Shah *et al.* (2019), which elucidate the alcoholic extract of *Quisqualis indica* leaves and flowers. These antioxidant effects of leaf and flower phenolic compound and flavonoids due to the high flavonoid concentration, which has a strong antioxidant effect and ability to scavenge free radical factors because the secondary metabolites like phenols and flavonoids neutralize, absorb, and scotch O_3 and other free radicals due to their redox activity, structure of the conjugated ring, and presence of a carboxyl group (Olugbami *et al.*, 2014).

Table 6. Scavenging activity of flowers and leaves compound

Concentration µg .mL^{-1}	Mean scavenging activity ± SE		
	Ascorbic acid	Flowers	Leaves
25	20.98 ±0.84 e	36.95 ±1.62 d	28.704 ±1.27 c
50	31.48 ±1.78 d	43.94 ±2.36 d	41.165 ±2.08 b
100	41.04 ±2.37 c	57.25 ±2.89 c	48.30 ±2.62 b
200	54.80 ±2.95 b	68.01 ±3.55 b	66.088 ±3.27 a
400	63.503 ±3.02 a	76.62 ±3.82 a	73.18 ±3.08 a
LSD value	7.0216 **	8.441 **	7.594 **
P-value	0.0001	0.0001	0.0001

Means having the different letters in the same column differed significantly, ** ($P\leq0.01$).

Conclusions

Active chemicals extracted from *Quisqualis indica* L. and other plants are being recognized as a new strategy for preventing and treating numerous human ailments. This research compares the chemical presence of alkaloids, terpenoids, tannins, flavonoids, and saponins in flowers and leaves of the same plant. High-performance liquid chromatography (HPLC) was used for quantitative analysis of phenolic compounds and flavonoids. The HPLC analysis revealed that the flowers contain a larger percentage of phenolic compounds and flavonoids compared to the leaves. The leaves and flowers exhibited considerable antioxidant activity against free radicals when compared to ascorbic acid. The antioxidant activity increased as the concentration increased, with flowers having an IC_{50} of 100 µg mL^{-1} and leaves having an IC_{50} of 200 µg mL^{-1}. This occurrence has led to a recent increase in demand for it.

Conflict of Interest

There are no disclosed conflicts of interest for authors.

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