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Original Article

Antioxidant and Antibacterial compounds from the leaves of *Psidium guajava*

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ABSTRACT

Psidium guajava Linn., family Myrtaceae, commonly known as guava, is an important food crop and medicinal plant in tropical and subtropical countries. Previous studies reported major contribution of the ethyl acetate fraction of *P. guajava* leaf extract to possess antioxidant and antibacterial activities. Therefore, the ethyl acetate fraction of *P. guajava* was subjected to chromatographic separation to explore the bioactive molecules responsible for these activities. Five known compounds were isolated and characterized using physical, chemical and spectral analysis. Quercetin-3-*O*-β-arabinopyranoside was isolated for the first time from this plant. Alongside this compound, the other four compounds viz., pyrogallol, quercetin, quercetin-3-*O*-β-D-xylopyranoside (reynoutrin) and quercetin-3-*O*-α-L-arabinofuranoside (avicularin) exhibited an exceptional antioxidant activity using $ABTS^{\bullet+}$ assay. The isolated pure compounds were further investigated for their antibacterial activity against both *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) bacteria. Pyrogallol didn't display any significant antibacterial activity against both types while, quercetin showed remarkable activity compared to ampicillin as a positive control assuming a promising antibacterial drug. Moreover, the three quercetin glycosides exhibited moderate antibacterial activity when compared to ampicillin.

Keywords: Guava; Pyrogallol; Quercetin; Reynoutrin; Quercetin-3-*O*-β-D-arabinopyranoside; Avicularin; Antioxidant; Antibacterial

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INTRODUCTION

Psidium guajava Linn. (Myrtaceae), commonly known as guava, a plant native to the Middle East region and it extends throughout Africa, South America, Europe and Asia. *P. guajava* is a small tree which is about 10 meter high with thin, smooth, patchy, peeling bark. The leaves are opposite, short-petiolate, the blade oval with prominent pinnate veins, from 5 to 15 cm in long. The flowers are slightly showy, petals whitish in color up to 2 cm long, the stamens are numerous. The fruits are fleshy yellow globose to ovoid berry about 5 cm in diameter with an edible mesocarp containing numerous small hard white seeds [1].

P. guajava leaves extract medicinal usage has been reported in indigenous system of medicines all over the world from traditional uses to recent use. The scientific records of the medicinal properties of *P. guajava* leaf products began in 1940's and reports maintain a tradition of repeating the data each decade. Different parts of the plant are used in the original system of medicine for the treatment of various human ailments. Many people used to take medicinal decoction of *P. guajava* leaf extract for its antispasmodic and antimicrobial properties in the treatment of diarrhea and dysentery. Therefore, the safety of *P. guajava* leaves have empirically been confirmed. The literature showed that *P. guajava* leaf extract contains plenteous amounts of phenolic phytochemicals which inhibit peroxidation reaction in the living body. Therefore, it can be expected to prevent various chronic diseases such as diabetes, cancer, and heart-disease. Moreover, reducing the free-radicals concentration has antioxidizing effect in the body, therefore the *P. guajava* leaf polyphenols can prevent or protect from arterial sclerosis, thrombosis, cataract and inhibit aging of the body and skin [2].

It was reported that the leaves of *P. guajava* contain an essential oil which is rich in cineol. Additionally, tannins and triterpenes are also important constituents of the plant leaves. Mature leaves contain flavonoids such as quercetin, myricetin, kaempferol and luteolin which were found with greatest concentrations in July. A lot of the medicinal properties of *P. guajava* are credited to these flavonoids, which are well-known for their multi-directional biological activities [3].

Major attention is focused on the protective biochemical functions of naturally occurring antioxidants. Restriction is being imposed on the use of synthetic antioxidants because of their carcinogenicity. So that, the need for natural antioxidants become urgent, imperious and desirable. Subsequently, as sources of natural antioxidants much attention is being paid to plants and other organisms [4].

Natural products from microorganisms have been the primary source of antibiotics. With increasing the acceptance of herbal medicine as a good alternative for health care, the screening of medicinal plants for antimicrobial compounds has become very important as they may serve as promising sources of novel antibiotic. Furthermore, The rapid appearance of multiple drug resistant strains of pathogens to current antimicrobial agents has created an urgent intensive search for new antibiotics from medicinal plants [5].

Previous Studies of *P. guajava* leaves extract reported that the ethyl acetate fraction exhibited a high potency against bacterial infections [6]. Hence, in this study, the ethyl acetate fraction of *P. guajava* aqueous leaf extract was subjected to extensive chromatographic investigation to isolate, identify and characterize the biologically active molecules present in the plant which are responsible for both antioxidant and antibacterial activities.

$ABTS^{\bullet+}$ assay was used to evaluate the antioxidant activity of the isolated compounds. This antioxidant assay is a rapid and precise technique which well known for its safe and low limitations. In addition, two microorganisms; *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) were used to evaluate the antibacterial activity of the isolated pure compounds in order to recognize the active biomolecules present in the plant.

MATERIALS AND METHODS

Materials.

Plant material: The dried leaves of *P. guajava* were purchased from professional local herbal market, the plant was taxonomically identified. Voucher specimens were deposited at the Department of Pharmacognosy, Mansoura University, Mansoura, Egypt.

Chemicals and solvents: The solvents used for extraction, chromatographic separation and crystallization {methanol and methylene chloride (EL-Nasr Company for Pharmaceutical Chemicals, Egypt)} were of reagent grade. Normal phase chromatography was carried out using silica gel G 60- 230 (Merck, Germany) packed by the wet method in the stated solvent. Size exclusion gel "Sephadex LH-20, GE healthcare company, USA". Azino-bis-(3-ethyl benzthiazoline-6-sulfonic acid) (ABTS) (Sigma Chemicals, St. Louis, USA). Manganese dioxide (MnO_2) (DBL chemicals, Germany). Ascorbic acid (Cevarol®) tablets

(Memphis Pharmaceutical Co., Cairo, Egypt) used as control. Nutrient agar media consists of peptone, beef extract, sodium chloride and agar. Microorganisms; *Staphylococcus aureus* and *Escherichia coli* were obtained from Microbiology Department, Faculty of Pharmacy, Mansoura University, Egypt. Ampicillin (Alexandria Pharmaceutical Co., Egypt) and Dimethylsulfoxide (DMSO), (Al-Gomheria Co., Mansoura, Egypt) were used in the antimicrobial assay.

Methods.

Extraction and fractionation: The Plant was thoroughly washed with distilled water and shade dried, ground in a tooth miller, 500 gm. Plant powder was extracted with distilled water (500 mL x 3) and combined together. The combined aqueous extract was partitioned with ethyl acetate for three successive times (1 L x 3). The ethyl acetate fraction was combined, evaporated using rotary evaporator till dryness and stored at 4°C for further use.

Chromatographic method: The ethyl acetate fraction of *P. guajava*, was dissolved in the least amount of methanol, then mixed with suitable amount of silica gel, dried, loaded on to the top of silica gel – packed column, and gradiently eluted with methylene chloride - methanol. The effluents were collected and concentrated separately to small volumes. The collected fraction were further purified by Sephadex LH-20 column using gradient elution of methylene chloride – methanol solvent system.

ABTS assay: Typically, 2 mL of ABTS solution (1mg/mL) and 3 mL of MnO_2 solution (25 mg/mL) were mixed, the mixture was shaken, centrifuged, and decanted. Absorbance ($A_{control}$) of the resulting green-blue solution ($ABTS^{\bullet+}$ solution) is recorded at λ_{max} 734 nm. Absorbance (A_{test}) is measured upon the addition of 50 µL of (1 mg/mL) solution of the test sample (pure compounds dissolved in DMSO) to the 1000 µL $ABTS^{\bullet+}$ mixture solution. The decrease in absorbance is expressed as % inhibition which is calculated from the equation:

% inhibition = $[A_{control} - A_{test} / A_{control}] \times 100$.

Ascorbic acid (50 µL, 2 mM solution) was used as a standard antioxidant and blank sample is run using solvent without $ABTS^{\bullet+}$ [7].

Antibacterial assay: the method of Aboaba *et al*., was used. All test compounds and ampicillin as a positive control were presented as solution in DMSO (100 µL, 1 mg/mL) within rounded reservoirs (cups) which were created on a seeded plate of nutrient agar. The test compounds were allowed to diffuse through agar, which will form a zone of inhibition. Two different strains of bacteria used, *Staphylococcus aureus* and *Escherichia coli*. Therefore, it is often assumed that the larger the diameter of the inhibition zones the more potent the antibacterial compound [8].

RESULTS

Five compounds were isolated from the ethyl acetate fraction of *P. guajava*. The isolated compounds were identified as pyrogallol **G1**, quercetin **G2**, quercetin-3-*O*-β-D-xylopyranoside **G3** (Reynoutrin), quercetin-3-*O*-β-arabinopyranoside **G4** and quercetin-3-*O*-α-L-arabinofuranoside **G5** (Avicularin) which are shown in Figure 1. The identification of the isolated compounds were achieved using spectroscopic methods including ^{1}H- and ^{13}C-NMR, see Table 1 & 2. All the isolated compounds exhibited remarkable antioxidant activity. The experiment was repeated three independent times for each pure compound and the standard deviation is calculated. The mean absorbance and % inhibition for each compound are represented in Table 3. Beside the antioxidant efficacy of the isolated compounds, they also possessed relative antibacterial activity. All inhibition zones data are represented for the isolated compounds **G1**-**G5** against both *S. Aureus* and *E. coli* in Table 4.

DISCUSSION

Chromatographic separation of the components of the ethyl acetate fraction of *P. guajava* was done using normal silica gel column chromatography. The collected fractions were further purified using Sephadex gel column to obtain five pure compounds (Figure 1). The identification process was achieved using spectroscopic NMR analysis. The pure compounds were coded from **G1** to **G5** depending on their order of elution.

Compound **G1** was isolated as white crystalline powder with melting point 133 - 134 °C**.** It is soluble in methanol, the R_f value is 0.65 (methylene chloride/methanol 9/1, silica gel plates GF_{254}) and gave a violet color with $FeCl_3$ indicating its phenolic nature. The proton signal at δ 6.29 ppm (2H, d, *J* = 8.0 Hz) assigned to two equivalent *ortho*-coupled aromatic protons H-4 and H-6. Also, the ^{1}H-NMR signal at δ 6.46 (1H, d, *J* = 8.0 Hz**)** could be assigned for H-5 proton. The ^{13}C-NMR (APT) spectrum revealed the presence of four signals assigned for six carbons. The carbon signal at δ 147.9 ppm could be assigned to two equivalent oxygenated carbon (C-1 and C-3) due to the presence of symmetry. Similarly, the carbon signal at δ 109.1 ppm could be assigned to two primary carbons (C-4 and C-6). The signals at δ_C 134.8 and 120.9 could be assigned to C-2 and C-5,

respectively. Thus, a tri-substituted symmetric benzene ring was suggested for **G1**. The previous data indicated that **G1** is pyrogallol which was reported before from *P. guajava* [9].

Compound **G2** was isolated as yellow needles, with melting point 314 - 317 °C. It is soluble in methanol, the R_f value is 0.58 (methylene chloride/methanol 9/1, silica gel plates GF_{254}), gave a yellow color with 5% alc. $AlCl_3$, 5% alc. KOH and NH_4OH visualizing reagents, indicating its flavonoidal nature. ^{1}H-NMR showed two doublets at δ_H 6.17 (1H, d, *J*=2 Hz) and δ 6.38 (1H, d, *J*=2.0 Hz) assigned for the protons H-6 and H-8 positions respectively and indicated free 6 and 8 positions. ^{1}H-NMR signals at δ 7.62 (1H, dd, *J*=8.5 & 2.0 Hz), 7.72 (1H, d, *J*=2.0 Hz) and 6.88 (1H, d, *J*=8.5 Hz) could be assigned for protons present at C-6`, C-2` and 5` positions respectively, of a di-substituted B-ring. From the ^{1}H-NMR data and R_f value and color produced with visualizing reagent against authentic sample. It could be concluded that **G2** is quercetin which was isolated before from *P. guajava* [3].

Compound **G3** was also isolated as yellow amorphous powder. It is soluble in methanol, Its R_f value is 0.30 (silica gel GF_{254}, chloroform/methanol 9:1), gave a yellow color with 5% alc. $AlCl_3$, 5% alc. KOH and NH_4OH visualizing reagent indicating its flavonoidal nature and gave a positive Molisch's test indicating its glycosidic nature. The ^{1}H NMR and APT spectra suggested a flavonol nucleus for **G3** as indicated from the signals at δ_C 180.18 and 136.2 assigned to C-4 and C-3, respectively, and from the absence of H-3 signal from the ^{1}H-NMR spectrum. The two carbon signals at δ 100.7 and 95.5 were assigned to C-6 and C-8 indicating these positions are unsubstituted. This was confirmed from the *meta*-coupled doublets at 6.19 δ (1H, d, *J*= 2.5) and 6.38 (1H, d, *J*= 2.5) which are characteristic for H-6 and H-8, respectively. The two signals at δ 163.9 and 166.9 were assigned to two oxygenated quaternary carbons, C-5 and C-7, respectively. ^{1}H-NMR signals at δ 7.61 (1H, d, *J*= 2.0), 6.84 (1H, d, *J*= 8.0) and 7.57 (1H, dd, *J*= 8.0, 2.0), corresponding to the ABX splitting pattern, were assigned to H-2`, H-5` and H-6` of a di-substituted B-ring respectively. This was confirmed from the carbon signals at δ 116.8, 117.9 and 123.8 corresponding to C-2`, C-5` and C-6`, respectively. Five carbon signals at δ 105.3, 76.1, 78.1, 71.8 and 68.1 suggested a xylopyranose moiety and were assigned to C-1``, C-2``, C-3``, C-4`` and C-5``, respectively [10]. The above ^{1}H-NMR and APT signals were in full agreement with those reported for reynoutrin [10]. A *beta* configuration was confirmed from the ^{1}H-NMR proton signals at δ 5.25 (1H, d *J*=7.5 Hz) for the anomeric proton of xylose. The ^{1}H-NMR signals were also in full agreement with those reported for xylose; δ 3.11 (1H, m, H-2``), 3.41 (1H, m, H-3``), 3.52 (1H, m, H-4``), 3.03 (1H, s, H-5``a) and 3.77 (1H, m, C-5``b). Therefore, **G3** was identified as quercetin-3-*O*-β-D-xylopyranoside (Reynoutrin) which is previously reported its isolation from *P. guajava* leaves [11].

Compounds **G4** & **G5** were isolated as yellow amorphous powder and were soluble in methanol, their R_f values are 0.32, 0.34 (silica gel plates GF_{254}, methylene chloride/methanol 9:1) for **G4** & **G5,** respectively. They produced a yellow color with 5% alc. $AlCl_3$, 5% alc. KOH and NH_4OH visualizing reagent indicating their flavonoidal nature and gave a positive Molisch's test indicating their glycosidic nature. They possessed spectral data more or less similar to **G3** (Reynoutrin). They shared the same aglycone moiety which was confirmed to be quercetin. Regarding compound **G4**, the ^{1} H-NMR doublet at δ 5.14 (1H, d, *J*= 7.0) was assigned to the anomeric H-1`` of a sugar moiety and confirmed that it is *beta*-linked. The presence of other proton signals equivalent to 5H; 1H (d, *J*=3.0 Hz) at δ 3.93 assigned to H-2``, 1H (m) at δ 3.93 assigned to H-3``, 1H (m) at δ 3.46 assigned to H-4``, 1H (m) at δ 3.89 assigned to H-5a`` and 1H (m) at δ 3.47 assigned to H-5b``, indicated the presence of a pentose sugar. The methine carbon signal at δ_C 105.4 was assigned to the anomeric carbon C-1``. Furthermore, signals at δ 74.9, 73.6, 69.6 and 67.8 assigned to C-3``, C-2``, C-4`` and C-5``, respectively and suggested an arabinopyranose sugar [10]. From these data, compound **G4** was confirmed to be quercetin-3-*O*-β-D-arabinopyranoside which according to our best knowledge, is isolated for the first time from *P. guajava*.

On the other hand, the difference between the H^1-NMR and C^{13}-NMR spectra of compounds **G4** and **G5** was mainly in their anomeric proton and carbon signals. For compound **G5**, the carbon signal for C-1`` appeared at $\delta_C$ 110.3 and for its $^1$H-NMR signal, it appeared as singlet at $\delta_H$ 5.46 indicating an *alpha*-linked sugar. Furthermore, the carbon signals at $\delta_C$ 88.8, 84.1, 79.5 and 63.3, assigned to C-4``, C-2``, C-3`` and C-5``, respectively and indicated an arabinofuranose moeity [10]. Therefore, the structure of **G5** was confirmed to be quercetin 3-*O*-α-L-arabinofuranoside which is reported previously from *P. guajava* [2].

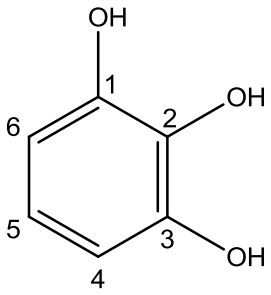
The aqueous extract *P. guajava* was reported to possess a high activity towards free radical scavenging. Previous reports showed that ascorbic acid was a substantially more powerful antioxidant

than the extracts of *P. guajava* leaves [2]. Consequently, this study investigated the isolated compounds from the ethyl acetate fraction of the leaves extract *P. guajava* for their antioxidant potential using ABTS free radical assay. The obtained results showed that all the isolated compounds exhibited promising antioxidant activity when compared to the reported commercial drug (Ascorbic acid). Compound **G1** (Pyrogallol) showed the highest antioxidant activity with 81.15 %inhibition which is comparable to the positive control (Ascorbic acid, 82.09 % inhibition), Table 3. This remarkable antioxidant activity of pyrogallol may be due to the structural similarity to gallic acid which is well known for its potent activity towards free radical scavenging. Compound **G2** (Quercetin) showed high antioxidant activity with % inhibition reached 80.41. This pronounced activity of quercetin towards free radical scavenging could be explained by its polyphenol groups which are further conducted with unsaturated conjugation. These results agree with the reported data for the antioxidant activity of quercetin [2]. Compound **G3** (Reynoutrin) showed a relatively similar antioxidant activity with 79.47 % inhibition which is slightly lower than that exhibited by pyrogallol and quercetin. Additionally, both quercetin-3-*O*-arabinoside (α & β) isomers possessed significant antioxidant activity with % inhibition of 76.67 and 76.11 for **G4** and **G5**, respectively. The reduced activity of the flavonoid glycoside (**G3**-**G5**) compared to their aglycone (**G2**) highlight the value of the presence of a free OH group at position C-3.

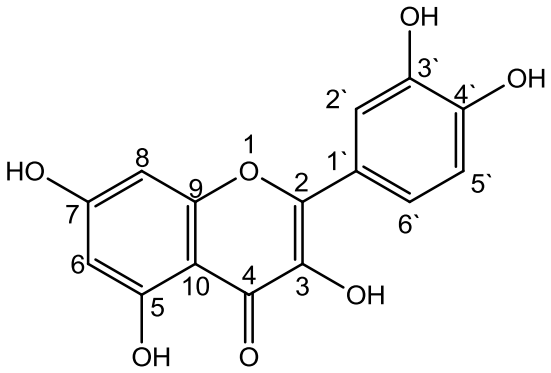
Many researchers studied the antimicrobial activity of the *P. guajava* leaves extract. Geidam et al. (2015), examined the antibacterial efficacy of ethyl acetate fraction of the aqueous extract of *P. guajava* leaves. They concluded that the ethyl acetate soluble fraction of leaf extract of *P. guajava* effectively has antibacterial activity towards *E. coli* gram negative bacteria [6]. In this study, the activity of the five isolated pure compounds from ethyl acetate fraction of guava were examined for their antibacterial activity against the Gram-positive bacteria, *Staphylococcus aureus* and the Gram-negative bacteria, *Escherichia coli*. Depending on the assumption that the larger the diameter of the inhibition zone, the more potent the antibacterial compound. The results showed that the highest antibacterial activity was attributed to quercetin (**G2**) with an inhibition zone equals 11 mm against *Staph. aureus* and 15 mm against *E. coli*. These results for quercetin were very comparable to that of the positive control ampicillin, a commercial antibiotic that showed an inhibition zone equals 18 mm against *Staph. aureus* and 16 mm against *E. coli*. (Table 4). The lowest antibacterial activity achieved among the five isolated compounds was for pyrogallol (**G1**), which didn't show any significant activity towards both types of bacteria. The three quercetin glycosides (**G3**-**G5**) showed a moderate activity towards both of *Staph. aureus* and *E. coli*. The inhibition zones of reynoutrin (**G3**), avicularin (**G5**) and its isomer quercetin-3-*O*-β-D-arabinopyranoside (**G4**) were 12, 8 and 11 mm, respectively, against *Staph. aureus* (Gram positive bacteria). However, they showed a much lower antibacterial activity against *E. coli* (Gram negative bacteria) with inhibition zones 4, 3 and 4 mm respectively, compared to the positive control ampicillin. Finally, it could be concluded that quercetin has superior potential antibacterial activity than its glycosides against *Staph. aureus* and *E. coli* strains compared to ampicillin. However, pyrogallol has no significant antibacterial activity.

CONCLUSION

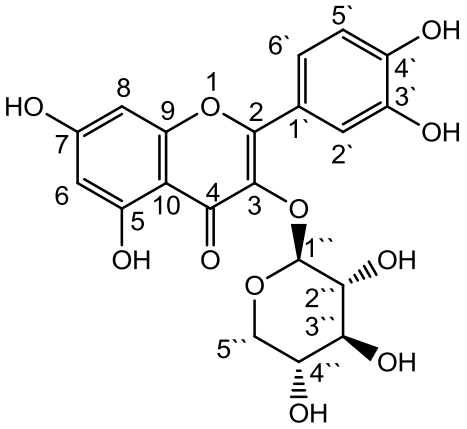
Five compounds were isolated and characterized from the ethyl acetate fraction of *Psidium guajava*. They were identified using physical, chemical and spectral analysis. Pyrogallol, quercetin, avicularin and reynoutrin are previously reported from *P. guajava*. However, quercetin-3-*O*-β-D-arabinopyranoside is isolated for the first time herein from *P. guajava*. All compounds were shown to exhibit remarkable antioxidant activity towards free radicals ($ABTS^{\bullet+}$ assay). Pyrogallol exhibited the highest antioxidant activity which was comparable to that of the positive control, ascorbic acid. Quercetin glycosides viz., reynoutrin, quercetin-3-*O*-β-D-arabinopyranoside and avicularin exhibited lower antioxidant activity than their aglycone "quercetin". Thus indicating the importance of the free hydroxyl group at position-3 of the flavonol structure in the antioxidant activity. The ethyl acetate fraction of *P. guajava* was reported to have potential antibacterial activity. We investigated the antibacterial activity of the major components of this extract. Our results indicated that quercetin has the major antibacterial effect against both *Staph. aureus* & *E. coli* while, its glycosides exhibited moderate antibacterial activity compared to ampicillin as a positive control. Furthermore, pyrogallol showed no significant antibacterial activity towards both gram positive and gram negative bacteria.



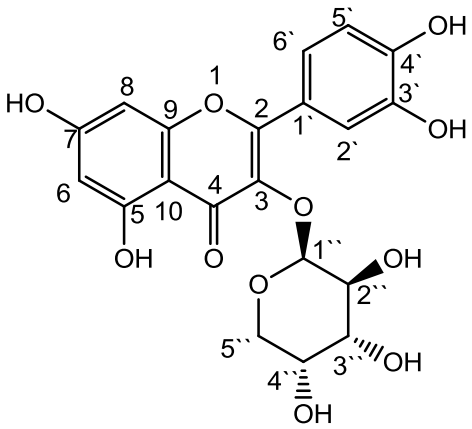
Pyrogallol (**G1**)



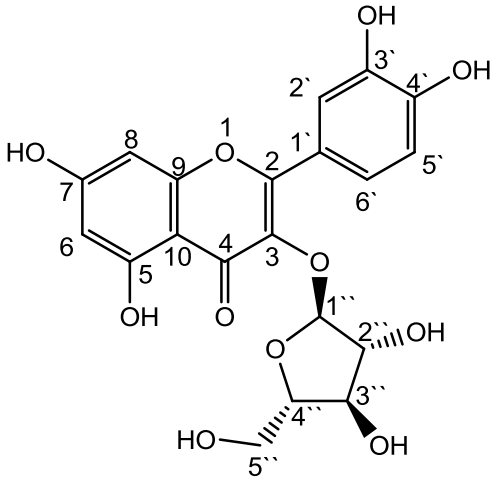
Quercetin (**G2**)



Quercetin-3-*O*-β-D-xylopyranoside (**G3**)



Quercetin-3-*O*-β-D-arabinopyranoside **(G4)**



Quercetin 3-*O*-α-L-arabinofuranoside (**G5**)

Figure 1. Chemical structure of the five isolated pure compounds.

Table 1. ^{1}H-NMR (500 MHz, CD_3OD, coupling constants *"J"* in Hz) data of compound **G3-G5**.

H #	G3	G4	G5
1			
2	---	---	---
3	---	---	---
4	---	---	---
5	---	---	---
6	6.19, 1H, d (2.5)	6.18, 1H, d (1.5)	6.19, 1H, d (1.5)
7	---	---	---
8	6.38, 1H, d (2.5)	6.37, 1H, d (1.5)	6.38, 1H, d (1.5)
9	---	---	---
10	---	---	---
1`	---	---	---
2`	7.61, 1H, d (2.0)	7.73, 1H, d (2.0)	7.52, 1H, d (2.5)
3`	---	---	---
4`	---	---	---
5`	6.86, 1H, d (8.0)	6.86, 1H, d (8.0)	6.86, 1H, d (8.0)
6`	7.55, 1H, dd (8.0, 2.0)	7.55, 1H, dd (8.0, 2)	7.49, 1H, dd (8.5, 2.5)
1``	5.17, 1H, d (7.5)	5.14, 1H, d (7.0)	5.46, 1H, s
2``	3.11, 1H, m	3.91, 1H, d (3.0)	4.32, 1H, d (2.0)
3``	3.41, m	3.81, m	3.90, m
4``	3.52, m	3.46, m	3.64, m
5``	(a) 3.03, m (b) 3.77, m	(a) 3.89, m (b) 3.47, m	(a) 3.85, m (b) 3.49, m

Table 2. ^{13}C-NMR (APT) data of compound **G3-G5** (125 MHz, CD_3OD):

C #	G3	G4	G5
1			
2	159.2, qC	159.1, qC	159.6, qC
3	136.2, qC	136.3, qC	135.7, qC
4	180.2, qC	180.2, qC	180.8, qC
5	163.8, qC	163.7, qC	163.9, qC
6	100.7, CH	100.6, CH	100.7, CH
7	166.9, qC	166.7, qC	166.9, qC
8	95.5, CH	95.49, CH	95.6, CH
9	159.7, qC	159.4, qC	160.4, qC
10	106.4, qC	106.3, qC	106.4, qC
1`	124.1, qC	123.7, qC	123.8, qC
2`	116.8, CH	116.9, CH	117.2, CH
3`	136.2, qC	146.7, qC	147.2, qC
4`	150.7, qC	150.7, qC	150.7, qC
5`	117.9, CH	118.2, CH	117.6, CH
6`	123.8, CH	123.8, CH	123.9, CH
1``	105.4, CH	105.4, CH	110.3, CH
2``	76.1, CH	73.6, CH	84.1, CH
3``	78.3, CH	74.9, CH	79.5, CH
4``	71.8, CH	69.9, CH	88.8, CH
5``	68.1, CH_2	67.8, CH_2	63.3, CH_2

Table 3. Results of ABTS$^{\bullet+}$ antioxidant assay showed % inhibition of ABTS superoxide ions by the isolated compounds (**G1**-**G5**) at concentration of (1 mg/mL).

Test samples	**Mean absorbance** (734 nm**)**	**Inhibition (%)**
Control	0.536	0
G1	0.053	81.15
G2	0.054	80.41
G3	0.051	79.47
G4	0.173	76.67
G5	0.168	76.11
Ascorbic acid (2 mM)	0.096	82.09

Table 4. Results of antibacterial activity (inhibition zones) of the isolated compounds (**G1**-**G5**) at concentration of (1 mg/mL).

Compound	**Inhibition zone actual diameter (mm)**	
	Staphylococcus aureus **(Gm +ve)**	***Escherichia coli*** **(Gm -ve)**
G1	1.5	-
G2	11	15
G3	12	7
G4	11	4
G5	8	3
Ampicillin	18	16

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