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BIFLAVONOIDS FROM THE ROOTS OF *RHUS RUSPOLII* AND EVALUATIONS OF THEIR ANTIOXIDANT ACTIVITIES

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ABSTRACT. Biflavonoids are C-C or C-O-C linked flavonoid dimers with highly restricted presence in plant species. They are extensively reported to possess interesting pharmacological properties. The chromatographic fractionation and purification of CH_2Cl_2/MeOH (1:1) extract of the root of *Rhus ruspolli* led to the isolation of a new biflavonoid (**1**) along with four other known biflavonoids (**2-5**). The structure of the compounds were identified based on the analysis of NMR spectroscopic and mass spectrometric data and also in comparison with reported literature data. Compounds **2-5** were assayed for their antioxidant activity using DPPH and displayed potent *in vitro* antioxidant activities. The percentage radical scavenging activities were 78.32, 68.90, 93.22 and 92.00 for compounds **2-5**, respectively. The highest activity was observed for compound **4** and **5** with IC_{50} values of 7.90 and 8.40, respectively, which are even greater than that of ascorbic acid (IC_{50} 9.90). The high antioxidant activity of the compounds could be due to the presence of free hydroxyl groups in the flavonoids. The antioxidant activities of these compounds support the traditional uses of the plant in treatment of wound, ectoparasite and as antibacteria and indicates the potential use of these compounds as drug lead candidates.

KEY WORDS: *Rhus ruspolii*, Roots, Biflavonoids, Chalcones, Antioxidant activity

INTRODUCTION

The genus *Rhus* (family, Anacardiaceae), consists of around 250 species with mostly small trees and shrubs widely distributed in subtropical and temperate regions of the world [1]. Plants of this genus have attracted a lot of attentions as they produce several biologicaly active compounds [1-3]. For instance, they produce polyphenolic caompounds, mainly monoflavonoids and biflavonoids as major constituents that have tremondous biological activities [4, 5]. *R. ruspolii* is belongs to the Anacardiaceae family mostly small trees 2-8 m and shrubs 1-2 m tall flowering plant. It is one of the species that has several medicinal applications traditionally. Its fresh leaves crushed and rubbed on affected part (Hyena bite), the root powdered mixed with water given orally for the treatment of tropical diseases and wounds. Biological activities reported from extracts of these plant species include antimicrobial, cytotoxicity, anticancer, antioxidant, antiviral, anti-inflammatory and antimalarial activities [6-8]. Despite the wider use of this plant by the communities, the investigations of phytochemical and bioactivity of pertaining to it is not exhaustive. Thus, as part of our ongoing search for biologically active molecules from Ethiopian medicinal plants, herein, the isolation and antioxidant activities compounds from *R. ruspolii* are reported.

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EXPERIMENTAL

General

Solvents and reagents used for extraction and isolation of the compounds were of analytical and HPLC grade. Analytical TLC pre-coated sheets ALUGRAM®Xtra SIL G/UV_{254} (layer: 0.20 mm silica gel 60 with fluorescent indicator $UV_{F254/365}$) was used for purity analysis. For column chromatography, silica gel 60-120 mesh was used. Chromatograms were visualized on TLC under UV (UV-VIS Shimadzu) light at 254 and 365 nm to detect UV absorbing or fluorescing bands and by spraying with 10% H_2SO_4 acid and heating on hot plate, UV-Visible (DU-8800D). NMR spectra were recorded on an Avance 500 MHz spectrometer (Bruker, Billerica, MA, USA, at 500 MHz (^{1}H) and 125 MHz (^{13}C)). Chemical shifts are expressed in ppm, J values are given in Hz and referenced to the central peak of the appropriate duetrated solvent's resonances (residual $CDCl_3$, $(CD_3)_2CO$, CD_3OD and $(CD_3)_2SO$ at δ_H 7.26, 2.20, 3.35, 2.52 for protons and δ_C 77.2, 205.9, 49.7, 40.8 for carbons, respectively).

Plant materials

The roots of *R. ruspolii* were collected from Horro Buluk, Horro Guduru Wollega zone, Oromia regional state, Ethiopia in September, 2019. The plant material was identified by botanist (Dr. Fekadu Gurmessa) and the voucher specimen (DAD001Rr) has been deposited at Wollega University Herbarium. The collected plant part was washed thoroughly with tap water and cut into smaller pieces and air dried under shed. The dried plant material was grinded to smaller pieces using mortar and pestle.

Extraction and isolation

The powdered roots of *R.ruspolii* (1 kg) were extracted with equal ratio of CH_2Cl_2/MeOH (3 times for 24 h each) at room temperature with occasional shaking and filtered using Whatman no.1 filter paper to separate the extract from the marc. The filtrate was collected and the solvents were evaporated under reduced pressure using rotary evaporator at 40 °C to yield dark brown extract 22.8 g (2.28%). A 20 g of the crude extract was adsorbed on silica gel 20 g (mesh size 60-120) and subjected to silica gel column chromatography (250 g of silica gel, using n-hexane for packing). The column was eluted with *n*-hexane with increasing gradient of ethyl acetate to afford 50 major fractions *ca*. 100 mL each. The purity of each fraction was checked by using TLC. Fractions that showed similar R_f values and the same characteristic color on TLC (visualized in UV lamp at 254 and 356 nm) were combined. Fractions15-20 were combined together based on their TLC analysis and chromatographed on Sephadex LH-20 (eluted with CH_2Cl_2/MeOH; 1:1) to give compound **5** (13.5 mg). Fractions 23-25 were combined and purified on Sephadex LH-20 (eluted with CH_2Cl_2/MeOH (1:1)) and gave compound **4** (12.3 mg). Fractions 28-30 were also combined together and purified further by small silica gel column chromatography (eluted with (4:1) hexane: ethyl acetate solvent ratio) to give compound **3** (8.5 mg). Fractions 31-35 showed single spot on TLC (3:2 n-hexane/EtOAc as eluent) to afforded compound **2** (10 mg). Fractions 41-45 were combined and purified by Sephadex LH-20 (eluted with CH_2Cl_2/MeOH; 1:1) result in compound **1** (30 mg).

Antioxidant activity assay

The radical scavenging activities of compounds **(2-5)** were evaluated using DPPH and ascorbic acid was used as reference [9]. 0.1 mM solution of DPPH was prepared in methanol (99.8%) and 1 mL was added in to 3 mL of different solutions of isolated compounds in methanol at different

concentrations 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/mL for the compounds (**2-5**). The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. After 30 min reaction time the absorbance was measured at 517 nm using UV-Vis spectrophotometer [10]. The experiments were done in triplicate and the IC_{50} values were calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percent DPPH scavenging effect was calculated by using following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{A0 - A1}{A0} x\ 100$$

where A_0 was the Absorbance of control reaction and A_1 was the Absorbance of test sample.

RESULTS AND DISCUSSION

Extraction of the CH_2Cl_2/MeOH (1:1) root extract of *R. ruspolii* yields 22.8 g (2.28%) dark brown extract. Chromatographic separations of the root extract of *R. ruspolii* and gel filtrations of the fractions using Sephadex LH-20 resulted a new compound (**1**) along with four known compounds (**2-5**) (Figure 1).

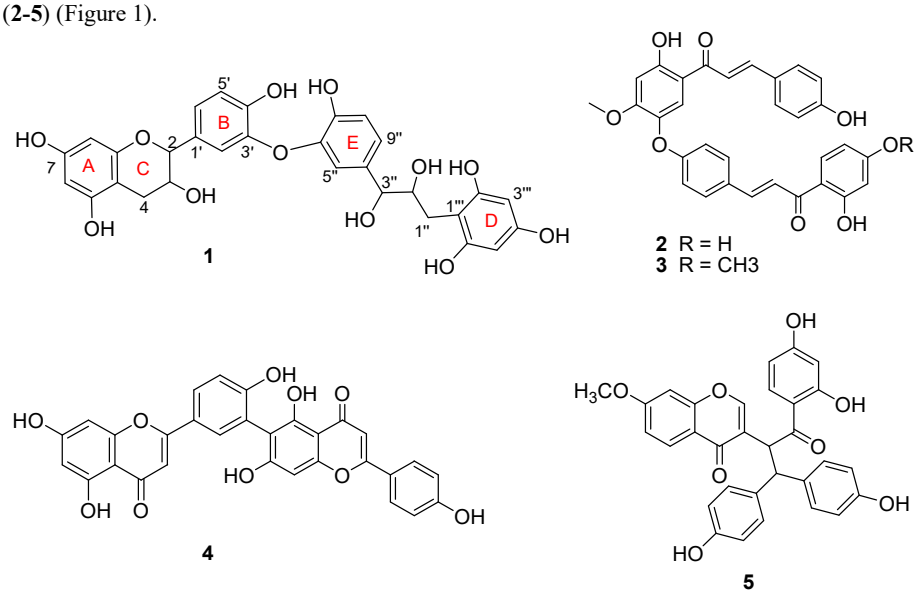

Figure 1. Structures of the isolated compounds from roots of *R. ruspolii*.

Compound **1** was isolated as yellow powder solid with a melting points of 203-205 °C. Its ESI-MS showed ion peak at *m/z* at 581.1602 $[M-H]^+$ and sodium adduct at *m/z* 603.1441 $[M+Na]^+$ both consistent with a molecular formula of $C_{30}H_{28}O_{12}$ indicating seventeen degrees of unsaturation.

The ^{1}H-NMR spectrum (Table 1) showed the presence of up-field shifted *meta*-coupled aromatic protons at δ_H 5.92 (1H, d, *J* = 2.3 Hz) and 5.89 (1H, d, *J* = 2.3 Hz) due to *di-ortho* oxygnations and were assigned to H-6 and H-8, respectively of tetrasubstitued benzene ring A. These were colaborated with three aromatic (at 6.92 (1H, d, *J* = 1.2 Hz), 6.80 (1H, d, *J* = 8.2 Hz) and 6.77 (1H, dd, *J* = 8.2, 1.2 Hz)) and four aliphatic (4.56 (1H, d, *J* = 7.8 Hz), 4.02 (1H, dd, *J* = 5.0, 6.0 Hz), 2.92 (1H, dd, *J* = 16.1, 5.5 Hz) and 2.52 (1H, dd, *J* = 16.0, 8.0 Hz) with the later

two were for methylene protons) protons indicated a flavan skeleton. The spectrum also revealed the presence of another set of proton signals for tetrasubstituted benzene ring D (at 6.02 (d, J = 2.2 Hz) intigerated for two protons for H-3''' and H-5'''), *ortho-meta*-coupled trisubstituted protons (δ_H 7.06 (1H, d, J = 2.0 Hz), 6.81 (1H, d, J = 8.2 Hz) and 6.84 (1H, dd, J = 8.2, 2.0 Hz) for H-5'', H-8'' and H-9'') and aliphatic protons (2.89 (1H, dd, J = 13.0, 6.0 Hz), 2.76 (1H, dd, J = 13.0, 6.0 Hz), 4.89 (1H, br. s) and 4.20 (1H, d, J = 4.2 Hz) for H-1''α, H-1''β, H-2'' and H-3', respectively) indicating that the compound is dimeric.

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compound **1** (acetone-d_6).

Carbon No.	Appearance	^{13}C NMR	δ_H (int., mult., J in Hz)	HMBC (H→C)
2	CH	81.8	4.56(1H, d, 7.8 Hz)	C-3, C-4, C-1', C-2',C-6',C-8a
3	CH	67.5	4.02(1H, dd, 5.0, 6.0 Hz)	C-2, C-4a, C-1'
4	CH_2	27.9	2.92(1H,dd,16.1,5.5Hz) 2.52 (1H,dd,16.0,8.0Hz)	C-2, C-3, C-4a, C-5
4a	C	99.7		
5	C	155.9		
6	CH	94.8	5.92(1H, d, 2.3 Hz)	C-4a, C-5, C-8
7	C	156.3		
8	CH	94.5	5.89(1H, d, 2.2 Hz)	C-4a, C-6, C-7
8a	C	156.2		
1'	C	131.5		
2'	CH	114. 8	6.92(1H, d, 1.2 Hz)	C-2, C-6', C-3'
3'	C	144.9		
4'	C	144.4		
5'	CH	114.4	6.80(1H, d, 8.2 Hz)	C-2, C-1', C-2'
6'	CH	119.2	6.77(1H, dd, 8.2,1.20 Hz)	C-4', C-5'
1''	CH_2	28.1	2.89(1H, dd, 13.0,6.0 Hz) 2.76 (1H, dd, 13.0,6.0 Hz)	C-2'', C-3'', C-1''', C-6'''
2''	CH	78.7	4.89(1H, br.s)	C-1'', C-4'', C-8'', C-9''
3''	CH	66.1	4.20(1H, d, 4.2 Hz)	C-4''
4''	C	131.2		
5''	CH	114.6	7.06 (1H, d, 2.0 Hz)	C-2'', C-8'', C-9'', C-3'
6''	C	144.8		
7''	C	144.5		
8''	CH	114.4	6.82(1H, d, 8.2 Hz)	C-2'', C-7''
9''	CH	118.4	6.86(1H, dd, 8.2, 2.0 Hz)	C-2'', C-7'', C-8''
1'''	C	98.9		
2'''	C	156.6		
3'''	CH	95.3	6.02(1H, d, 2.2 Hz)	C-1''', C-4''', C-5'''
4'''	C	156.8		
5'''	CH	95.2	6.02(1H, d, 2.2 Hz)	C-1''', C-3''' , C-6'''
6'''	C	156.7		

The ^{13}C-NMR spectrum (Table 1) showed a total of 30 carbons signals with 24 aromatic and six aliphatic carbons. The HSQC analysis confirmed 16 of them were protonated, of which six of them are aliphatic carbons (δ_C 27.9 (C-4), 28.1 (C-1''), 66.1 (C-3'') 67.5 (C-3), 78.7(C-2'') and 81.8 (C-2)). Further analysis of DEPT-135 spectrum supported the presence of two CH_2 carbon atoms at δ_C 27.9 (C-4), 28.1(C-1''), and 14 quaternary carbons. The long range HMBC, H-H COSY and NOE correlations showed that the compounds is a dimer of catechin and 2''',4''',6'''- benzentriol-(7''-hydroxylphenyl)-2'',3''-dihydroxylpropyl. The HMBC correlations from proton at δ_H 4.56 (H-2) with carbons at δ_C 27.9 (C-4), 67.5 (C-3), 114.8 (C-2'), 131.5 (C-1'), 119.2 (C-6') and 156.2 (C-8a); δ_H 2.92/2.52 (H-4) with 67.5 (C-3), 81.8 (C-2), 99.7 (C-4a), and 155.9 (C-5); δ_H 5.92 (H-6)

with carbons at δ_C 99.7 (C-4a), 155.9 (C-5) and 94.8 (C-8); δ_H 5.89 (H-8) with carbons at δ_C 94.8 (C-6), 99.7(C-4a), and 156.3(C-7); δ_H 6.92 (H-2') with carbon at 81.8 (C-2), 119.2 (C-6') and 144.9 (C-3'); δ_H 6.80 (H-5') with carbon at 81.1 (C-2), 131.5 (C-1'), 114.8 (C-2') and δ_H 6.77 (H-6') with carbon at 144.4 (C-4') and 114.4 (C-5') suggested catechin skeleton [11-13]. Key HMBC correlations of proton at δ_H 6.92 (H-2') with 81.8 (C-2), 119.2 (C-6') and 144.8 (C-6") confirm the position of the side chain at C-3'. The side chain on ring-B was identifies as filiferol analogue (2'",4'",6'"-benzentriol-(7"-hydroxylphenyl)-2",3" dihydroxylpropyl) based on its ^{1}H NMR at δ_H 2.89/2.76 (1H, dd, J = 13.0, 6.0 Hz each, H-1"α, H-1"β), 4.89 (1H, br. s, H-2"), 4.20(1H, d, J = 4.2 Hz, H-3"), 7.06 (1H, d, J = 2.0 Hz, H-5"), 6.82 (1H, d, J = 8.2 Hz, H-8"), 6.86 (1H, dd, J = 8.2, 2.0 Hz, H-9") and 6.02 (1H, d, J = 2.2 Hz each , H-3'"/5'") suggested 2'", 4'", 6'"-benzentriol-(7"-hydroxylphenyl)-2", 3"-dihydroxylpropyl which differ from filiferol [14] in the absence of one OH group that was supported by ESI-MS showed a monomer ion peak at *m/z* 291[M+H]$^+$. The long rage HMBC coupling of δ_H 7.06 (H-5") with carbons at δ_C (78.7 (C-2"), 118.4 (C-9"), 114.4 (C-8") and 144.8 (C-3")) confirmed the point of dimeriation involing 3'-*O*-6" linkage. Based on these spectroscopic data compound **1** was identified as catechin-(3'-*O*-6")-2'",4'",6'"-benzentriol-(7"-hydroxylphenyl)-2",3"-dihydroxylpropyl dimer. The position of the dimer is also confirmed from the carbon chemical shift value that it is downfield shifted than the value of C-6" (δ_C 144.8) for 2'",4'",6'"-benzentriol-(7"-hydroxylphenyl)-2",3"-dihydroxylpropyl monomer. It is worth to mention that biflavonoids including those with C–*O*–C linkage are common to the genus [15-18].

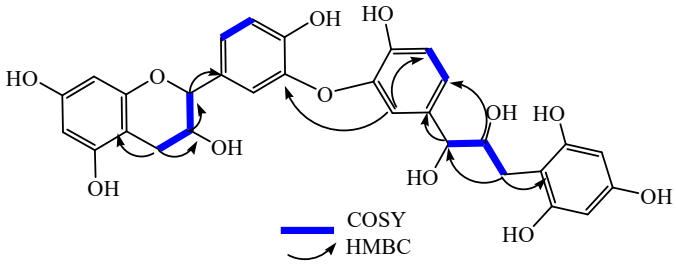

Figure 2. Key COSY and HMBC correlations.

The known compounds were identified based on their spectroscopic data and comparison with reported literature values.

Compound **2** was isolated as yellow amorphous solid with a melting point of 159-160 °C. ^{1}H-NMR: 7.99 (1H, d, 9.0 Hz, H-6'), 7.94 (1H, s, H-6'"), 7.86 (1H, d, 15.3 Hz, H-β'), 7.82 (1H, d, 15.4 Hz, H- β), 7.71 (2H, d, 8.6 Hz, H-2"/6"), 7.69 (1H, d, 15.4 Hz, H-α), 7.63 (1H, d, 8.7 Hz, H-2/6), 7.59 (1H, d, 15.3 Hz, H-α'), 6.92 (2H, d, 8.6 Hz, H-3"/5"), 6.83 (1H, d, 8.7 Hz, H-3/5), 6.71 (1H, s, H-3'"), 6.43 (1H, dd, 8.9, 2.4 Hz, H-5'), 6.31 (1H, d, 2.4 Hz, H-3') and 3.83 (3H,s, H-OCH_3(4')). ^{13}C-NMR: 191.9 (C=O), 191.8 (C=O), 166.2 (C-4'), 165.2 (C-2'), 163.8 (C-4'"), 161.2 (C-4"), 160.4 (C-4), 158.9 (C-2'"), 145.3 (C-β'), 143.4 (C-β), 135.1 (C-5'"), 132.0 (C-6'), 130.7 (C-2"/6"), 130.1 (C-2/6), 128.7 (C-1), 126.4 (C-1"), 123.2 (C-6'"), 118.5 (C-α), 116.5 (C-α'), 115.5 (C-3"/5"), 115.3 (C-3), 115.3 (C-5), 113.3 (C-1'"), 112.0 (C-1'), 107.8 (C-5'), 102.4 (C-3'), 101.0 (C-3'"), 55.3(-OMe) identified as 2',4',4",2'"-tetrahydroxy-4'"-methoxy-4-O-5'"-bichalcone [19].

Compound **3** was isolated as yellow powder with a melting points of 230-232 °C. ^{1}H-NMR: 8.30 (1H, d, 7.8 Hz, H-6'), 8.27(1H, s, H-6'"), 8.07 (1H, d, 15.2 Hz, H-β'), 7.93 (1H, d, 15.4 Hz, H-β), 7.90 (2H, d, 8.6 Hz, H-2"/6"), 7.88 (1H, d, 15.3 Hz, H-α), 7.82 (1H, d, 8.4 Hz, H-2/6), 7.79 (1H, d, 15.2 Hz, H-α'), 6.95 (2H, d, 8.6 Hz, 3"/5"), 6.83 (1H, d, 8.0 Hz, H-3/5), 6.77 (1H, s, H-3'"), 6.57 (1H, dd, 8.4, 2.2 Hz, H-5'), 6.52 (1H, d, 2.2 Hz, H-3'), 3.85(3H, s, H-4'), 3.81(3H, s, H-

4'''). ^{13}C-NMR: 192.3 (C=O), 192.1 (C=O), 166.4 (C-4'), 166.2 (C-2'), 164.0 (C-4'''), 161.3 (C-4''), 160.9 (C-4), 159.1 (C-2'''), 145.8 (C-β'), 144.4 (C-β), 134.9 (C-5'''),133.1 (C-6'), 132.1 (C-2''/6''),131.6 (C-6), 131.6 (C-2), 128.8 (C-1), 126.2 (C-1''),124.7 (C-6'''), 119.7 (C-α), 117.8 (C-α'), 116.2 (C-3''/5''), 115.7 (C-5), 115.7 (C-3), 114.3 (C-1'''), 113.2 (C-1'), 107.9 (C-5'), 102.1 (C-3'), 101.3 (C-3'''), 56.8 (-OMe) and 56.2 (-OMe) identified as rhuschalcone I [20].

Compound **4** was isolated as yellow solid substance with melting points of 296-298 °C. ^{1}H-NMR: 7.97 (1H, d, 2.4 Hz, H-6'), 7.92 (1H, d, 8.2 Hz, H-2'''/6'''), 6.64 (1H, s, H-3), 7.55 (1H, d, 8.4 Hz, H-2'), 7.15 (1H, d, 8.4 Hz, H-3'), 6.75 (1H, d, 8.3 Hz, H-3'''/5'''), 6.64 (1H, s, H-3''), 6.42 (1H, d, 2.3 Hz, H-6), 6.45 (1H, d, 2.3 Hz, H-8) and 6.21 (1H, s, H-8''). ^{13}C-NMR: 184.2 (C-4''), 183.8 (C-4), 166.2 (C-2), 166.0 (C-7), 165.9 (C-5''), 163.5 (C-5), 163.2 (C-2''), 162.5 (C-7''), 161.0 (C-4'), 159.5 (C-9/9''), 156.7 (C-4'''), 132.9 (C-6'), 129.3 (C-2'), 129.1 (C-5'), 128.8 (C-6'''), 128.2 (C-2'''), 123.2 (C-1'), 121.5 (C-1'''), 117.6 (C-3'), 116.8 (C-3'''/5'''), 105.4 (C-6''), 105.3 (C-10/10''), 104.1 (C-3), 103.7 (C-3''), 100.3 (C-8''), 99.9 (C-6) and 95.3 (C-8) identified as robustaflavone [21-22].

Compound **5** was isolated as an amorphous yellow powder with a melting point of 246-248 °C. ^{1}H-NMR: 8.31 (1H, s, H-2), 8.15 (1H, d, 9.1 Hz, H-5), 7.96 (1H, d, 9.0 Hz, H-`18), 7.18 (1H, d, 8.5 Hz, H-21/25), 7.14 (1H, d, 8.6 Hz, H-27/31), 6.99 (1H, dd, 9.0, 2.4 Hz, H-17), 6.95 (1H, d, 2.4 Hz, H-8), 6.61(1H, d, 8.5 Hz, H-28/30), 6.56 (1H, d, 8.5 Hz, H-22/24), 6.35 (1H, dd, 9.0, 2.4 Hz, H-6), 6.14 (1H, d, 2.4 Hz, H-15), 6.02 (1H, d, 12.2 Hz, H-11), 4.69 (1H, d, 12.1 Hz, H-19). ^{13}C-NMR: 203.2 (C-12), 175.6 (C-4), 165.7 (C-14), 165.4 (C-7), 164.6 (C-16), 157.9 (C-9), 156.2 (C-2), 155.3 (C-29), 155.2 (C-23), 134.4 (C-13), 133.4 (C-20/26), 132.9 (C-5), 130.3 (C-27/31), 128.9 (C-21/25), 128.4 (C-18),121.3 (C-3), 116.6 (C-17), 115.5 (C-24), 114.9 (C-30), 114.7 (C-22), 114.6 (C-28), 107.9 (C-6), 102.1 (C-15), 99.8 (C-8), 55.1 (OCH_3), 52.9 (C-19), 43.2 (C-11) and 12.8 (C-10) identified as (3-(1-(2,4-dihydroxyphenyl)-3,3-bis(4-hydroxyphenyl)-1-oxo-propan-2-yl)-7-methoxy-4H-chromone-4-one [23].

The free radical scavenging activities of compounds (**2-5**) were evaluated by DPPH assays using ascorbic acid as standard (Table 2). The result showed that all compounds (**2-5)** showed significant DPPH scavenging activity with inhibition percent 78.32, 68.90, 93.22 and 92.00, respectively compared with 84.02% inhibition for ascorbic acid. This scavenging activity was also confirmed by the IC_{50} values of the compounds. The IC_{50} values were found to be 10.80, 26.03, 7.90 and 8.40 µg/mL for compounds **2-5**, respectively compared with IC_{50} value for ascorbic acid was 9.90 µg/mL [24]. As displayed in the (Table 2) compound **4** exhibited the strongest activity for the DPPH radical scavenging activity [**25**], followed by compound **5**. Correspondingly, their IC_{50} values were 7.90 µg/mL and 8.40 µg/mL, respectively, which were both lower than the positive control (ascorbic acid) 9.90 µg/mL. The high antioxidant activity of the compounds were due to the presence of free hydroxyl groups in the structures that can donate H· (radical hydrogen) to reduce free radicals [26] and polyphenols are potent antioxidants [27]. The result of the study showed that all compounds (**2-5)** exhibited a DPPH free radical scavenging activity is in agreement with the wide range of pharmacological activities of biflavonoids, including anti-inflammatory, antioxidant, antibacterial, antiviral, antidiabetic, antitumor, and cytotoxic properties [28, 29].

Table 2. Percentage inhibition of DPPH radical scavenging activities of compounds (**2-5**).

Compound	% DPPH inhibition at different concentration							IC_{50} value
	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25µg/mL	3.13 µg/mL	1.56 µg/mL	
2	78.32±0.02	70.11±0.02	60.50±0.03	52.61±0.01	37.34±0.02	18.25±0.03	3.25± 0.01	10.8
3	68.90±0.01	55.35±0.02	46.62±0.01	35.98±0.02	29.48±0.01	18.41±0.01	1.61± 0.01	26.03
4	93.22±0.01	90.00±0.01	87.10±0.03	70.21±0.01	53.33±0.01	33.25±0.01	12.13± 0.01	7.90
5	92.31±0.01	80.01±0.03	69.85±0.02	59.21±0.01	50.11±0.02	30.50±0.03	11.05±0.01	8.40
Ascorbic acid	84.02±0.01	83.22±0.01	81.01±0.02	70.80±0.01	29.66±0.01	2.61±0.01	1.45±0.01	9.90

CONCLUSION

Phytochemical investigation of DCM/MeOH root extract of *R. ruspolli* gave one new biflavonoids, named catechin-(3'-*O*-6")-2'",4'",6'"-benzentriol-(7"-hydroxylphenyl)-2",3"-dihydroxylpropyl (**1**), along with four known biflavonoids. The isolated compounds (**2-5**) exhibited antioxidant activity with compounds **4** and **5** showed excellent activity with IC_{50} values of 7.90 and 8.40, respectively. The isolation of these bioactive compounds from this plant and antioxidant activities of these compounds suggest the potential use of these compounds as drug lead candidates.

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