

Original Research Article**IN-VITRO ANTIVIRAL ACTIVITY OF *PISTACIA CHINENSIS* FLAVONOIDS AGAINST HEPATITIS C VIRUS (HCV)*****Khaled Rashed¹, Noémie Calland², Gaspard Deloison³, Yves Rouillé², Karin Séron²**

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ABSTRACT

The present study was designed to evaluate anti-HCV activity of *Pistacia chinensis* aerial parts and identify the responsible bioactive constituents of anti-hepatitis ethnomedicinal plant *P. chinensis* methanol 80% extract. This has led to the isolation and characterization of gallic acid and eight flavonoids, apigenin, diosmetin, myricetin, apigenin 7-*O*- β -glucoside, quercetin 3-*O*- β -glucoside myricetin 3-*O*- α -rhamnoside, myricetin 3-*O*- β -glucuronide and quercetin 3-*O*- β -glucoside-7-*O*- α -rhamnoside from the plant, using various chromatographic procedures and the interpretation of spectral data in comparison with already existing data reported in the literature. Methanol 80% extract of *P. chinensis* and some isolates were tested for their anti-HCV activity using HCV cell culture (HCVcc) system. The results have shown that diosmetin and apigenin, significantly reduce HCV infection while myricetin sugars (myricetin 3-*O*- α -rhamnoside and myricetin 3-*O*- β -glucuronide) had no significant effect on HCV infection. The half maximal inhibitory concentration (IC₅₀) of diosmetin and apigenin were calculated to be 42.5 μ M and 39.9 μ M respectively. However, cell viability assays demonstrated that apigenin was toxic in cell culture in the same range of concentrations that show HCV inhibition. This is the first time report for anti-HCV activity of diosmetin isolated from *P. chinensis* and this could become a molecular template for the development of new anti-HCV drugs.

Keywords: *Pistacia chinensis*, aerial parts, flavonoids, antiviral activity, HCV, diosmetin

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INTRODUCTION

Hepatitis C virus (HCV) is a major cause of chronic liver disease which can lead to permanent liver damage, hepatocellular carcinoma and death [1]. Egypt reports the highest prevalence of HCV worldwide [2,3]. The presently available treatment with pegylated interferon plus ribavirin, has limited benefits due to adverse side effects and high cost. The recent addition to the therapy of direct acting antivirals (DAA), specific inhibitors of the viral protease NS3/4A, concerns only

HCV genotype 1 infected patients and are also accompanied by numerous adverse effects [4]. It is important to note that, in Egypt, HCV genotype 4 is the most prevalent. Hence, there is a need to develop anti-HCV agents that are less toxic and cost-effective [5,6]. In spite the highly vigorous and extensive research in this field, a protective vaccine and effective treatment for HCV genotype 4 infected patients are not yet available [7,8].

Herbal medicines have been used for centuries against different ailments including viral diseases and become a focal point to identify, isolate and purify new entities to treat diseases like hepatitis C. In the course of our studies on development of HCV inhibitors from naturally occurring products, we focused on medicinal plants. *Pistacia chinensis* is a deciduous and small to medium-sized tree from Anacardiaceae family. It is widely distributed in China and North America. It is well known as a landscape and shade tree [9]. In Chinese traditional medicine, the oil from its seeds is used for biodiesel production in China and it shows a high resistance to various pests in the United States [9]. There are very few reports about chemical constituents and biological activities from *P. chinensis* plant. Two 4-arylcoumarin moieties (neoflavone) dimers were isolated from of *P. chinensis* leaves with estrogen-like activity [10]. Also some phenolic compounds such as gallic acid, m-digallic acid, quercetin, 6-O-galloyl arbutin-quercitrin and quercetin-3-O(6"-galloyl)- β -D-glucosides were isolated from the leaves [11] and a new pyrrolidone derivative was isolated from *P. chinensis* tender burgeon and anthotaxy [12]. *P. chinensis* has the ability to inhibit NO production as anti-inflammatory potential of this plant [13]. This research was carried out to evaluate antiviral activity of naturally derived extract and bio-active compounds on HCV from *P. chinensis* plant.

MATERIALS AND METHODS

General experimental procedures

UV/VIS: Shimadzu UV-visible recording spectrophotometer model-UV 240 (NRC, Egypt). $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ (Varian Unity Inova). MS (Finnigan MAT SSQ 7000, 70 ev). (Silica gel (0.063-0.200 mm for column chromatography) and Sephadex LH-20 (Pharmacia Fine Chemicals). Thin layer chromatography (TLC) F₂₅₄ plates. Solvent mixtures, BAW (*n*-butanol:acetic acid:water 4:1:5 upper phase, 15% acetic acid: water: glacial acetic acid: 85:15). Paper Chromatography (PC) Whatman No.1 (Whatman Led.Maid Stone, Kent, England) sheets for qualitative detection of flavonoids and sugars were used in this study.

Plant identification and collection

Aerial parts of *P. chinensis* were collected from Al-Zohiriya garden, Giza, Egypt in May 2012. The plant was identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Tereza Labib consultant of plant taxonomy at the Ministry of Agriculture and director of Orman botanical garden, Giza, Egypt. A voucher specimen is deposited in the herbarium of Al-Zohiriya garden, Giza, Egypt.

Chemicals

Dulbecco's modified Eagle's medium (DMEM), phosphate-buffered saline (PBS), glutamax-I, goat, and fetal calf sera were purchased from Invitrogen (Carlsbad, CA). 4',6-Diamidino-2-phenylindole (DAPI) was from Molecular Probes (Invitrogen). Epigallocatechin-3-gallate (EGCG) was from Calbiochem (Merck Chemicals, Darmstadt, Germany), and for cytotoxicity

experiments apigenin and diosmetin were purchased from Extrasynthèse (Lyon, France). Stocks were resuspended in dimethylsulfoxide (DMSO) at 0.5 M.

Antibodies, cells and culture conditions

Mouse anti-E1 monoclonal antibody (MAb) A4 [14] was produced *in vitro*. Cy3-conjugated goat anti-mouse IgG was from Jackson Immunoresearch (West Grove, PA). Huh-7 [15] and HEK 293T (ATCC number CRL-11268) cells were grown in DMEM supplemented with glutamax-I and 10% fetal calf serum.

Virus

A modified Japanese Fulminant Hepatitis-1 (JFH1) virus containing titer-enhancing mutations [16] and in which the A4 epitope of HCV glycoprotein E1 of strain H77 was reconstituted [17].

Preparation of the extract

Air-dried powder of *P. chinensis* aerial parts (800 g) was extracted with methanol 80% several times at room temperature until exhaustion by maceration method. The extract was concentrated under reduced pressure to give 42 g of the crude extract.

Isolation of bioactive compounds from methanol 80% extracts of *P. chinensis* aerial parts

40 g of methanol extract was subjected to silica gel column chromatography eluting with hexane, dichloromethane, ethyl acetate and methanol gradually.

One hundred and eighty fractions of 100 ml conical flask were collected. The fractions that showed similar Paper Chromatography (PC) in Butanol–Acetic acid–Water 4:1:5 (BAW) and 15% acetic acid were combined to give 4 fractions (I, II, III, and IV). Fraction I (1.2 g) was subjected to sub-column of silica gel eluted with dichloromethane: ethyl acetate (60:40) gave compound 1 and elution with dichloromethane: ethyl acetate (80:20) gave compound 2. Fraction II (928 mg) was subjected to sub-column of silica gel eluted with dichloromethane: ethyl acetate (95:5) yielded compound 3 and elution with ethyl acetate solvent gave compound 4. Compound 5 yielded from elution with ethyl acetate : methanol (95: 5) and compound 6 was obtained from elution with ethyl acetate: methanol (90:10) and also compound 7 was obtained by elution with ethyl acetate: methanol (85:15) from fraction III (1.45 g). Compound 8 yielded by elution with ethyl acetate: methanol (75:25) and compound 9 was obtained by elution with ethyl acetate: methanol (60:40) from fraction IV (1.35 g). All the isolated compounds were purified on sephadex LH-20 column using different systems of methanol and distilled water.

General method for acid hydrolysis of flavonoid glycosides

5 mg of each flavonoid glycoside 5, 6, 7, 8 and 9 in 5 ml 10% HCl was heated for 5 h. The aglycones were extracted with ethyl acetate and identified by co-TLC with authentic standards. The sugars in the aqueous layer were identified by co-paper chromatography (co-PC) with authentic markers on Whatman No. 1 sheets in solvent system (*n*-BuOH-AcOH-H₂O 4:1:5 upper layer).

Viability assay

Subconfluent cell cultures grown in 96-well plates were incubated in culture medium. An MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] based viability assay (Cell Titer 96 aqueous non radioactive cell proliferation assay from Promega) was conducted as recommended by the manufacturer.

Anti-HCV inhibition study

The day before infection, Huh-7 cells were plated in 96-well plates at a density of 6.000 cells/well. Huh-7 cells were infected with HCVcc at a multiplicity of infection of 0.7 in the presence of given concentrations of plant extracts or molecules. The inoculum was removed and cells were overlaid with fresh medium. After 28 h, infected cells were processed for immunofluorescent detection of E1 envelope glycoprotein as previously described by Rouillé *et al.*, 2006 [18]. Quantity of cell/well and multiplicity of infection (MOI) were adjusted to obtain 20-40% of infected cells at 30 h post infection, allowing the automated quantification. The plates were analyzed with a High Content Screening (HCS) Operetta machine (Perkin Elmer) and the signals quantified with the Harmony software.

RESULTS AND DISCUSSION

The present investigation was focused on the evaluation of anti-HCV activity of *P. chinensis* methanol 80% extract and of flavonoids isolated from the extract. We investigated the presence of phytochemicals and bioactive constituents in *P. chinensis* aerial parts methanol extract. The major bioactive components of *P. chinensis* are gallic acid and eight flavonoids, apigenin, diosmetin, myricetin, apigenin 7-*O*- β -glucoside, quercetin 3-*O*- β -glucoside, myricetin 3-*O*- α -rhamnoside, myricetin 3-*O*- β -glucuronide and quercetin 3-*O*- β -glucoside-7-*O*- α -rhamnoside. The chemical structures of the bio-active components were elucidated by different spectroscopic analyses and shown in Figure 1.

Structure Elucidation of the isolated compounds

Gallic acid (1): 21 mg, white amorphous powder. UV λ_{\max} (MeOH): 273. $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ 7.15 (2H, s, H-2, 6). $^{13}\text{C-NMR}$ (DMSO- d_6 , 100 MHz): δ 167.2 (-COOH), 145 (C-3,5), 137.7 (C-4), 121.2 (C-1), 109.1 (C-2,6).

Apigenin (2): 27 mg, yellow powder. $^1\text{H-NMR}$ (CD₃OD, 500 MHz): δ ppm 7.85 (2H, d, $J = 8.5$ Hz, H -2', H 6'), 6.95 (2H, d, $J = 8.5$ Hz, H-3', H-5'), 6.54 (1H, s, H-3), 6.42 (1H, s, H-8), 6.28 (1H, s, H-6). EI-MS: m/z 270.

Diosmetin (4'-methoxy luteolin) (3): 22 mg, yellow powder. UV λ_{\max} (MeOH): 247, 264, 345, (NaOMe): 249, 302, 388, (AlCl₃): 273, 352, 400 (AlCl₃/HCl): 272, 352, 400, (NaOAc): 252, 382, (NaOAc/H₃BO₃): 252, 382. $^1\text{H-NMR}$ (DMSO- d_6 , 500 MHz): δ ppm 7.65 (1H, d, $J = 2.4$ Hz, H2'); δ 7.35 (1H, dd, $J = 7.6, 2.4$ Hz, H-6'); δ 6.85 (1H, d, $J = 7.6$ Hz, H-5'); δ 6.68 (1H, d, $J = 2.4$ Hz, H-8); δ 6.45 (1H, d, $J = 2.2$ Hz, H6); δ 6.65 (1H, s, H3); δ 3.9 (3H, s, OCH₃). $^{13}\text{C-NMR}$ (DMSO- d_6 , 100 MHz): δ ppm 162.9 (C-2), 103.8 (C-3), 182.6 (C-4), 161.4 (C-5), 98.7.0 (C-6), 164.7 (C-7), 94.2 (C-8), 157.6 (C-9), 104.2 (C-10), 123.4 (C-1'), 112.8 (C-2'), 147.3 (C-3'), 151.4 (C-4'), 112.7 (C-5'), 119.1 (C-6') and 55.6 (OCH₃).

Myricetin (4): 22 mg, yellow powder. UV λ_{\max} (MeOH): 254, 272sh, 374; (NaOMe): 262sh, 285sh, 322sh, 423(Dec.); (AlCl₃): 271, 316sh, 450; (AlCl₃/HCl): 266, 275sh, 308sh, 360sh, 428; (NaOAc): 269, 335; (NaOAc/H₃BO₃): 258, 304sh, 392. EI-MS: m/z 318.

Apigenin 7-*O*- β -glucoside (5): 24 mg, yellow powder. UV λ_{\max} (MeOH): 269, 332, (NaOMe): 272, 387, AlCl₃: 277, 299, 346, 383, AlCl₃/HCl: 277, 298, 342, 383, NaOAc: 268, 338 NaOAc/H₃BO₃: 268, 335. $^1\text{H-NMR}$ (DMSO- d_6 , 300 MHz): δ ppm 7.94 (2H, d, $J = 8.5$ Hz, H-2', H-6'), δ 6.92 (2H, d, $J = 8.5$ Hz, H-3', H-5'), δ 6.87 (1H, s, H-3), δ 6.84 (1H, d,

$J= 2.0\text{Hz}$, H-8), δ 6.42 (1H, d, $J= 2.0\text{Hz}$, H-6), δ 5.08 (1H, d, $J= 7.2$ Hz, H-1''), δ 3.1-4 (rest of sugar protons, H-2'' to H-6''). ¹³C-NMR: (DMSO- d_6 , 100 MHz): δ ppm 182.2 (C-4), 164.5 (C-2), 163.8 (C-7), 162.6 (C-5), 161.4 (C-4'), 157.5 (C-9), 129.4 (C-2',6'), 121.6 (C-1'), 116.8 (C-3',5'), 105.6 (C-10), 103.7 (C-3), 100.6 (C-1''), 100.2 (C-6), 95.8 (C-8), 77.6 (C-5''), 76.9 (C-3''), 73.8 (C-2''), 70.4 (C-4''), 61.2 (C-6'').

Quercetin 3-*O*- β -glucoside (6): 21 mg, yellow crystals, ¹H-NMR (DMSO- d_6 , 400 MHz): δ ppm 7.78 (1H, dd, $J=2$, 8.5 Hz, H-6'), 7.54 (1H, d, $J=2$ Hz, H-2'), 6.82 (1H, d, $J=8.5$ Hz, H-5'), d 6.42 (1H, d, $J= 2$ Hz, H-8), 6.24 (1H, d, $J= 2$ Hz, H-6), 5.5 (1H, d, $J=7.5$ Hz, H-1''). (-) ESI-MS: m/z 463 [M-H]⁻.

Myricetin 3-*O*- α -rhamnoside (7): 30 mg, yellow amorphous powder. UV λ_{max} (MeOH): 260, 296sh, 352; (NaOMe): 273, 321, 392; (AlCl₃): 272, 312, 420; (AlCl₃/HCl): 270, 310, 404; (NaOAc): 270, 317, 364; (NaOAc/H₃BO₃): 260, 303, 376. ¹H-NMR (DMSO- d_6 , 400 MHz): δ ppm 6.89 (2H, s, H-2'/6'), δ 6.2 (1H, d, $J = 2.5$ Hz, H-6), δ 6.37 (1H, d, $J = 2.5$ Hz, H-8), 5.2 (1H, s, H-1''), 3.9-3.2 (m, remaining sugar protons), 0.8 (CH₃-rhamnosyl, d, $J = 6$ Hz, H-6'').

Myricetin 3-*O*- β -glucuronide (8): 26 mg, yellow amorphous powder. UV λ_{max} (MeOH): 262, 298sh, 349; (NaOMe): 272, 324, 392; (AlCl₃): 272, 312, 428; (AlCl₃/HCl): 270, 310, 404; (NaOAc): 270, 318, 366; (NaOAc/H₃BO₃): 260, 300, 374. ¹H-NMR (CD₃OD, 400 MHz): δ ppm 7.42 (2H, s, H-2',6'), 6.45 (1H, d, $J = 1.2$ Hz, H-8), 6.22 (1H, d, $J = 1.2$ Hz, H-6), 5.47 (1H, d, $J = 7.5$ Hz, H-1''). ¹³C-NMR (CD₃OD, 100 MHz): δ ppm 177.5 (C-4), 174 (C-6''), 165.8 (C-7), 162.6 (C-5), 158.4 (C-9), 148.2 (C-2), 146.9 (C-3',5'), 137.5 (C-3), 137.1 (C-4'), 123.3 (C-1'), 108.8 (C-2',6'), 104.7 (C-10), 104 (C-1''), 99.5 (C-8), 94.6 (C-6), 78.2 (C-3''), 78 (C-5''), 75.6 (C-2''), 73.4 (C-4'').

Quercetin 3-*O*- β -glucoside-7-*O*- α -rhamnoside (9): 26 mg, yellow powder. UV λ_{max} (MeOH): 258, 295 sh, 359 (NaOMe): 267, 409; (AlCl₃): 274, 303 sh, 339 sh, 427 (AlCl₃/HCl): 270, 298 sh, 359, 403; (NaOAc): 260, 396sh, 368, 416 (NaOAc/H₃BO₃): 262, 295 sh, 379. ¹H-NMR (DMSO- d_6 , 500 MHz): δ ppm 7.64 (2H, multiplet, H-2', H-6'), δ 6.85 (1H, d, $J= 8.5$ Hz, H-5'), δ 6.79 (1H, d, $J= 2.0$ Hz H-8), δ 6.45 (1H, d, $J= 2.0\text{Hz}$, H-6), δ 5.45 (1H, d, $J= 7.5\text{Hz}$ H-1''), δ 5.52 (1H, d, $J= 2.0$ Hz, H-1'''), δ 1.08 (3H, d, $J= 6.2$ Hz, CH₃ of Rhamnose sugar), δ 3.1-4 (9H, m, glucose protons, H-2''- H-6'', rhamnose protons, H-2'''- H-5''').

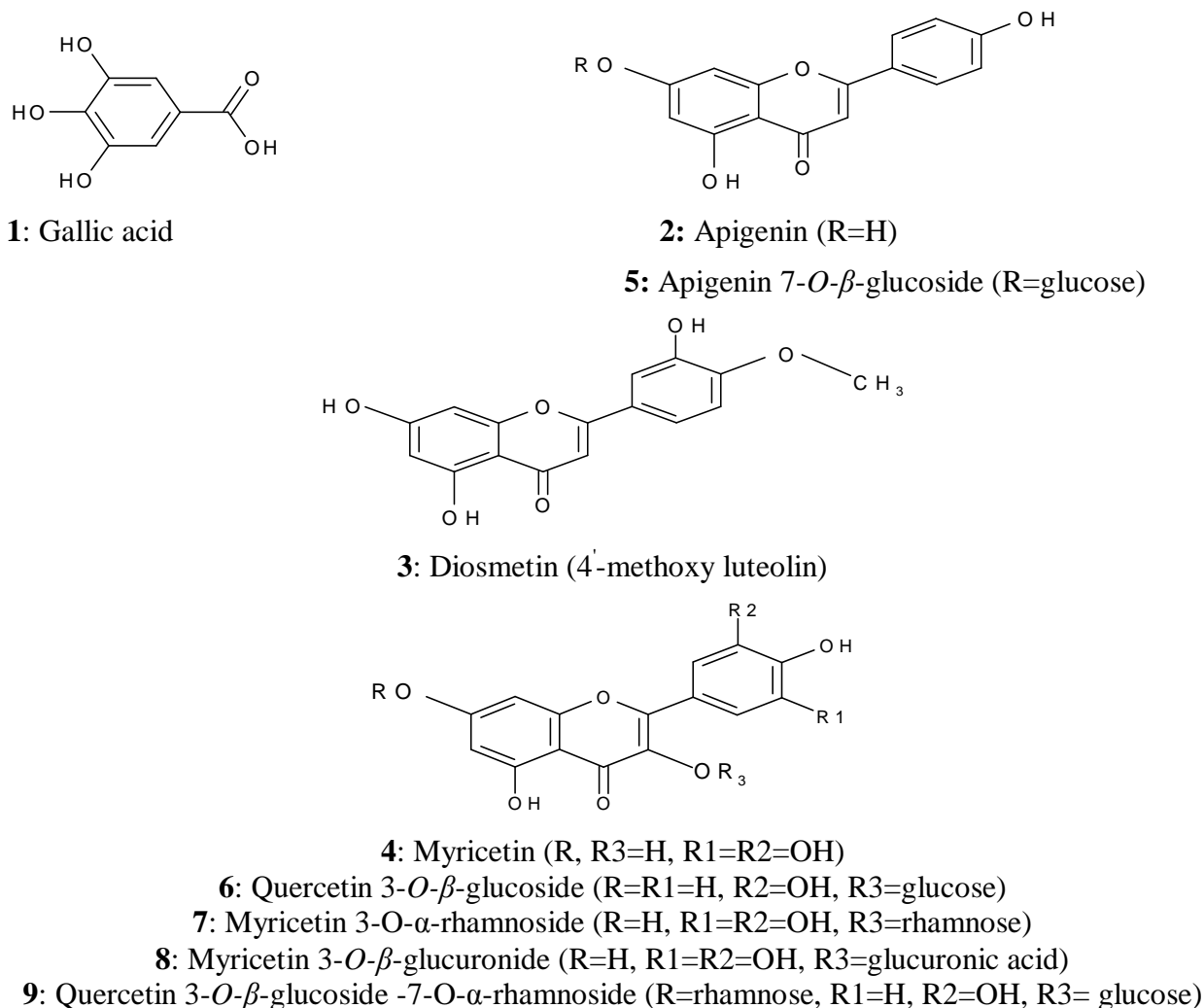


Figure 1. Chemical structures of the compounds isolated from *P. chinensis* methanol extract

Identification of the isolated compounds of *P. chinensis* methanol extract

Compound 1 (gallic acid) showed a single violet spot under short UV light and it gave black green colour with ferric chloride reagent confirming the presence of the phenolic gallic moiety [19] and confirmation of the chemical structure of compound 1 was proved by comparison of spectral data with spectra of Naira and Karvekar 2010 [20]. Compound 2 (apigenin) was obtained as a deep purple spot and the compound gave yellow colour when exposed to ammonia vapour and gave a bright yellow colour when spraying with AlCl₃ [21], spectral data of this compound is very close to spectra of Fatemeh *et al.* 2006 [22]. Compound 3 (Diosmetin, 4'-methoxy luteolin) has appeared as a single deep purple spot when exposed to ammonia vapour, this compound gave yellow colour and also it gave a bright yellow colour when spraying with AlCl₃ (Mabry *et al.*, 1970). The compound spectral data was very similar to spectra of Lunesa *et al.* 2011 [23]. Compound 4 (myricetin) was obtained as yellow green spot and gave a bright yellow colour when spraying with AlCl₃ [21] and its spectral data is identical to that of Chhagan *et al.* 2011 [24]. Compound 5 (apigenin 7-O-β-glucoside) is obtained as a deep purple spot and

the compound gave yellow colour when exposed to ammonia vapour and gave a bright yellow colour when spraying with AlCl_3 [21], acid hydrolysis of the compound gave apigenin as an aglycone and glucose as sugar moiety. Spectral data of this compound is very close to spectra of Ahmad *et al.* (2011 [25]). Compound 6 (quercetin 3-*O*- β -glucoside) is obtained as deep purple spot and the compound gave yellow colour when exposed to ammonia vapour and gave a bright yellow colour when spraying with AlCl_3 [21], acid hydrolysis of the compound gave quercetin as an aglycone and glucose as sugar moiety. Spectral data of this compound is very close to spectra of Ning *et al.* 2007 [26]. Compound 7 (myricetin 3-*O*- α -rhamnoside) and compound 8 (myricetin 3-*O*- β -glucuronide) both gave deep purple spot and both compounds gave yellow colour when exposed to ammonia vapour and also gave a bright yellow colour when spraying with AlCl_3 [21], acid hydrolysis of the two compounds gave myricetin as an aglycone and rhamnose and glucuronic acid as sugar moieties, respectively, and spectral data of the two compounds are very close to spectra of Rashed *et al.* 2012 [27]. Compound 9 (quercetin 3-*O*- β -glucoside-7-*O*- α -rhamnoside) was isolated as yellow powder with a deep brown spot under UV light and this compound gave yellow orange colour when exposed to ammonia vapour and gave a bright yellow colour when spraying with AlCl_3 [21]. Complete acid hydrolysis of compound 9 yielded quercetin (Co-PC) as an aglycone and glucose and rhamnose as the sugar moieties. UV spectral data suggested that compound 9 is quercetin with substitution in positions 3 and 7 with free hydroxyl groups at 5, 3', 4' positions [21]. $^1\text{H-NMR}$ spectral data of compound 9 showed very similar proton signals to quercetin 3-*O*- β -glucoside-7-*O*- α -rhamnoside [28].

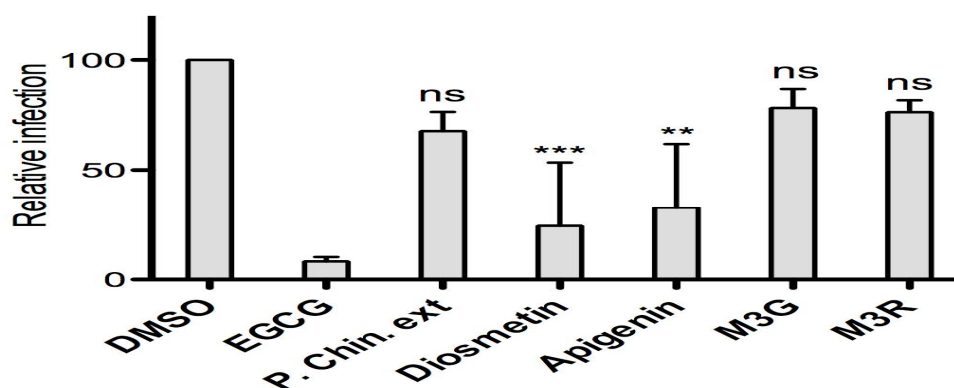


Figure 2A. Anti-HCV effect and toxicity in hepatoma cells of *P. chinensis* methanol extract and purified compounds. Anti-HCV inhibition in Huh-7 cells. Huh-7 cells were inoculated with HCVcc in the presence of *P. chinensis* extract at 25 $\mu\text{g}/\text{ml}$ or purified compounds at 50 μM for 2h. DMSO was used as a control. M3G: myricetin 3-*O*- β -glucuronide; M3R: myricetin 3-*O*- α -rhamnoside. Cells were further incubated in medium containing plant extract or molecules for 28 h. Cells were fixed and infectivity quantified by immunofluorescence by calculating the number of infected cells. Statistical analyses were performed using Mann-Whitney non parametric test

(*= $p < 0.05$; **= $p < 0.01$; ns: not significant)

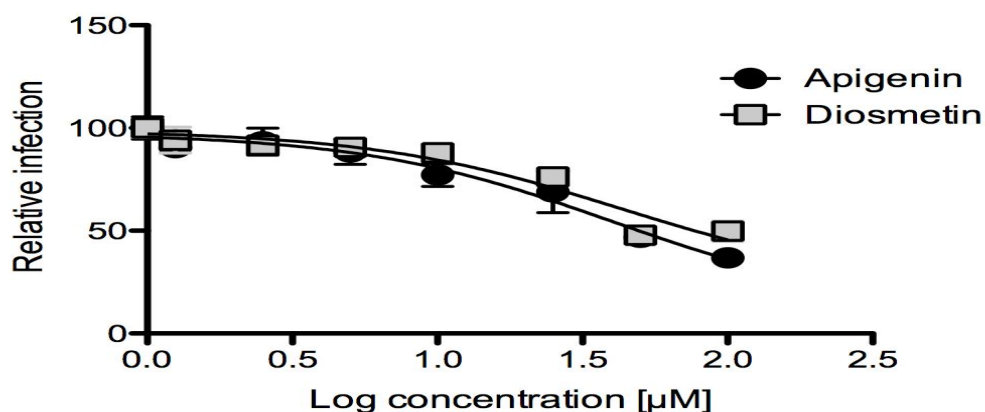


Figure 2B. Dose-response inhibition study of apigenin and diosmetin. Huh-7 cells were infected with HCVcc for 2 h in the presence of given concentrations of apigenin and diosmetin and further incubated in molecule-containing medium for 28 h. Cells were fixed and infectivity quantified by immunofluorescence

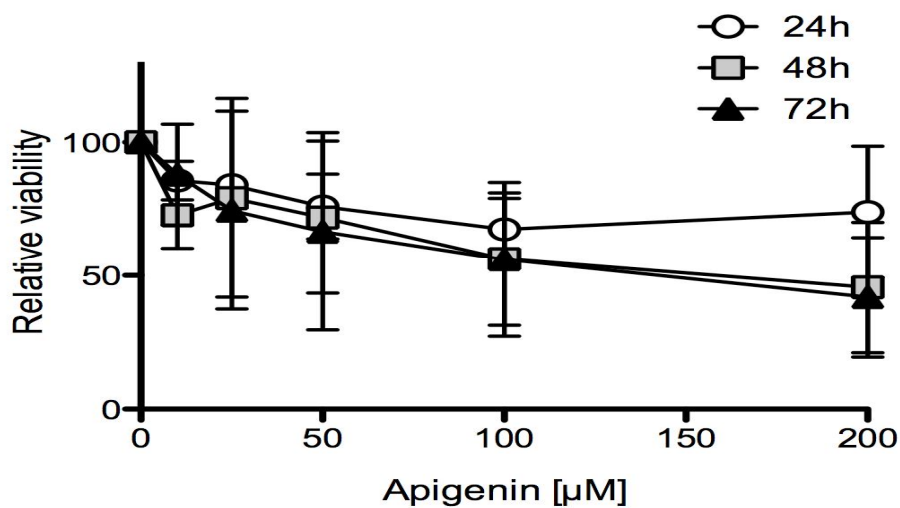


Figure 2C. Cellular toxicity study of apigenin

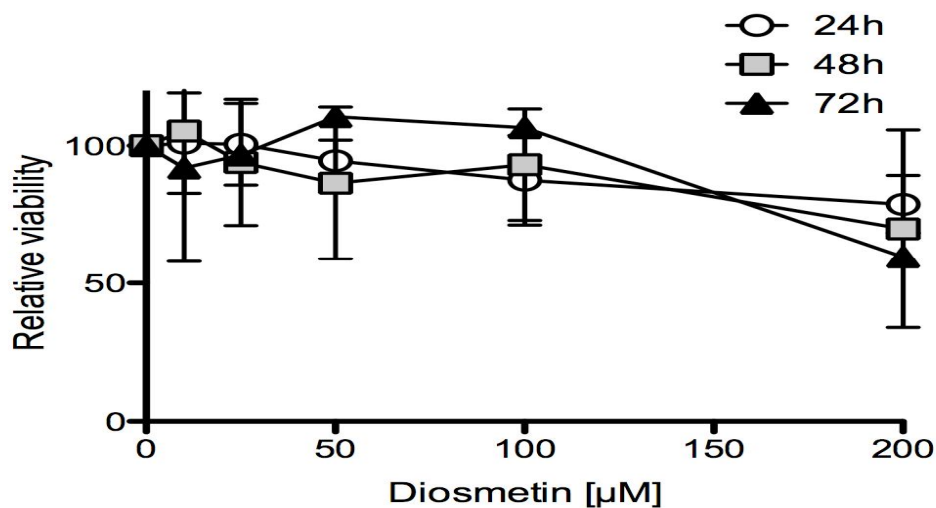


Figure 2D. Cellular toxicity study of diosmetin. Huh-7 cells were cultured in the presence of given concentrations of apigenin or diosmetin. The viability was monitored using a MTS-based viability assay after 24 h, 48 h and 72h by determining the OD at 490 nm. Values relative to the condition without molecules are given for which a value of 1 was attributed. Data are means of values obtained in 3 independent experiments performed in triplicate. Error bars represent SD of the mean of the 3 experiments.

Anti-HCV activity of *P. chinensis* methanol extract and some isolated compounds

To determine the anti-HCV capacity of *P. chinensis* methanol extract, it was added at 25 µg/ml during inoculation of Huh-7 hepatoma cells with HCVcc and during the 28h post-inoculation. The number of infected cells was quantified at 30 h post infection. EGCG was used as a positive control, since it was recently identified as a potent inhibitor of HCV entry [29,30]. Our results show that *P. chinensis* extract at 25 µg/ml reduces HCV infection from 100% in the control to 70% (Figure 2A). Although this decrease was not statistically significant, it nonetheless suggested the presence of active compounds in the extract. Then, the purified compounds apigenin, diosmetin, myricetin 3-*O*- α -rhamnoside and myricetin 3-*O*- β -glucuronide were challenged for their anti-HCV capacities. Compounds were added at 50 µM during all the infection process as described above. Apigenin and diosmetin reduced significantly HCV infection, whereas myricetin 3-*O*- α -rhamnoside and myricetin 3-*O*- β -glucuronide had no effect on HCV infection (Figure 2A). To further characterize the anti-HCV activity of apigenin and diosmetin, a dose-response study was performed (Figure 2B). The half maximal inhibitory concentrations (IC_{50}) of the two compounds were calculated as $IC_{50}=39.9$ µM for apigenin, and $IC_{50} = 42.5$ µM for diosmetin. Because a number of dead cells were observed in the first experiment (data not shown), the cytotoxicity of these two compounds was also determined. The cellular toxicity experiments showed that apigenin is toxic for the cells at low concentration. Only 66 % and 41% of the cells were viable after 72h treatment with 50 µM and 200 µM of apigenin, respectively (Figure 2C). On the other hand, diosmetin was not toxic for Huh-7 cells at concentrations up to 100 µM, and had a limited effect at 200 µM (Figure 2D). Taken together,

these results indicate that even if apigenin inhibits HCV infection, its IC₅₀ is very close to its half-lethal dose and could not be further used for anti-HCV inhibition studies. In contrast, diosmetin has a very low toxicity in cell culture and is worth further investigation, even if its IC₅₀ is quite high. This molecule might not be used directly as an anti-HCV agent but could be further investigated in structure-function studies together with other polyphenols inhibiting HCV infection to better understand the mode of action of this class of compounds.

CONCLUSION

It can be fairly concluded that *P. chinensis* aerial parts methanol 80% extract is a potential source for compounds with specific antiviral properties for hepatitis C virus.

CONFLICT OF INTEREST

There is no conflict of interest associated with the authors of this paper

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