Anticancer activity of flavane gallates isolated from *Plicosepalus curviflorus*

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ABSTRACT

Background: Previous investigation of the methanol extract of *Plicosepalus curviflorus* leaves led to the isolation of two new flavane gallates (1, 2), together with other compounds including quercetin (3). The stems of *P. curviflorus* are used traditionally for the treatment of cancer in Yemen. **Objective:** The aim of this study was to evaluate the anticancer activity of the plant methanol extract as well as isolated compounds (1-3). **Materials and Methods:** The human cancer cell lines used were; MCF-7, HepG-2, HCT-116, Hep-2, HeLa and normal, Vero cell line using the Crystal Violet Staining method (CVS). **Results:** Quercetin (3) possessed the highest anticancer effect against all five cell lines (IC_{50} ranging from 3.6 to 16.2 µg/ml). It was followed by *2S*, *3R*-3, 3′, 4′, 5, 7-pentahydroxyflavane-5-*O*-gallate **(1)**, with IC_{50} ranging from 11.6 to 38.8 µg/ml. The weakest anticancer activity was given by *2S*, *3R*-3,3′,4′,5,5′, 7-hexahydroxyflavane-3′,5-di-*O*-gallate (2) with IC_{50} ranging from 39.8 to above 50 µg/ml, compared to vinblastine sulphate as reference drug. Colon, liver and breast cell lines seemed to be more sensitive to the tested compounds than the cervical and laryngeal cell lines. Concerning the cytotoxic effect on Vero cell line, the pentahydroxyflavane-5-*O*-gallate (1) showed the highest IC_{50} (138.2 µg/ml), while quercetin exhibited the lowest IC_{50} to Vero cells (30.5 µg/ml), compared to vinblastine sulphate as reference drug (IC_{50}: 39.7 µg/ml). **Conclusion:** The results suggest the possible use of compounds 1 and 3 as anticancer drugs especially against colon and liver cancers.

Key words: Anticancer, flavane gallates, *plicosepalus curviflorus*, quercetin

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INTRODUCTION

Cancer is among the major causes of death in the world. However, several classes of anticancer agents have been developed and many of them are from natural origin.[1] Moreover, traditional medicine has aroused interest in the search for safe, potent and selective anticancer compounds.[2]

Family Loranthaceae, is a large family that includes about 73 genera and almost 900 species living on branches, twigs or roots of host plants.[3] Genus *Plicosepalus* comprises ca. 11 species scattered throughout Africa, Arabian Peninsula and the Middle East. In Saudi Arabia, it is represented by two species, *Plicosepalus curviflorus* and *Plicosepalus acaciae*.[4] Earlier investigations on genus *Plicosepalus* reported various types of biological activities such as antihepatotoxic,[5] anti-diabetic and cytotoxic activities.[6] Moreover, the stems of *P. curviflorus* are used for the treatment of cancer in Yemen.[7]

In our previous study on *P. curviflorus* methanol extract,[8] we reported the isolation and structure elucidation of two new flavane gallates namely; *2S*, *3R*-3, 3', 4', 5, 7-pentahydroxyflavane-5-*O*-gallate (1) and *2S*, *3R*-3, 3', 4', 5, 5', 7-hexahydroxyflavane-3', 5-di-*O*-gallate (2). This is in addition to isolation of seven known compounds (-)-catechin (3), quercetin (4), lupeol (5), β-sitosterol (6), pomolic acid (7), β-sitosterol 3-*O*-β-D-glucopyranoside (8) and 4-methoxycinnamic acid (9). In continuation of our work, this study aims to compare the anticancer activity of the *P. curviflorus* methanol extract and the major isolated compounds, which were the two new flavane gallates (1,2) as well as quercetin (3), on five human cancer cell lines using CVS method.

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MATERIALS AND METHODS

Plant material

Dried powdered leaves of *P. curviflorus* (1.0 kg) were collected from South Hijaz, Saudi Arabia in March 2008 and were identified by Dr. M. Atiqur Rahman, Prof. of Taxonomy, College of Pharmacy, King Saud University. Voucher specimen (No. 127) was deposited in Department of Pharmacognosy, College of Pharmacy, King Saud University (Riyadh, Saudi Arabia).

Source of tested compounds

The tested compounds (1-3) as well as the total methanol extract of the plant were obtained as explained under the experimental section of our previous paper[8] [Figure 1].

Cytotoxicity assay

Cell culture

Human cell lines: MCF-7 cells (breast cancer cell line), HepG-2 (liver cancer cell line), HCT-116 (colon cancer cell line), Hep-2 (laryngeal cancer cell line), HeLa (cervical cancer cell line), and Vero (cell line was initiated from kidney of a normal adult African green monkey) were obtained from The Holding Company for Biological Products and Vaccines (VACSERA) Tissue Culture Unit. The cells were propagated in Dulbeccos modified Eagles Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (Sigma Chemical Co., St. Louis, Mo, U.S.A), 1% L-glutamine, HEPES buffer and 50 μg/ml gentamycin (Sigma Chemical Co., St. Louis, Mo, U.S.A). All cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were subcultured two times a week.

Evaluation of cellular cytotoxicity

The cytotoxicity was evaluated by the crystal violet staining (CVS) method described by Saotome *et al.*[9] and modified by Itagaki *et al.*[10] Briefly, in a 96-well tissue culture microplate, the cells were seeded at a cell concentration of 1×10^4 cells per well in 100 μl of growth medium. Fresh medium containing different concentrations of tested compounds were added after 24 h of seeding at 37°C. Serial two-fold dilutions of the tested compounds were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells was found not to affect the experiment. After the 48 h incubation period, the viable cells yield was determined by a colorimetric method. In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 min. The stain was removed and the plates were rinsed using distilled water. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly. The quantitative analysis (colorimetric evaluation of fixed cells) was performed by absorbance measurements in an automatic Microplate reader (TECAN, Inc.) at 595 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The effect on cell growth was calculated as the difference in absorbance percentage in presence and absence of the tested compounds and illustrated in a dose-response curve. The concentration at which the growth of cells was inhibited to 50% of the control (IC_{50}) was obtained from this dose-response curve. The standard antitumor drug used was vinblastine sulfate [Tables 1-6].

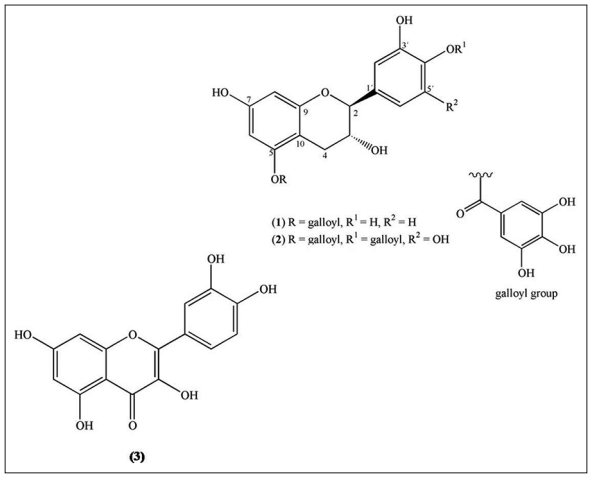

Figure 1: Chemical structures of tested compounds 1-3

Statistical analyses

Data were expressed as means ± S.D. For multi-variable comparisons, one-way ANOVA was conducted, followed by Tukey-Kramer testing using the GraphPad InStat (ISI Software) computer program. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The anticancer activity of total methanol extract and compounds 1 to 3 against five human carcinoma cell lines was determined using CVS method and vinblastine sulphate as a reference drug. The response parameter (IC_{50}) was calculated for each cell line. From the results it could be seen that all tested compounds possessed a dose dependent cytotoxic effect against all five cell lines; however, we found a differential

Table 1: *In vitro* anticancer activities on MCF-7

Tumor cell line MCF-7 Sample conc.(µg/ml)	Vinblastine sulfate	Mean of surviving fraction±S.D.#			
		Methanol extract	1	2	3
50	7.82±0.98	21.64±5.13[c]	36.82±3.56[a]	40.94±4.14[a]	7.84±2.14
25	15.18±1.23	42.11±3.24[a]	59.44±2.42[a]	63.12±3.65[a]	18.96±3.34
12.5	29.26±2.74	65.92±1.28[a]	71.23±1.26[a]	76.98±2.89[a]	47.25±2.95[b]
6.25	42.35±2.21	74.23±1.34[a]	82.16±1.13[a]	85.43±3.05[a]	62.18±1.64[a]
3.125	56.54±1.96	82.94±0.98[a]	89.48±0.98[a]	92.72±1.42[a]	74.86±0.98[a]
1.56	67.24±2.94	93.32±0.46[a]	93.61±0.46[a]	98.38±0.36[a]	91.53±0.86[a]
*IC_{50}	4.6 µg/ml	20.9 µg/ml	35.4 µg/ml	39.8 µg/ml	11.3 µg/ml

#Mean of surviving fraction±standard deviation: Mean of three assays. [a]*P*<0.001, [b]*P*<0.01, [c]*P*<0.05 compared to reference drug. *IC_{50}: Concentration of extract required to reduce cell survival by 50%, S.D.: Standard deviation

Table 2: *In vitro* anticancer activities on HepG-2

Tumor cell line HepG-2 Sample conc.(µg/ml)	Vinblastine sulfate	Mean of surviving fraction±S.D.#			
		Methanol extract	1	2	3
50	14.38±1.41	18.56±3.42	39.12±3.12[a]	43.18±2.12[a]	9.47±1.82[c]
25	16.13±2.24	36.18±2.86[a]	63.34±1.86[a]	62.49±3.56[a]	23.16±1.56[b]
12.5	24.25±2.96	45.82±3.14[a]	78.25±2.11[a]	80.26±1.98[a]	45.38±3.16[a]
6.25	45.13±2.04	60.93±2.53[b]	86.78±1.42[a]	89.37±0.98[a]	63.54±1.28[a]
3.125	55.00±2.33	74.34±1.54[a]	94.22±1.16[a]	94.24±0.46[a]	79.82±1.19[a]
1.56	72.13±3.06	80.61±1.18[b]	97.73±0.53[a]	98.12±0.18[a]	84.93±0.96[b]
*IC_{50}	4.6 µg/ml	10.8 µg/ml	38.8 µg/ml	41.2 µg/ml	10.9 µg/ml

#Mean of surviving fraction±standard deviation: Mean of three assays. [a]*P*<0.001, [b]*P*<0.01, [c]*P*<0.05 compared to reference drug. *IC_{50}: Concentration of extract required to reduce cell survival by 50%, S.D.: Standard deviation

Table 3: *In vitro* anticancer activities on HCT-116

Tumor cell line HCT-116 Sample conc.(µg/ml)	Vinblastine sulfate	Mean of surviving fraction±S.D.#			
		Methanol extract	1	2	3
50	16.27±1.12	10.58±2.12[b]	23.56±3.86[c]	46.13±2.38[a]	6.38±1.42[a]
25	21.68±2.38	21.74±3.46	39.17±2.98[a]	61.76±3.12[a]	12.24±2.64[b]
12.5	28.2±4.56	38.17±3.65[c]	48.32±1.88[b]	78.85±1.84[a]	19.61±3.18
6.25	38.06±5.32	49.25±2.73[c]	60.58±2.13[b]	86.47±2.13[a]	28.97±3.45
3.125	47.54±4.04	65.58±1.88[b]	72.21±1.02[a]	91.08±0.98[a]	53.64±5.12
1.56	53.42±3.96	86.49±2.34[a]	84.99±0.85[a]	97.52±1.06[a]	61.43±1.98[c]
*IC_{50}	2.6 µg/ml	6.1 µg/ml	11.6 µg/ml	43.8 µg/ml	3.6 µg/ml

#Mean of surviving fraction±standard deviation: Mean of three assays. [a]*P*<0.001, [b]*P*<0.01, [c]*P*<0.05 compared to reference drug. *IC_{50}: Concentration of extract required to reduce cell survival by 50%, S.D.: Standard deviation

Table 4: *In vitro* anticancer activities on Hep-2

Tumor cell line Hep-2 Sample conc.(µg/ml)	Vinblastine sulfate	Mean of surviving fraction±S.D.#			
		Methanol extract	1	2	3
50	15.38±1.84	28.17±1.98[a]	25.26±3.12[a]	53.98±2.11[a]	15.24±1.85
25	27.35±2.89	51.34±2.14[a]	61.48±2.85[a]	70.24±1.85[a]	34.89±2.34[c]
12.5	43.59±2.12	68.26±1.96[a]	70.93±1.41[a]	86.32±2.56[a]	56.28±3.56[b]
6.25	53.85±3.11	82.44±0.82[a]	83.42±0.98[a]	94.13±1.04[a]	72.68±1.24[a]
3.125	69.23±2.54	89.52±0.64[a]	95.29±1.02[a]	99.02±0.24[a]	87.31±0.87[a]
1.56	76.82±1.36	96.73±0.51[a]	98.74±0.44[a]	100	96.12±0.52[a]
*IC_{50}	9.8 µg/ml	26.4 µg/ml	32.9 µg/ml	>50 µg/ml	16.2 µg/ml

#Mean of surviving fraction±standard deviation: Mean of three assays. [a]*P*<0.001, [b]*P*<0.01, [c]*P*<0.05 compared to reference drug. *IC_{50}: Concentration of extract required to reduce cell survival by 50%, S.D.: Standard deviation

Table 5: *In vitro* anticancer activities on HeLa

Tumor cell line HeLa Sample conc.(µg/ml)	Vinblastine sulfate	Mean of surviving fraction±S.D.#			
		Methanol extract	1	2	3
50	12.46±2.35	38.49±3.14[a]	30.87±4.12[b]	59.22±1.28[a]	13.78±2.56
25	29.60±1.88	62.18±2.11[a]	53.12±2.89[a]	74.16±1.42[a]	32.42±3.86
12.5	48.95±1.32	83.22±1.54[a]	71.84±3.17[a]	89.38±0.88[a]	51.97±1.54
6.25	62.37±2.32	91.74±0.98[a]	84.19±1.24[a]	95.20±0.72[a]	69.24±2.13[c]
3.125	78.24±1.55	97.28±0.28[a]	92.73±0.98[a]	98.74±0.43[a]	78.16±1.28
1.56	84.38±0.95	98.97±0.43[a]	98.06±0.46[a]	100	89.72±0.84[b]
*IC_{50}	11.6 µg/ml	37.9 µg/ml	28.5 µg/ml	>50 µg/ml	13.8 µg/ml

#Mean of surviving fraction±standard deviation: Mean of three assays. [a]*P*<0.001, [b]*P*<0.01, [c]*P*<0.05 compared to reference drug. *IC_{50}: Concentration of extract required to reduce cell survival by 50%, S.D.: Standard deviation

Table 6: Evaluation of cytotoxicity against Vero cell line

Vero cell line Sample conc.(µg/ml)	Vinblastine sulfate	Mean of surviving fraction±S.D.#			
		Methanol extract	1	2	3
200	26.16±2.48	32.12±2.36[c]	44.38±3.12[a]	64.82±2.9[a]	18.79±3.56[c]
100	33.88±2.54	49.28±1.82[a]	53.47±2.86[a]	73.18±4.22[a]	29.36±2.84
50	45.97±4.11	64.36±2.68[b]	61.98±1.88[a]	84.20±2.86[a]	43.82±4.35
25	58.04±2.64	79.13±1.46[a]	73.26±0.98[a]	90.34±1.02[a]	51.74±2.89
12.5	70.36±1.88	91.22±1.84[a]	84.15±1.13[a]	95.13±1.16[a]	68.48±1.84
6.25	82.42±1.94	97.94±0.62[a]	93.27±0.98[a]	98.82±0.46[a]	79.23±0.86
3.125	94.86±0.95	100	98.21±0.26[b]	100	88.17±0.42[a]
1.56	100	100	100	100	96.32±0.32
*IC_{50}	39.7 µg/ml	97.6 µg/ml	138.2 µg/ml	No cytotoxic activity against Vero cells	30.5 µg/ml

#Mean of surviving fraction±standard deviation: Mean of three assays. [a]*P*<0.001, [b]*P*<0.01, [c]*P*<0.05 compared to reference drug. *IC_{50}: concentration of extract required to reduce cell survival by 50%, S.D.: Standard deviation

effect for each compound. Quercetin (3) possessed the highest anticancer effect against all five cell lines (IC_{50} ranging from 3.6 to 16.2 µg/ml). This is in agreement with literature.[11,12] It was followed by *2S*, *3R*-3, 3', 4', 5, 7-pentahydroxyflavane-5-*O*-gallate (1), with IC_{50} ranging from 11.6 to 38.8 µg/ml. This is also in agreement with previously reported studies.[13,14] The weakest anticancer activity was given by *2S*,*3R*-3, 3',4', 5, 5', 7-hexahydroxyflavane-3', 5-di-*O*-gallate (2) with IC_{50} ranging from 39.8 to above 50 µg/ml, compared to vinblastine sulphate as reference drug. Moreover, the total methanol extract exhibited a significant anticancer effect against the tested cell lines with IC_{50} ranging from 6.1 to 37.9 µg/ml, proving the possible synergistic effect between the compounds. Colon, liver and breast cell lines seemed to be more sensitive to the tested compounds than the cervical and laryngeal cell lines. As for the cytotoxic effect on Vero cell line, the pentahydroxyflavane-5-*O*-gall.late (1) showed the highest IC_{50} (138.2 µg/ml), followed by the methanol extract (97.6 µg/ml) and quercetin (30.5 µg/ml) compared to vinblastine sulphate as reference drug (IC_{50}: 39.7 µg/ml), thus proving their safety to normal cells, while being cytotoxic to cancerous cells. The hexahydroxyflavane-3', 5-di-*O*-gallate (2) did not show any cytotoxicity against Vero cell line (up to 200 µg/ml), but it also exhibited weak anticancer activity against the five cancer cell lines. As far as literature is concerned, this is the first report of anticancer activity for these two new flavane gallates.

CONCLUSION

The results of this study are in agreement with literature as many studies have demonstrated that catechins and their derivatives possess significant anticancer activities. The galloylated catechins have shown a stronger anti-proliferative activity and apoptotic effect than the one produced by non galloylated catechins. Additionally, the position of the galloyl moiety and stereochemistry of the compound may affect its anticancer activity.[15]

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