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*Original Article*

# Effective compounds screening from *Rabdosia serra (Maxim) Hara* against HBV and tumor *in vitro*

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**Abstract:** The aim of this study was to screen and investigate the anti-HBV and anti-tumor activities of separated compounds from *Rabdosia serra (Maxim.) Hara* to lay the basis for further isolate active entity. Three kinds of extractions from *Rabdosia serra* using different solvents (petroleum ether, acetidin, butyl alcohol) were prepared and used to analyze their anti-HBV activity in HepG2.2.15 cells for further separation. The cytotoxicity of each extraction was tested by MTT assay, the levels of HBsAg, HBeAg and HBV DNA in supernatants from HepG2.2.15 cells were detected by ELISA and real-time quantitative polymerase chain reaction (PCR). Then, the most effective extraction was further separated, the anti-HBV activities of separated compounds were also tested by MTT and ELISA, and three compounds with highest cytotoxicity were selected to further identify their anti-tumor activities on MCF-7, BGC-823 and HepG2 cells. Acetidin extraction $C_2$ had the most effective anti-HBV activity that was used to be further separated, it led to statistically significant reduction in HBsAg and HBeAg secretion and HBV DNA. The separation of $C_2$ resulted in 14 compounds, $A_3$ and $A_5$ markedly inhibited HBsAg secretion, while $A_9$ inhibited HBeAg secretion in a dose-dependent manner with higher TI comparing with $C_2$. $A_6$, $A_7$, $A_{11}$ had different anti-tumor activity against different tumor cells. These data showed that the extraction and their separated effective compounds had strong inhibitory effect on HBV replication so as to have anti-HBV activity, and further separation and purification could enhance anti-HBV activity. Meanwhile, some compounds have high cytotoxicities on different tumor cells. Our study could provide a theoretical basis for the next clinical use and the development of potential and efficient drugs for HBV and tumor therapy from *Rabdosia serra.*

**Keywords:** *Rabdosia serra (Maxim.) Hara*, effective compounds, anti-HBV, anti-tumor

## Introduction

*Rabdosia serra (Maxim) Hara (Isodon lophanthoides (Buch.-Ham.exD.Don) H. Hara),* belongs to the Labiatae family, which has been popularly used in china for years on the treatment of arthritis, enteritis, jaundice, hepatitis, acute icteric hepatitis, lepromatous leprosy and ascariasis [1-3]. The treatment of 68 hepatitis B patients with a decoction contained *Rabdosia serra* obtained a recovery rate of 83.8% [4]. And there was also a clinical report made by Qin Xuefeng [5] about the use of *Rabdosia serra* for the treatment of 300 acute icteric hepatitis patients. The genus Rabdosia is well known to be abundant in diterpenoids, which have diverse bioactivities, such as antibacterial, anti-inflammatory and antitumor activities [6-9]. In particular, *Rabdosia serra* extract has a well-documented antioxidant property [10], which is probably a major underlying mechanism for its anti-inflammatory, anti-tumor effects. Although *Rabdosia serra* has been shown to have different pharmacologic actions, the information regarding the ant-viral and anti-tumor capacity of main active compounds from *Rabdosia serra* in vitro is limited. Thus far there has been one report [11] about its anti-HBV activity on HepG2.2.15 which only investigated the influence on secretion of the HBsAg and HBeAg, and several reports about anti-tumor activities on Hela cells [12, 13] and CaEs-17 cells [14].

Hepatitis B is a serious disease caused by hepatitis B virus (HBV), patients infected HBV can

develop acute and chronic infection, which could lead to cirrhosis, liver failure, liver cancer even or death. Several anti-viral drugs have been approved for the treatment of hepatitis B, including interferon and nucleoside analogues *Lamivudine*, *Adefovir*, *Entecavir* [15-17], but unresolved significant issues remain with current drugs, such as dose-dependent side effects, hepatic decompensation, viral mutations and drug resistance during long-term therapy [17, 18], and "rebound phenomenon" [19, 20]. Therefore, there exists a imperative medical need for safe and efficacious new anti-HBV drugs. It has been widely accepted that natural products [21], like flavonoids, terpenoids, alkaloids [22, 23], are good sources for potential anti-viral drugs based on their molecular diversity and functional multiplicity, synergistic hepatoprotective effect and low toxicity [24-26]. To screen and investigate effective compounds from *Rabdosia serra* and explore their new potential clinical indications, in this study we have extracted and separated effective compounds from *Rabdosia serra*, investigated their anti-HBV activities such as inhibitory effects on HBsAg and HBeAg secretion and HBV DNA replication in vitro, and identified their anti-tumor effects on three different tumor cells (MCF-7 cells, BGC-823 cells, HepG2 cells). The study could provide the foundation for the further anti-HBV and anti-tumor study of *Rabdosia serra.*

## Materials and methods

### *Plant and reagents*

*Rabdosia serra (Maxim.) Hara (Isodon lophanthoides (Buch.-Ham.exD.Don) H. Hara)* was collected from Changsha, Hunan Province in China, and authenticated by Professor Chen Yuxiang (XiangYa Medical College of Central South University, Changsha, China). Ethanol, petroleum ether, acetidin, butyl alcohol, methanol, chloroform were from Chemical Plant of Hunan Normal University (Changsha, China), lamivudine (3TC) was provided by the cancer research institute of Central South University. All the chemicals used were of analytical grade.

3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) was purchased from Sigma (St. Louis, MO, USA). RPMI-1640 medium was purchased from Invitrogen (USA), Trypsin, Fetal bovine serum (FBS), Dimethyl sulfoxide (DMSO) and Geneticin (G418) were acquired from Gibco BRL (Grand Island, NY, USA), glutamine, penicillin and streptomycin were obtained from XiangYa cell center of Central South University (Changsha, China).

### *Extraction and separation*

The dried whole plant *Rabdosia serra (Maxim) Hara* was crushed into powder and extracted with 50% ethanol under reflux twice, and 3 hours each time, ratio of solvent to material was 20:1. The resulting ethanol extract was filtered and concentrated by a rotary evaporate to crude extract sicca. The triplicate extracta sicca 40.2 g was dissolved by distilled water, and then further extracted by petroleum ether, acetidin and butyl alcohol respectively using a separatory funnel, the extractions were collected and concentrated to petroleum ether extraction ($C_1$), acetidin extraction ($C_2$) and butyl alcohol extraction ($C_3$) respectively, the yield of each extraction was calculated as follows: Extraction yield % = weight of extraction / weight of extracta sicca × 100%.

According to the result of following anti-HBV study of different extraction, the most effective extraction was further separated by silica gel column chromatography. 10.2 g of extraction was precisely weighted and dissolved with chloroform (20 mL), after the sample solution was added into silica gel column carefully, 50 mL of chloroform was added, and then was gradient eluted with different chloroform-methanol-water mixtures (**Table 1**). The fractions were identified by thin layer chromatography (TLC) and observed by UV spectrophotometer. The same fractions were combined and then concentrated under reduced pressure. Percentage (%) of each compound was calculated as follow: Percentage % = weight of compound / weight of active extraction × 100%.

### *Preparation of samples*

The petroleum ether extraction ($C_1$), acetidin extraction ($C_2$), butyl alcohol extraction ($C_3$), separated compounds were dissolved by 0.1% DMSO respectively to make stock solution. The stock solution was further diluted to concentration of 0.5, 2, 4, 8, 16 mg/ml respectively with RPMI-1640 medium (supplemented with 10% FBS, 100 U/ml penicillin, 100 U/ml streptomycin, 100 mg/L G418, 2 mmol/L glutamine, pH

**Table 1.** Composition of chloroform-methanol-water mixture

| Chloroform | Methanol | Water |
|---|---|---|
| 9 | 1 | 0.1 |
| 8 | 2 | 0.1 |
| 7 | 3 | 0.1 |
| 6 | 4 | 0.1 |
| 4 | 6 | 0.1 |
| 3 | 7 | 0.1 |
| 2 | 8 | 0.1 |
| 1 | 1 | 0.1 |

**Table 2.** Weights (g) and yield (%) of different extracts from *Rabdosia serra (Maxim) Hara*

| Extraction | Weight (g) | Yield (%) |
|---|---|---|
| Petroleum ether extraction ($C_1$) | 3.4 | 8.5 |
| Acetidin extraction ($C_2$) | 10.2 | 25.4 |
| Butyl alcohol extraction ($C_3$) | 10.7 | 26.5 |

7.0). These drug concentrations were used in subsequent assays, and all the samples were filtered by syringe filter (0.22 μm).

*Cell culture*

The HepG2.2.15 cell line (purchased from XiangYa cell center of Central South University, Changsha, China) were cultured in RPMI-1640 medium (supplemented with 10% FBS, 100 U/ml penicillin, 100 U/ml streptomycin, 100 mg/L G418, 2 mmol/L glutamine, pH 7.0) in the 5% $CO_2$ incubator at 37°C.

Human breast carcinoma cell line MCF-7 cells, human gastric cancer cell line BGC-823 cells, Human hepatocellular liver carcinoma cell-HepG2 cells (all purchased from Xiangya Cell Center of Central South University, Changsha, China) were cultured as same as HepG2.2.15 cells described above.

*Anti-HBV study*

The cytotoxicities of each extraction ($C_1$, $C_2$, $C_3$) and separated compounds from *Rabdosia serra* towards HepG2.2.15 were evaluated using the MTT assay. HepG2.2.15 cells ($2\times10^4$ cells/well) were seeded in 96-well plates in a volume of 200 μl/well for 24 h. After cells adhered, the supernatants in each well were replaced very carefully with 200 μl of different concentrations (0.5, 2, 4, 8, 16 mg/ml) of samples. Cells treated with the culture medium (contained 0.1% of DMSO) were used as negative control, 3TC (0.1 mg/ml) was used as positive control. Each group contained 4 parallel wells. After cells were treated or untreated with drug samples for 6 days, the supernatant was removed, 20 μl of MTT (5 mg/mL) was added to each well. After incubation for 4 h, all the supernatants were replaced with 200 μl of DMSO. After mixed thoroughly to dissolve purple formazan of MTT, absorbance (OD value) was detected by a microplate reader at 490 nm. The cells inhibition ratio was calculated as follow: Inhibition ratio % = $(OD_{negative} - OD_{sample}) / (OD_{negative} - OD_{blank}) \times 100\%$.

The median toxic concentration ($TC_{50}$) was the concentration that achieved 50% cytotoxicity against culture cells, and was determined from dose-response data from at least three independent experiments by origin.

The levels of HBsAg and HBeAg were determined by ELISA. All the supernatants were collected in the sixth day and kept at -20°C for analysis. HBsAg and HBeAg in culture supernatants of HepG2.2.15 cells were quantified using quantitative ELISA test kits (Rongsheng, Shanghai, China) follow manufacturer's instructions, and the absorbance (OD value) was measured with a microplate reader at 490 nm. The antigen inhibition ratio was calculated as follow: Inhibition ratio % = $(OD_{negative} - OD_{sample}) / (OD_{negative} - OD_{blank}) \times 100\%$.

The median effect concentration ($EC_{50}$) was the concentration that achieved 50% inhibition against antigens, and was determined from dose-response data by Origin software.

Therapeutic index (TI) of sample was calculated as follow: $TI = TC_{50}/EC_{50}$.

TI > 2 means the drug is effective (Positive), 1 < TI < 2 means the drug has high cytotoxicity but low effect (Weakly positive), TI < 1 means the drug is noneffective (Negative).

According to the TI of different extraction, the most effective one was employed for the quantification of HBV DNA by real-time quantitative PCR. After cells were incubated with extract solution of different concentrations (0.5, 2, 4, 8, 16 mg/ml) for 6 days, the supernatant were collected, and cells treated with 3TC was used as positive control. The HBV DNA was extracted

**Table 3.** Influence of different concentrations of two extractions on HepG2.2.15 cell viability

| Extract | Concentration (mg/mL) | Day 6 | | $TC_{50}$ (mg/ml) |
|---|---|---|---|---|
| | | $A_{490}$ IR (%) | | |
| Acetidin extraction ($C_2$) | 16 | 0.293±0.056** | 59.52 | 10.471 |
| | 8 | 0.407±0.048** | 43.81 | |
| | 4 | 0.530±0.051* | 26.73 | |
| | 2 | 0.689±0.089 | 5.26 | |
| | 0.5 | 0.749±0.063 | -3.45 | |
| Butyl alcohol extraction ($C_3$) | 16 | 0.352±0.058** | 51.34 | 14.563 |
| | 8 | 0.472±0.054* | 34.75 | |
| | 4 | 0.613±0.037 | 15.31 | |
| | 2 | 0.732±0.065 | 1.13 | |
| | 0.5 | 0.793±0.056 | -9.57 | |
| Negative control | | 0.724±0.051 | 0 | |
| Positive control | 0.1 | 0.236±0.082** | 67.35 | |

Note: IR means Inhibition rate (%), $TC_{50}$ is 50% cytotoxic concentration in HepG2.2.15 cells. **P < 0.01, *P < 0.05 compared with negative control group.

**Table 4.** Inhibitory effect of two extractions on secretion of HBsAg from HepG2.2.15 cells

| Extract | Concentration (mg/mL) | Day 6 | | $EC_{50}$ (mg/ml) | TI ($TC_{50}/EC_{50}$) |
|---|---|---|---|---|---|
| | | $A_{490}$ IR (%) | | | |
| Acetidin extraction ($C_2$) | 16 | 0.203±0.041** | 90.52 | 2.351 | 4.453 |
| | 8 | 0.449±0.052** | 79.29 | | |
| | 4 | 0.921±0.068* | 57.10 | | |
| | 2 | 1.360±0.049* | 36.61 | | |
| | 0.5 | 1.682±0.052 | 21.57 | | |
| Butyl alcohol extraction ($C_3$) | 16 | 0.810±0.053** | 62.24 | 8.211 | 1.773 |
| | 8 | 1.004±0.092** | 53.21 | | |
| | 4 | 1.466±0.085* | 31.65 | | |
| | 2 | 1.606±0.112 | 25.12 | | |
| | 0.5 | 1.965±0.0089 | 8.36 | | |
| Negative control | | 2.145±0.132 | 0 | | |
| Positive control | 0.1 | 0.319±0.083 | 85.13 | | |

Note: IR means Inhibition rate (%), $EC_{50}$ is 50% inhibitory concentration on secretion of HBsAg. TI means therapeutic index (TI = TC50/EC50). **P < 0.01, *P < 0.05 compared with negative control group.

from culture supernatants using DNA Extraction Kit (CASarray, Shanghai, China), and a real-time quantitative PCR was performed in an Applied Biosystems Prism 7000 instrument (ABI 7000, USA) using HBV Fluorescent Quantitative PCR Detection Kit (RongSheng biological technology Co., LTD, Shanghai, China).

*Anti-tumor activity study*

According to the result of cytotoxicity assay of separated compounds on HepG2.2.15 cells, 3 compounds with highest cytotoxicities were selected for the detection of anti-tumor activity. And MCF-7 cells, BGC-823 cells, HepG2 cells were used. The growth inhibitory rates of MCF-7 cells, BGC-823 cells, HepG2 cells were also evaluated by the MTT assay.

These three cells were incubated in 96-well plates at approximately $1\times10^4$/well in 200 μl volume respectively. After cells adhered, the supernatants in each well were replaced with 200 μl of different concentrations (0.5, 2, 4, 8, 16 mg/ml) of samples. Each group contained 4 parallel wells. And the following experimental procedures were as same as described previously in 1.5.1. The inhibition ratio was calculat-

**Table 5.** Inhibitory effect of two extractions on secretion of HBeAg from HepG2.2.15 cells

| Extract | Concentration (mg/mL) | Day 6 | | $EC_{50}$ (mg/ml) | TI ($TC_{50}/EC_{50}$) |
|---|---|---|---|---|---|
| | | $A_{490}$ IR (%) | | | |
| Acetidin extraction ($C_2$) | 16 | 0.076±0.028** | 97.32 | 1.490 | 7.028 |
| | 8 | 0.266±0.062** | 87.53 | | |
| | 4 | 0.571±0.043** | 64.62 | | |
| | 2 | 0.882±0.051** | 47.37 | | |
| | 0.5 | 1.128±0.079* | 31.13 | | |
| Butyl alcohol extraction ($C_3$) | 16 | 0.674±0.060** | 58.25 | 11.316 | 1.287 |
| | 8 | 0.995±0.064** | 38.36 | | |
| | 4 | 1.249±0.092** | 22.72 | | |
| | 2 | 1.343±0.071* | 16.83 | | |
| | 0.5 | 1.578±0.088 | 2.32 | | |
| Negative control | | 1.615±0.134 | 0 | | |
| Positive control | 0.1 | 0.416±0.016 | 74.25 | | |

Note: IR means Inhibition rate (%), $EC_{50}$ is 50% inhibitory concentration on secretion of HBeAg. TI means therapeutic index (TI = $TC_{50}/EC_{50}$). **P < 0.01, *P < 0.05 compared with negative control group.

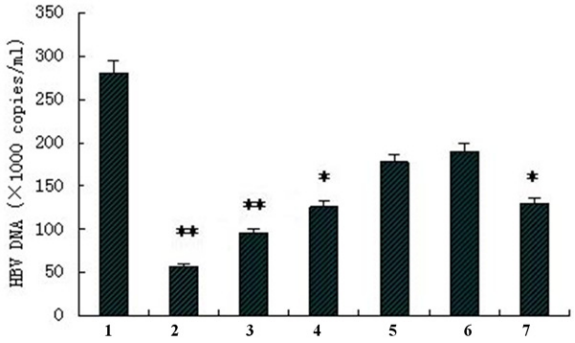


**Figure 1.** Inhibitory effect of $C_2$ on HBV DNA level in supernatant from HepG2.2.15 cells. When cells were incubated with $C_2$ at various concentrations, the HBV DNA was significantly reduced at a dose-dependent manner, and consistent with the inhibitory effect on HBsAg and HBeAg secretion, $C_2$ treatment groups of 4, 8, 16 mg/ml led to statistically significant reduction in extracellular HBV DNA compared with the no drug control (P < 0.05). 1. Control; 2. $C_2$ 16 mg/mL; 3. $C_2$ 8 mg/mL; 4. $C_2$ 4 mg/mL; 5. $C_2$ 2 mg/mL; 6. $C_2$ 0.5 mg/mL; 7. Standard control: 0.1 mg/mL.

ed as follow: Inhibition ratio % = ($OD_{negitive}$ - $OD_{sample}$) / ($OD_{control}$ - $OD_{blank}$) × 100%.

The median inhibitory concentration ($IC_{50}$) was the concentration that achieved 50% inhibition against culture cells, and was determined from dose-response data from at least three independent experiments by Origin software.

*Statistical analysis*

The data were expressed as mean ± SD. Statistical differences between control and treated groups were determined by t-test using SPSS13.0 software. P < 0.05 were considered statistically significant.

## Results and discussion

*Anti-HBV activity on Hep 2.2.15 cells of three extractions*

The triplicate suspension of extracta sicca (40.2 g) was extracted respectively by petroleum ether, acetidin and butyl alcohol, weight and yield of extraction $C_1$, $C_2$, $C_3$ were stated in **Table 2**. It showed that the yield of $C_1$ was the lowest, while $C_2$ and $C_3$ were almost equal.

HBsAg, HBeAg and HBV DNA are general markers for HBV replication. The eliminations of HBsAg and HBeAg are generally accepted as indications of a complete cure state of HBV epidemiology along with the eradication of HBV DNA. In our study, we firstly detected anti-HBV activity of three extractions by the detection of levels of HBsAg, HBeAg and HBV DNA, and then further separated the most effective extraction to screening effective anti-HBV and anti-tumor compounds.

*The cytotoxicities of three extractions*

The growth inhibitory rates of three extractions ($C_1$, $C_2$, $C_3$) on HepG2.2.15 were measured by MTT assay on day 6. It found that $C_1$ had no cytotoxicity and inhibition on secretion of HBsAg and HBeAg, and the growth of Hep

**Table 6.** Weight, weight percentages and $TC_{50}$ of compounds from acetidin extraction

| compounds | Weight (g) | Percentage (%) | $TC_{50}$ (mg/ml) |
|---|---|---|---|
| $A_1$ | 0.20 | 1.961 | 7.983 |
| $A_2$ | 1.00 | 9.80 | 6.107 |
| $A_3$ | 0.06 | 0.59 | 5.216 |
| $A_4$ | 0.03 | 0.29 | - |
| $A_5$ | 1.21 | 11.86 | 5.236 |
| $A_6$ | 0.93 | 9.12 | 0.471 |
| $A_7$ | 0.61 | 6.27 | 1.208 |
| $A_8$ | 1.23 | 12.06 | 5.856 |
| $A_9$ | 0.02 | 0.20 | 8.062 |
| $A_{10}$ | 2.10 | 20.59 | 6.535 |
| $A_{11}$ | 1.78 | 17.45 | 0.658 |
| $A_{12}$ | 0.32 | 3.14 | 13.129 |
| $A_{13}$ | 0.03 | 0.29 | - |
| $A_{14}$ | 0.01 | 0.09 | - |

Note: Percentage (%) = weight of compound / weight of acetidin extraction × 100%, $TC_{50}$ is 50% cytotoxic concentration in HepG2.2.15 cells.

G2.2.15 cells treated with different concentrations of $C_2$ and $C_3$ was inhibited at a dose-dependent manner (**Table 3**). It presented the $TC_{50}$ values of $C_2$ and $C_3$ were 10.471 and 14.563 mg/ml respectively. Consistent with its reported anti-tumor activity, *Rabdosia serra* extracts exhibited inhibitory effects on proliferation of HepG2.2.15 cells, when treated with $C_2$ and $C_3$ of 16 mg/ml, the inhibit values were 59.52% and 51.34% respectively ($P < 0.01$). And the cytotoxicity was measured to calculate the treatment index on Hep G2.2.15 cells.

*Inhibitory effect of different extraction on secretion of HBsAg and HBeAg*

Levels of HBsAg and HBeAg were quantified by ELISA. As shown in **Tables 4** and **5**, treatment of HepG2.2.15 cells with $C_2$ at different concentrations for 6 days resulted in significant reduction of HBsAg and HBeAg secretion in a dose-dependent manner, with $EC_{50}$ values of 2.351 mg/ml to HBsAg and 1.490 mg/ml to HBeAg, and TI was 4.453 to HBsAg and 7.028 to HBeAg respectively (TI > 2, means positive). $C_3$ was less efficient than $C_2$, while $EC_{50}$ values of $C_3$ were 8.211 mg/ml to HBsAg and 11.316 mg/ml to HBeAg, TI to HBsAg and HBeAg were both smaller than 2 (weakly positive, high toxic and low effective). Furthermore, noted that for both HBsAg and HBeAg, $C_2$ was more potent than 3TC and was highly efficacious, achieving maximal (90-100%) inhibition at 16 mg/ml.

In contrast to the results of MTT and ELISA of $C_1$ and $C_3$, the cytotoxicity of $C_2$ was higher, and inhibitory effect on secretion of HBsAg and HBeAg was stronger (TI > 2). These data (**Tables 3**-**5**) demonstrated that $C_2$ has the most efficient anti-HBV activity. To further confirm the anti-HBV activity of $C_2$, the effect of $C_2$ treatment on HBV DNA level was evaluated.

*Influence of acetidin extraction ($C_2$) on HBV DNA level*

Real-time PCR was performed with virus DNA in the supernatant from the HepG2.2.15 cells to determine whether $C_2$ could inhibit HBV DNA replication. When cells were incubated with $C_2$ at various concentrations, the HBV DNA was significantly reduced at a dose-dependent manner, and consistent with the inhibitory effect on HBsAg and HBeAg secretion, $C_2$ treatment groups of 4, 8, 16 mg/ml led to statistically significant reduction in extracellular HBV DNA compared with the no drug control ($P < 0.05$) (**Figure 1**). The result showed that $C_2$ could markedly inhibit the HBV DNA replication, which further confirmed that extraction $C_2$ has efficient anti-HBV activity.

*Anti-HBV study of separated compounds from acetidin extraction ($C_2$)*

Because of the most efficient anti-HBV activity of extraction $C_2$, $C_2$ was selected to further separate and identify by silica gel column chromatography and TLC. And 14 compounds were obtained, their cytotoxicities on HepG2.2.15 cells were also examined by MTT, weight, their weight percentages and $TC_{50}$ values were stated in **Table 6**. Thereinto, $A_4$, $A_{13}$ and $A_{14}$ had no cytotoxicity.

Influences of 14 compounds from $C_2$ on secretion of HBsAg and HBeAg from HepG2.2.15 cells were investigated by ELISA. The results were stated in **Tables 7** and **8**. Among these compounds, $A_3$ and $A_5$ had the better inhibitory effects on secretion of HBsAg, with the TI of 5.934 and 2.655 respectively. And $A_9$ inhibited the secretion of HBeAg better, with the TI of 9.485. The inhibitory effect was all in a dose-independent manner (**Tables 7**, **8**). In addition, treatments of HepG2.2.15 cells with $A_3$ and $A_5$

**Table 7.** Inhibitory effect of $A_3$, $A_5$ on secretion of HBsAg from HepG2.2.15

| compound | Concentration (mg/mL) | Day 6 | | $EC_{50}$ (mg/ml) | TI ($TC_{50}/EC_{50}$) |
|---|---|---|---|---|---|
| | | $A_{490}$ IR (%) | | | |
| $A_3$ | 16 | 0.041±0.009** | 97.86 | 0.879 | 5.934 |
| | 8 | 0.083±0.032** | 96.72 | | |
| | 4 | 0.285±0.051** | 89.39 | | |
| | 2 | 0.735±0.043** | 62.27 | | |
| | 0.5 | 1.215±0.052** | 37.61 | | |
| $A_5$ | 16 | 0.073±0.021** | 96.27 | 1.972 | 2.655 |
| | 8 | 0.173±0.020** | 91.11 | | |
| | 4 | 0.544±0.053** | 72.05 | | |
| | 2 | 1.143±0.045** | 41.32 | | |
| | 0.5 | 1.683±0.118** | 13.58 | | |
| Negative control | | 1.947±0.115 | 0 | | |
| Positive control | 0.1 | 0.319±0.083* | 87.13 | | |

Note: IR means Inhibition rate (%), $EC_{50}$ is 50% inhibitory concentration on secretion of HBsAg. TI means therapeutic index (TI = $TC_{50}/EC_{50}$). **P < 0.01, *P < 0.05 compared with negative control group.

**Table 8.** Inhibitory effect of $A_9$ on secretion of HBsAg from HepG2.2.15

| compound | Concentration (mg/mL) | Day 6 | | $EC_{50}$ (mg/ml) | TI ($TC_{50}/EC_{50}$) |
|---|---|---|---|---|---|
| | | $A_{490}$ IR (%) | | | |
| $A_9$ | 16 | 0.010±0.013** | 99.36** | 0.850 | 9.485 |
| | 8 | 0.045±0.023** | 97.21 | | |
| | 4 | 0.167±0.018** | 89.65 | | |
| | 2 | 0.510±0.044** | 68.41 | | |
| | 0.5 | 0.958±0.056** | 40.70 | | |
| Negative control | | 1.615±0.174 | 0 | | |
| Positive control | 0.1 | 0.416±0.046* | 71.25 | | |

Note: IR means Inhibition rate (%), $EC_{50}$ is 50% inhibitory concentration on secretion of HBeAg. TI means therapeutic index (TI = $TC_{50}/EC_{50}$). **P < 0.01, *P < 0.05 compared with negative control group.

resulted in over 90% inhibition of HBsAg secretion at 8 and 16 mg/ml compared with untreated control (P < 0.01), while the treatment of 3TC resulted in only 87.13% inhibition. And $A_9$ inhibited HBeAg secretion over 80% at 4, 8, 16 mg/ml (P < 0.01), while 3-TC only 71.25%. And comparing the ELISA results with the extraction $C_2$, it showed that with the further separation and purification to carry on, the TI of effective compounds increased accordingly. $A_3$, $A_5$, $A_9$ were the final effective anti-HBV compounds from *Rabdosia serra* with high effect and low toxic. $A_3$, $A_5$ and $A_9$ were not efficient on both HBsAg and HBeAg, but only impacted on one kind antigen respectively. And so therefore, before the separation, the mixture of each compound could inhibit the secretion of both HBsAg and HBeAg, but separation could purify the most effective compounds in order to have better pertinency and maneuverability, and optimal combination of separated active compounds could gain better effect, which were in accordance with the theory "effective compounds group" [27, 28].

*Anti-tumor activity study*

Among these compounds of $C_2$, three compounds ($A_6$, $A_7$, $A_{11}$) had higher cytotoxicities on HepG2.2.15 cells with the lower $TC_{50}$ values of 0.471, 1.208, 0.658 mg/ml respectively. So some more anti-tumor studies using MTT assay were performed on different tumor cells (MCF-7 cells, BGC-823 cells, HepG2 cells) to investigate their anti-tumor activity for the further study of anti-tumor drug development from *Rabdosia serra*.

As shown in **Table 9**, HepG2 cells growth were significantly inhibited in a dose-dependent

**Table 9.** Influence of $A_6$, $A_7$ and $A_{11}$ on three cells viability (%, $\bar{X}$±SD)

| compound | Concentration (mg/mL) | MCF-7 | | BGC-823 | | HepG2 | |
|---|---|---|---|---|---|---|---|
| | | IR (%) | $TC_{50}$ (mg/ml) | IR (%) | $TC_{50}$ (mg/ml) | IR (%) | $TC_{50}$ (mg/ml) |
| $A_6$ | 16 | 32.56±3.27 | 45.042 | 33.83±4.93 | 40.201 | 100±0.00 | 0.597 |
| | 8 | 19.35±1.41 | | 25.28±3.71 | | 96.35±7.51 | |
| | 4 | 15.17±2.03 | | 18.01±2.61 | | 92.51±8.17 | |
| | 2 | 11.92±0.75 | | 10.79±1.86 | | 84.63±6.52 | |
| | 0.5 | 3.27±1.22 | | -5.93±0.81 | | 59.39±4.63 | |
| $A_7$ | 16 | 96.02±7.07 | 0.902 | 41.13±5.13 | 28.151 | 98.03±8.27 | 1.156 |
| | 8 | 93.32±6.51 | | 30.72±4.69 | | 93.16±7.49 | |
| | 4 | 75.46±4.34 | | 23.61±3.26 | | 76.09±5.33 | |
| | 2 | 68.91±4.03 | | 13.87±2.61 | | 59.63±3.95 | |
| | 0.5 | 38.64±3.84 | | 7.25±1.06 | | 32.76±2.19 | |
| $A_{11}$ | 16 | 98.78±6.89 | | 97.53±8.40 | | 100.00±0.00 | |
| | 8 | 96.03±5.52 | | 93.32±7.84 | | 96.12±6.81 | |
| | 4 | 81.29±5.36 | 0.795 | 74.03±6.72 | 1.368 | 87.26±7.57 | 0.744 |
| | 2 | 67.31±3.50 | | 57.76±4.65 | | 78.62±6.79 | |
| | 0.5 | 47.82±2.78 | | 24.45±3.32 | | 53.92±5.02 | |

manner by all the three compounds, and the most effective compound is $A_6$ with $TC_{50}$ value of 0.597, but $A_6$ had little effect on other two cells with $TC_{50}$ values over 40 mg/ml. $A_7$ could inhibit MCF-7 and HepG2 with $TC_{50}$ values of 0.902 and 1.156 mg/ml respectively, but had slight effect on BGC-823 cells with the $TC_{50}$ value of 28.151 mg/ml. And $A_{11}$ had markedly inhibitory effect on all the three cells with the $TC_{50}$ values of 0.795, 1.368 and 0.744 mg/ml respectively. Moreover, obvious enhancements of cytotoxicities comparing with acetidin extraction $C_2$ were observed, and influence of different active compound on different tumor cells was different. These data exhibited that further isolation was good for increasing effectiveness and target activity of separated compounds. Thus these active compounds derived from $C_2$ can be used for different cancer prevention due to their various pertinencies on different cells.

In conclusion, the present study demonstrated that the acetidin extraction and their separated compounds from *Rabdosia serra* had potent anti-HBV activity *in vitro*. And among the purified compounds, there were three active compounds with high anti-tumor activity on different tumor cells *in vitro*. And with the further separation and purification carried on, the effectiveness were accordingly increased apparently. The *in vitro* potency and efficacy of active compounds from *Rabdosia serra* support the necessity and significance of developing this natural product into a potential therapeutic agent for better management of hepatitis B and cancer. Our study filled the gaps in the studies of anti-HBV and anti-tumor effective compounds from *Rabdosia serra in vitro,* and the activity screening in our study could provide a basis for further development and clinical use of prospective anti-tumor and anti-HBV medicines from *Rabdosia serra.* Nevertheless, further studies in vivo are necessary to confirm the activity and safety of *Rabdosia serra* for its clinical apply.

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## Disclosure of conflict of interest

None.

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