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ORIGINAL ARTICLE

A New Method for Purifying Brazilin from Lignum Sappan – Cytotoxic and Anti-Proliferative Effects in Cell Lines and Improved Survival in Mice Bearing Urinary Bladder Carcinoma

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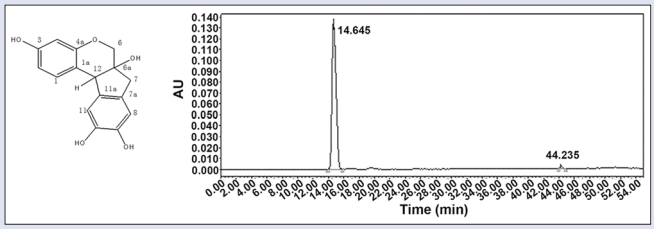
ABSTRACT

Background: Urinary bladder carcinoma is characterized by high post-operative recurrence rates. Sufu'ning lotion, prepared with Lignum Sappan, can reduce the recurrence of urinary bladder carcinoma. **Objectives:** The present study describes a methodology for extracting and obtaining high purity brazilin from Lignum sappan and evaluates its effects on urinary bladder carcinoma, *in vitro* and *in vivo*. **Materials and Methods:** The brazilin was extracted from Lignum sappan, purified and identified. The purified brazilin was assessed *in vitro* for its effects on tumor cell viability and growth using human (T24) and mouse urinary bladder (BTT) cancer cell lines. The role of brazilin in the inhibition of the cell cycle was determined by flow cytometry. The *in vivo* activity of brazilin was assessed based on the survival of tumor-bearing mice. **Results:** Brazilin was purified of 99.10% purity and was bioactive as it significantly ($P < 0.05$) inhibited the growth of T24 and BTT cells. The IC_{50} was 10.08 μg/ml for T24 cells and 8.76 μg/ml for BTT cells after 48 h of treatment. Brazilin blocked the cell cycle progression in the G2 phase. Brazilin (100, 200 and 300 mg/kg) significantly ($P < 0.05$) increased the survival of BTT tumor-bearing T739 mice and T24 tumor-bearing BALB/c nude mice. **Conclusion:** The methodology described for the extraction of brazilin from Lignum sappan yields high purity and bioactive brazilin. The extracted brazilin exerts marked inhibitory effects on urinary bladder cancer cells *in vitro* and *in vivo*.

Key words: Anti-tumor effect, brazilin, Lignum Sappan, purification, urinary bladder carcinoma

SUMMARY

- The study suggests the high purity and bioactive extraction of brazilin from Lignum sappan exhibits cytotoxic activity by inhibiting cell proliferation and viability of the human and mouse bladder cancer cell line. In addition, treatment with brazilin increases the survival of tumor-bearing mice.



Abbreviations used: TLC: Thin-layer chromatography; HPLC: High-performance liquid chromatography; NS: Normal saline; PI: Propidium iodide; LSD: Least significant difference; SD: Standard deviation; UV: Ultraviolet.

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INTRODUCTION

Urinary bladder carcinoma is the most common urogenital malignancy in China and is associated with high recurrence and mortality rates.[1] In China, there is an increasing trend in the incidence of bladder cancer; in 2008 alone, there were 54,927 new cases (estimated) and 21,024 deaths (reported) due to bladder cancer.[2] Without treatment, the survival of patients with bladder cancer is 16–20 months.[3,4] About 70% of bladder cancers are noninvasive and the recurrence rate is over 30% for patients undergoing partial resection. The remaining 30% of the reported urinary bladder cancers are muscle-invasive bladder carcinoma and the recurrence rates at 3–5 years are 50%–70%.[3,4]

The therapeutic strategy varies depending on the type, stage, and grade of cancer. Post-operative intravesical drugs can eliminate residual cancer cells and reduce the relapse rate.[5] The most commonly used perfusion drugs include a variety of anticancer drugs, Calmette's vaccine, and biological agents.[5,6] The common drawback associated with these drugs is high toxicity accompanied by severe side effects and relatively low efficacy since the 2-year relapse rate after intravesical treatments remains around 30%–50%,[5,7] even with the latest cytotoxic chemotherapeutics.[3,5,6] In addition, among all the cancers, the lifetime

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treatment cost per patient with bladder cancer is the highest, imposing a severe economic burden on health-care systems.[8] Therefore, there is an urgent need for the identification and characterization of new drugs effective for the treatment of bladder cancer.

Sufu'ning lotion, developed by our research group, is prepared with Lignum sappan and used for preventing the recurrence of bladder cancer.[9,10] Characterization of Sufu'ning lotion for the bioactive component identified brazilin as the primary agent with activity against cancer cells.[11,12]

Brazilin is reported to possess anti-inflammatory, antioxidative, and anti-proliferative activities.[12,13] Brazilin is a naturally occurring chemopreventive agent with strong anti-proliferative activity and has been reported to suppress cell proliferation and induce apoptosis in various cancer cells.[14] Moreover, brazilin can increase the activity of Poly Adp-Ribose Polymerase, resulting in proliferation inhibition of glioma cells in a time-dependent manner.[15] Brazilin is one of the most important bioactive components in the heartwood of Lignum sappan.[16,17] Isolation of brazilin in its purest form is crucial to study its anti-tumor mechanisms, but no effective methodology is available for the isolation and production of pharmacologically active brazilin.

Therefore, the present study aims to develop a methodology for the isolation and purification of brazilin from Lignum sappan. Furthermore, the purified brazilin was evaluated for its biological activity in an *in vitro* and *in vivo* assay assessing the anti-proliferative and anti-tumor effects on bladder cancer.

MATERIALS AND METHODS

Extraction and purification of brazilin

The heartwood of Lignum sappan was obtained from Wansheng Traditional Chinese Medication Decoction Pieces (Anhui, China). The procedure for the extraction of brazilin was in accordance with the previously described method for the purification of Protosappanin B.[11] The heartwood of Lignum sappan (1 kg) was extracted thrice with 8 L of boiling water for 60 min. The extracts were pooled together, concentrated with stills and filtered using an 80–120-μm membrane at normal pressure. An equivalent volume of petroleum ether was added to the filtrate to remove lipids. This procedure was repeated twice, and the aqueous phase was retained. An ethyl acetate fraction was obtained by adding an equivalent volume of ethyl acetate to the aqueous phase. This process was repeated thrice. The aqueous phase was dried under low pressure to obtain the sappan extract in a powdered form. For the purification of brazilin, 150 g of 160–200 mesh silica gel (Qingdao Marine Chemical Factory, Qingdao, China) were packed in a chromatographic column (4 cm × 40 cm). The powdered sappan extract (15 g) was dissolved in 100 mL of ethyl acetate along with 30 g of silica gel and mixed homogeneously. The solvent was removed by low-pressure distillation before the samples were subjected to chromatographic separation. The samples were eluted with ethyl acetate: Petroleum ether: Acetic acid (20:100:1) and analyzed using thin-layer chromatography (TLC) with developing solvent of chloroform-acetone-methanoic acid (8:4:1). The eluent was changed to ethyl acetate: Petroleum ether: Acetic acid (20:60:1) when spots of brazilin appeared and the distillate was collected until the spots of brazilin disappeared. The distillate was reapplied to the chromatographic column, eluted with ethyl acetate: Petroleum ether: Acetic acid (20:100:1) and analyzed by TLC. The distillates containing brazilin were pooled, dried under reduced pressure and the powder was collected as pure brazilin.

Identification of brazilin

Brazilin was identified by TLC and high-performance liquid chromatography (HPLC). For TLC, samples of the purified brazilin and a reference brazilin (Chengdu Mansite Biological Technology, Chengdu, China) were solubilized in ethanol and applied to the TLC plate (Qingdao Marine Chemical Factory, Qingdao, China), which was placed in a chromatography cylinder filled with the developing solvent chloroform: Acetone: Methanoic acid (8:4:1). The Rf values of the samples were evaluated by observing the plates under ultraviolet (UV) light.

For HPLC, purified brazilin was solubilized in methanol-water (18:82) to obtain a solution with a concentration of 0.2 mg/ml. HPLC was performed with a HPLC (1525, Waters, USA), and the amount of brazilin was quantified using a normalization method.

Structure analysis of brazilin

The structure of the purified brazilin was confirmed using elemental analysis (DR × 300 MHz NMR equipment, Bruker, USA), UV absorption spectrum analysis (UV-2550 UV absorption spectrometry, Shimadzu, Japan) and infrared absorption spectrum analysis (Fourier-transformation infrared spectroscopy, 380, Nicolet, USA).

Cell culture

T24 (human bladder carcinoma cell line; Shanghai Institute of Biological Sciences, Chinese Academy of Sciences, China) and BTT (mice urinary bladder cell line, a kind gift from Professor Xiaofeng Yang of the Department of Urology, the First Affiliated Hospital of Shanxi Medical University, China) cells were cultured in RPMI 1640 culture medium (Gibco, USA) containing 0.05 g/L streptomycin (North China Pharmaceutical Group Corp, Shijiazhuang, China), 0.05 g/L penicillin (North China Pharmaceutical Group Corp, Shijiazhuang, China), 0.8 g/L $NaHCO_3$, 3.6 g/L HEPES (Sangon Biotech (Shanghai) Co., Ltd., China), and 10% (fetal bovine serum, Hangzhou Sijiqing Biological Engineering Materials, China). The cells were grown in an incubator (NU-5500E, NuAire, USA) at 37°C, under 5% CO_2 and saturated humidity. The cells were detached using 0.25% trypsin (Sangon Biotech, Shanghai, China) to obtain a cell suspension.

Trypan blue exclusion assay

The T24 and BTT cells were adjusted to 2×10^6 cells/ml with normal saline (NS). The cell suspension (500 μL) was treated with 500 μL of brazilin solubilized in NS (final concentration of 2 mg/ml), 500 μL of mitomycin dissolved in NS (final concentration of 1.2 mg/ml, positive control), or 500 μL of NS (control group). The experiment was performed in duplicate. The cell suspension (100 μL) was collected at 0 (baseline control), 20, 40, 60, and 100 min and mixed with an equal volume of trypan blue (Sigma, St. Louis, MO). The number of dead (dark blue-stained) and viable (non-stained) cells in four large grids of the hemocytometer were recorded under an optical microscope (×100; Olympus, Tokyo, Japan). The death rates of T24 and BTT cells were calculated as cell death rate = dead cell count/(dead cell count + viable cell count) ×100%.

Cell proliferation assay

The T24 and BTT cells were seeded into 96-well plates (4×10^3 cells/well) with 100 μL of culture medium. The plate was incubated at 37°C, under 5% CO_2 and saturated humidity. After 24 h, the culture medium was replaced with 200 μL of fresh culture medium containing different concentrations of purified brazilin (final concentrations of 12.5, 25, 50, 100, and 200 μg/ml); an equal volume of culture medium was added to the negative control group; mitomycin was used for the positive control group. The culture medium alone was used as a blank. Five wells were analyzed for each group. After 48 h, 20 μL of MTT (5 mg/mL; Solarbio, Beijing, China) were added to each well and incubated for an additional 4 h. The culture medium was aspirated before adding 150 μL of dimethyl

sulfoxide (Beijing Chemical Works, Beijing, China). The plate was agitated at low speed for 10 min to allow the complete dissolution of the crystals and the absorbance was measured with a microplate reader at 570 nm (A_{570}). The growth inhibition ratio and IC_{50} values of purified brazilin for T24 and BTT cells were calculated as: Growth inhibition ratio (%) = (1–mean A_{570} in the treatment group/mean A_{570} in the control group) ×100%; $lgIC_{50}$ = Xm – I (P–(3–Pm–Pn)/4), where Xm: l g maximum dose; I: l g (maximum dose/adjacent dose); P: Sum of positive reaction rates; Pm: Maximum positive reaction rate; Pn: Minimum positive reaction rate.

Cell cycle analysis

The cells were seeded at 8×10^5 in a T25 culture flask. After 24 h, the medium was replaced with fresh medium containing different concentrations of brazilin (12.5, 25, and 50 μg/mL) and negative control was set. Post 24 h incubation, the cells were harvested by trypsin treatment and centrifugation (1000 rpm, 5 min), washed with phosphate-buffered saline (PBS) and fixed with 70% cold ethanol at 4°C overnight. The fixed cells were washed with PBS and filtered through nylon mesh and pellets were obtained by centrifugation (1000 rpm, 5 min). RNase A was added to the pellet and incubated at room temperature for 20 min. The cells were stained with propidium iodide (PI) in the dark for 20 min and analyzed by flow cytometry to determine the cell cycle distribution. The experiment was performed in duplicates.

Effects of brazilin purified on the survival of tumor-bearing mice

Thirty female T739 mice (6 weeks of age and weighing 18–22 g) were purchased from Beijing HuaFuKang Biotechnology, Beijing, China (production license number: SCXK (Jing) 2014–0008). Thirty female BALB/c nude mice (5 weeks of age and weighing 16–19 g) were purchased from the Vital River Laboratories, China (production license number: SCXK (Jing) 2016–0006). The T739 mice and the BALB/c nude mice were intraperitoneally injected with 2 mL of 5×10^5 logarithmic phase BTT and T24 cells, respectively.

The T739 mice and BALB/c nude mice were randomized into five groups: Control group, mitomycin (1 mg/kg; Zhejiang Hisun Chemical Co., Ltd, Zhejiang, China), brazilin (100 mg/kg), brazilin (200 mg/kg), and brazilin (300 mg/kg) (n = 6/group). The drugs or NS (0.2 mL) were administered intraperitoneally to each group 24 h after intraperitoneal injection of the cells. The drugs were administered daily for 6 days in the brazilin and control groups, while the mitomycin injections were administered twice on the 1st and 6th days.

Survival was evaluated over a 60-day observation period (60 days was recorded as the maximum survival time). Survival was compared among the different groups and the rate of extended survival was calculated. The mice were dissected immediately after death to harvest the bladder. The tissue was weighed and inspected with the naked eye by a trained investigator. The laboratory's animal use certification number was SYXK-2017–0003 and the study was approved by the Laboratory Animal Management Committee at the Shanxi Cancer Institute with reference to the National Research Council Guide for the Care and Use of Laboratory Animals in China.

Statistical analysis

The SPSS 17.0 (IBM Crop, Armonk, NY, USA)was used for all statistical analyses. The data were compared with one-way analysis of variances followed by the least significant difference *post hoc* test. The Student's *t*-test was used for comparisons between two groups. All data were expressed as mean ± standard deviation. P <0.05 was considered to indicate statistical significance.

RESULTS

Identification of brazilin

The purified brazilin extracted from Lignum sappan was qualitatively analyzed by TLC with reference to a standard preparation of brazilin. The Rf value of the purified brazilin was 0.67, which was identical to that of the reference brazilin, confirming the identity of the purified brazilin. The chemical structural formula of the purified brazilin is shown in Figure 1a. The purity of the brazilin extract was shown to be 99.10% by HPLC, and a normalization method [Figure 1b].

Elemental analysis of carbon, hydrogen, and oxygen content

Elemental analyses showed an error rate not exceeding ±3%, suggesting that the carbon, hydrogen, and oxygen contents of the purified brazilin were in accordance with the theoretical values of the reference brazilin ($C_{16}H_{14}O_5$) [Table 1].

Ultraviolet absorption spectra analyses

The purified brazilin showed maximum absorption peaks at the λ_{max}^{MeOH} of 201 nm and 287 nm in UV spectrum analysis [Figure 1c], which represents the E- and B-bands of aromatic compounds. The absorption characteristics were in accordance with the reference brazilin [Figure 1d]. Therefore, the UV absorption spectrum of purified brazilin was identical to the reference brazilin.

Infrared absorption spectra analyses

Infrared absorption spectrum analysis showed a wide, strong $\nu_{O\text{-}H}$ peak at 3389.05 cm^{-1} and strong νø-o peaks at 1301.53 cm^{-1} and 1230.10 cm^{-1}, indicating the presence of phenolic hydroxyl groups in the purified brazilin. The strong $\nu_{C\text{-}O}$ peak at 1158.81 cm^{-1} indicated the existence of tertiary alcohol hydroxyl groups, while the $\nu_{=C\text{-}H}$ peak at 3036 cm^{-1}, the $\nu_{C=C}$ peaks at 1622.20, 1598.49, and 1507.88 cm^{-1} and the $\gamma_{ø\text{-}H}$ peaks at 963.21, 867.58, and 776.39 cm^{-1} suggested the existence of 1, 2, 4 three substituted and 1, 2, 4, 5 four substituted phenyl groups. The infrared absorption spectrum of the purified brazilin was identical to the spectrum of the reference brazilin [Figure 1e and f].

These results clearly demonstrated that the structure of the purified brazilin extracted from Lignum sappan in the present study was identical to the reference brazilin, with a molecular formula of $C_{16}H_{14}O_5$ and molecular weight of 286.28.

Brazilin induced cytotoxicity in T24 and BTT cell lines

Brazilin exerted statistically significant ($P < 0.05$) inhibitory effects on the proliferation of T24 and BTT cells in a concentration-dependent manner, as assayed by the MTT cell proliferation assay. Compared with the control group, the inhibitory rates of brazilin at 12.5, 25, 50, 100, and 200 μg/mL in T24 cells were 74.5%, 96.7%, 98.0%, 97.7%, and 95.9%, respectively; those rates in BTT cells were 94.9%, 94.8%, 96.2%, 96.7%, and 97.8%, respectively. The IC_{50} of brazilin treating for 48 h was 10.08 μg/mL for T24 cells and 8.76 μg/mL for BTT cells. Brazilin caused

Table 1: Elemental analysis of carbon, hydrogen, and oxygen contents of the purified brazilin

Sample	Carbon (%)	Hydrogen (%)	Oxygen (%)
Purified brazilin	67.1	4.9	28.0
Reference brazilin	67.1	4.9	28.0

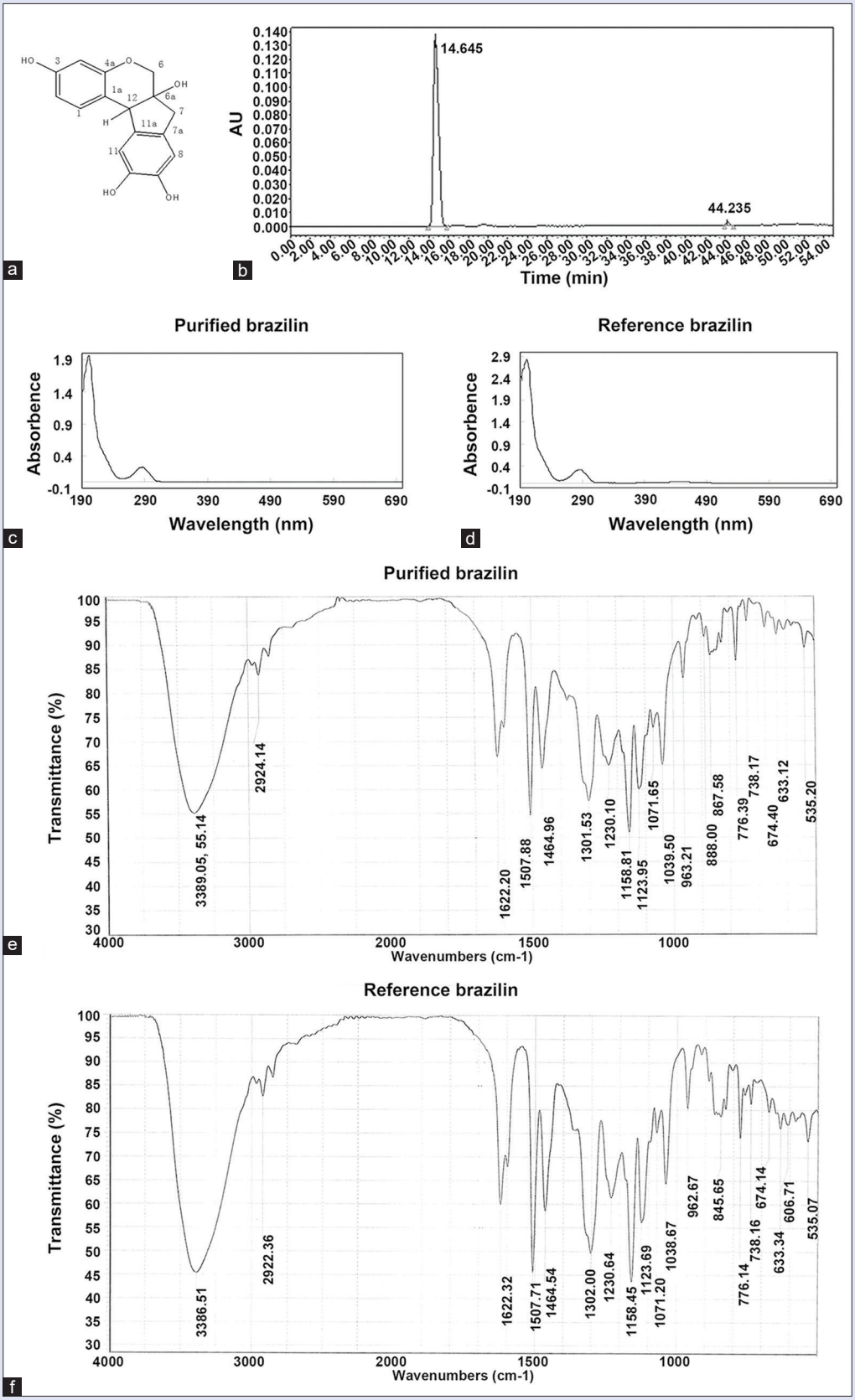

Figure 1: Identification of Brazilin. The structure of the purified brazilin was confirmed using elemental analysis, ultraviolet absorption spectra and infrared absorption spectra. The purity of the purified brazilin was determined from high-performance liquid chromatography analysis. (a) Chemical structure of the purified brazilin. (b) High-performance liquid chromatogram of the purified brazilin. (c) Ultraviolet absorption spectrum of the purified brazilin. (d) Ultraviolet absorption spectrum of the reference brazilin. (e) Infrared absorption spectrum of the purified brazilin. (f) Infrared absorption spectrum of the reference brazilin

a significant ($P < 0.05$) decrease in the viability of both T24 and BTT cells, as assayed by the trypan blue exclusion assay [Tables 2 and 3]. The effect of brazilin on the cell cycle of T24 cells was studied by flow cytometry. The flow cytometry data revealed that with the increase in concentration of brazilin, there was statistically significant ($P < 0.05$) increase in the cells accumulated in the G2 phase and significant ($P < 0.05$) decrease in the cells in G1 phase, resulting in cell arrest in G2 phase [Table 4 and Figure 2].

Effects of brazilin purified on the survival of tumor-bearing mice

The administration of brazilin or mitomycin into the abdominal cavity of BTT tumor-bearing T739 mice and T24 tumor-bearing BALB/c nude mice significantly ($P < 0.05$) increased the mean survival compared with the control group [Tables 5 and 6].

Table 2: Death rates (%) of T24 cells after treatment with brazilin *in vitro*

Group/time[18]	0	20	40	60	80	100
Control	2.21±0.35	1.69±0.35	1.66±0.37	1.65±0.35	1.52±0.31	1.33±0.02
Mitomycin	1.79±0.33	11.15±0.36*	13.06±3.50*	38.18±2.90*	55.91±2.29*	83.97±0.94*
Brazilin	2.41±0.29	97.71±0.95*	100.00±0.00*	100.00±0.00*	100.00±0.00*	100.00±0.00*

*$P<0.05$, versus the control group

Table 3: Death rates (%) of BTT cells after treatment with brazilin *in vitro*

Group/time[18]	0	20	40	60	80	100
Control	2.04±0.51	2.87±0.69	2.47±0.88	1.90±0.24	3.66±1.00	3.42±1.51
Mitomycin	2.22±0.45	15.45±1.52*	19.88±2.54*	40.25±3.35*	60.88±2.54*	85.36±1.32*
Brazilin	2.58±0.54	98.77±0.31*	100.00±0.00*	100.00±0.00*	100.00±0.00*	100.00±0.00*

*$P<0.05$, versus the control group

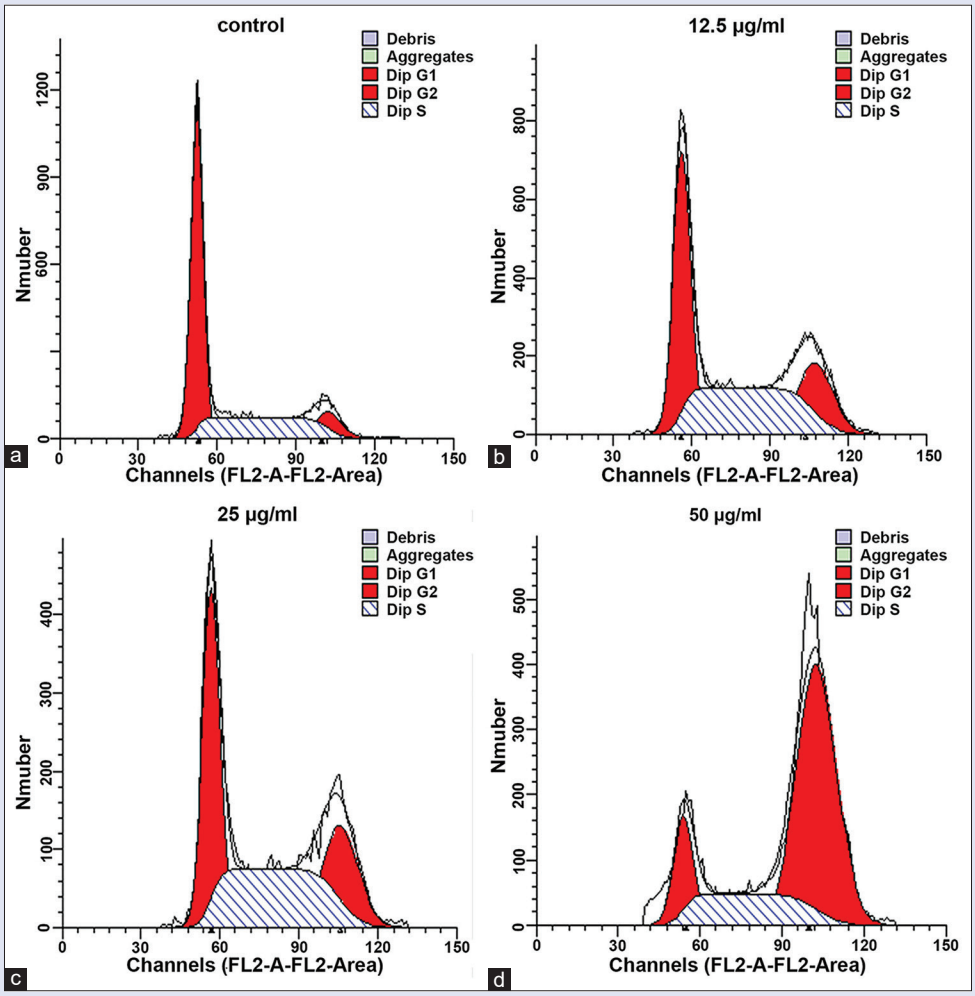

Figure 2: Effect of purified brazilin on cell cycle of T24 cells. Brazilin arrests the growth of T24 cells in the G2 phase. Flow cytometer data of T24 cells treated with no brazilin-Control. (a) 12.5 μg/ml brazilin, (b) 25 μg/ml brazilin, (c) and 50 μg/ml brazilin, (d) for 24 h

DISCUSSION

In the present study, we report a methodology for the extraction and purification of brazilin in a highly pure form. We demonstrate that the purified brazilin is bioactive and has cytotoxic effects on the human (T24) and mice urinary bladder (BTT) cancer cells. In addition, the purified brazilin increases the survival of BTT tumor-bearing T739 mice and T24 tumor-bearing BALB/c nude mice.

In the present study, we obtained high-purity brazilin (99.1%), as determined by TLC and HPLC. In addition, the UV and IR spectra revealed that the purified brazilin is optically similar to the reference brazilin. We successfully isolated brazilin from Lignum sappan, in the same manner, that some previous studies of brazilin.[18-22] Nevertheless, the optimal and most efficient method to extract brazilin should be investigated by comparing multiple methods. Finding an efficient method is a prerequisite for the pharmaceutical production of brazilin.

Brazilin exhibits anti-tumor activity through unknown mechanisms. Brazilin has an anti-inflammatory effect on macrophages and functions through a novel mechanism involving the action of heme oxygenase-1 (HO-1)[23] and iNOS gene expression.[24] Brazilin is an antioxidant that protects against oxidative injury through the expression of HO-1. It also protects cultured rat hepatocytes from $BrCCl_3$-induced toxicity.[25] Brazilin may increase glucose transport (GLUT) by recruitment of GLUT4 from intracellular pools to the plasma membrane in adipocytes via the activation of PI3-kinase,[26] inhibit the downstream signaling of cAMP pathways,[27] and inhibit hepatic gluconeogenesis by elevating the F-2 and 6-BP levels in hepatocytes.[27] Brazilin has been found to be a collagen receptor agonist in platelets.[18] Nevertheless, the understanding of the precise mechanism of the highly pure form of brazilin is another prerequisite for future clinical applications in patients with cancer.

Besides the metabolic effects described above, the ethyl acetate extracts of the heartwood of Lignum sappan L. have anti-cancer activity.[28] Characterization of its crude extracts revealed that the active component is brazilin.[28] Brazilin was shown to induce myeloma cancer cell (U266) death over a short duration and also to reduce the risk of metastasis.[14] A similar cytotoxic effect of brazilin in human glioblastoma cells was reported,[15] as well as in multiple myeloma cells.[14] Brazilin can inhibit the proliferation and induce apoptosis of tongue cancer cells through the AMPK/mTOR pathway.[29] In the present study, the purified brazilin was cytotoxic to T24 and BTT cells was anti-proliferative and induced cell death in both cell lines. We found that the IC_{50} was 10.08 μg/mL for T24 cells and 8.76 μg/mL for BTT cells after treatment with brazilin for 48 h. Therefore, we conclude that the purified brazilin was highly active in the urinary bladder cancer cells *in vitro*. Nevertheless, the exact molecular mechanisms responsible for those effects still have to be elucidated.

To assess the *in vivo* activity of brazilin, the survival of BTT tumor-bearing T739 mice and T24 tumor-bearing BALB/c nude mice treated with brazilin was studied. Over a follow-up period of 60 days, brazilin significantly increased the survival of the tumor-induced mice. Therefore, the brazilin extracted from Lignum sappan using the methodology described here exerts anti-tumor effects. This is supported by a previous study that showed that the intravesical treatment using Sufu'ning lotion in mice bearing xenografts inhibited cancer growth and improved survival.[10]

Table 4: Cell cycle distribution of T24 cells after brazilin administration

Group	G1 phase	S phase	G2 phase
Control	60.06±0.29	30.70±0.99	9.24±1.22
12.5 μg/mL	39.37±1.06*	40.20±0.43*	20.43±0.63*
25 μg/mL	37.30±0.83*	41.09±1.12*	21.98±0.87*
50 μg/mL	18.51±5.14*	22.10±1.19*	59.40±5.57*

The data are represented as percentage mean±SD. *P<0.05 compared to the control group. SD: Standard deviation

Table 5: Effect of brazilin on survival of BTT tumor-bearing T739 mice

Group	*n*		Drug administration times (d)	Mean survival (d)	Rate of extended survival (%)
	Baseline	End			
Control	6	0	-	14.52±2.32	-
Brazilin 300 mg/kg	6	0	6	22.35±2.41*	53.92
Brazilin 200 mg/kg	6	0	6	23.67±3.25*	63.02
Brazilin 100 mg/kg	6	0	6	23.58±2.35*	62.40
Mitomycin 1 mg/kg	6	0	2	25.66±4.21*	76.72

The data are represented as mean±SD. *P<0.05 versus the control group. SD: Standard deviation

Table 6: Effect of brazilin on survival of T24 tumor-bearing BALB/c nude mice

Group	*n*		Drug administration times (d)	Mean survival (d)	Rate of extended survival (%)
	Baseline	End			
Control	6	0	-	20.55±4.69	-
Brazilin 300 mg/kg	6	0	6	36.65±2.04*	78.34
Brazilin 200 mg/kg	6	0	6	35.08±3.25*	70.70
Brazilin 100 mg/kg	6	0	6	35.58±3.35*	73.14
Mitomycin 1 mg/kg	6	0	2	36.45±3.63*	77.37

The data are represented as mean±SD. *P<0.05 versus the control group. SD: Standard deviation

CONCLUSION

Brazilin exhibits cytotoxic activity by inhibiting cell proliferation and viability of the human bladder cancer cell line T24 and the mouse bladder cancer cell line BTT. In addition, treatment with brazilin increases the survival of tumor-bearing mice. The brazilin extracted using the described methodology has the potential to be an effective chemotherapeutic for the treatment of urinary bladder carcinoma. The underlying mechanisms are yet to be elucidated.

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Conflicts of interest

There are no conflicts of interest.

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