Original Article

Isocoreopsin: An active constituent of n-butanol extract of *Butea monosperma* flowers against colorectal cancer (CRC) ☆

CrossMark

Boopathi Subramaniyan, Navaneethakrishnan Polachi, Ganeshan Mathan *

Department of Biomedical Science, School of Basic Medical Science, Bharathidasan University, Tiruchirappalli 620 024, TN, India

ARTICLE INFO

ABSTRACT

The herb *Butea monosperma* constitutes several human health beneficial components, which are mostly studied for their anticancer effects. In this study, the activity of n-butanol fractions of *B. monosperma* floral extract was examined on inhibiting aberrant crypt foci (ACF) formation in azoxymethane induced Wistar albino rats. The n-butanol extracts (150 mg/kg) decreased the ACF formation (per rat) by 92% and 78% in short- and long-term in vivo treatments, respectively. All the compounds in the n-butanol extract were isolated and purified using column and reverse-phase high pressure liquid chromatography (HPLC). Their structures were characterized using UV–visible spectroscopy, nuclear magnetic resonance (NMR) and electrospray–ionisation mass spectrometry (ESI–MS) to determine important flavonoids, namely isocoreopsin, butrin and isobutrin. These compounds were studied for their free radical scavenging and anticancer activities. The compound isocoreopsin showed significantly greater efficacy in cell death on human colon and liver cancer cell lines (50 µg/mL in HT-29 and 100 µg/mL in HepG2) than butrin (100 µg/mL in HT-29 and 500 µg/mL in HepG2) and isobutrin (80 µg/mL in HT-29 and 150 µg/mL in HepG2). These results suggest that isocoreopsin, butrin and isobutrin are the important key compounds for the chemoprevention of colon cancer and isocoreopsin can be considered as a promising novel drug.
© 2016 Xi'an Jiaotong University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

In humans, colorectal cancer (CRC) and hepatocellular carcinoma (HCC) rank the second and third most common causes of cancer-related death in most developed countries around the world [1,2]. The incidence of liver cancer is steadily increasing due to obesity, diabetes, alcohol intake, cirrhosis and rise of hepatitis C virus infection [3]. Surgery and chemotherapy are available for HCC treatment but the multidrug resistance (MDR-1) gene properties and their related protein expression always pose a challenge for the treatment of cancer [4].

Aberrant crypt foci (ACF) in colon has been proposed as precancerous lesions for CRC and an appropriate biomarker for identifying modulatory effects of xenobiotic on colon carcinogenesis [5]. The simple interpretation is that the identification of preneoplastic lesions may lead to the execution of successful chemoprevention studies in cancer treatment [6]. Interestingly, nutrients derived from plant source play a vital role in prevention and treatment of many diseases by stimulating antioxidants. Over several years, the accumulating evidence suggested that phenolic compounds have potential antioxidant activity with the presence of flavonoids [7]. Earlier reports of medicinal plants and their prospective effects on many diseases and disorders have revealed a growing interest in the herbal drug for its divergent medicinal purpose [8].

Several studies suggest that the methanolic and acetone extract of *Butea monosperma* (*B. monosperma*) flowers have antibacterial, antifungal, anti-inflammatory and wound healing properties [9–11]. In addition to this, petroleum ether extracts and ethanolic extracts of *B. monosperma* flowers possess antidiabetic, antipyretic and radical scavenging activities with respect to in vivo and in vitro models [12,13].

Ethanolic extract of *B. monosperma* leaves increased the anticancer activity against Ehrlich ascites carcinoma (EAC) in Swiss albino mice [14]. Similarly, aqueous extract of *B. monosperma* flowers was found to be effective on HBV-related X15-*myc* mouse model of HCC [15]. Further, it was reported that the aqueous extract of *B. monosperma* flowers has anticancer activity by stimulating proapoptotic function and inhibiting cell growth, anti-oxidant and free radical scavenging activities in vitro study [16]. As mentioned previously, extracts of *B. monosperma* flowers exhibit potential anticancer activity due to its flavonoids such as butin, butein, butrin, isobutrin, palasitrin, coreopsin, isocoreopsin,

---

sulphurein, monospermoside and isomonospermoside [17]. Moreover, the earlier reports indicated that the overexpression of Wnt signaling pathway played a vital role in CRC and HCC development through the upregulation of early growth response genes [18–20]. However, the reports suggested that *B. monosperma* may have a suppressive effect on early growth response genes and thus mechanism needs further investigation for better understanding.

This paper details the effect of the n-butanol extract of *B. monosperma* flowers on colon cancer in azoxymethane (AOM) induced rat model and the isolation of all the three active compounds, namely isocoreopsin, butrin and isobutrin, by chromatography methods and structural validation using nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FTIR) and electrospray–ionisation mass spectrometry (ESI–MS). Further, it strived a clear idea on the n-butanol extract (NBE) of *B. monosperma* flowers and their individual effect on hepatocarcinoma and adenoma cancer cell lines.

2. Experimental

2.1. Chemicals and reagents

Naringenin (5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one), ellagic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and AOM were obtained from Sigma–Aldrich Co (Saint Louis, MO, USA). Sodium carbonate, aluminium chloride, potassium acetate, silica gel (60–120 mesh) and HPLC grade acetonitrile were obtained from Merck & Co (USA). Dulbecco's modified Eagle's medium (DMEM), 10% fetal bovine serum (10% FBS), penicillin/streptomycin and DMSO (cell culture grade) were purchased from Hi Media Laboratories, Mumbai, India. All other chemicals and solvents used were of analytical grade/better.

2.2. Instrumentation

The UV spectra were recorded with double beam UV-visible spectrophotometer. The FTIR spectra of the separated compounds were confirmed on JASCO FTIR Spectrophotometer (USA). NMR spectrum was revealed in DMSO-d_6 on a Bruker Avance 500 or 600 spectrometer (Switzerland). ESI–MS was recorded on Waters UPLC-TQD (USA). Silica gel (60–120 mesh) was used to separate the various compounds by column chromatography against methanol and ethyl acetate of analytical grade (Merck & Co, USA). Purification of the compounds was achieved by pre-coated silica gel F254 TLC. Adsorption data for the free radical scavenging activity was measured by using Shimadzu 800 spectroscopy (Japan).

2.3. Plant material

Butea monosperma (Lam.) Taub. flowers were collected from the Palkalai Perur campus of Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. A voucher specimen was confirmed and deposited in the Department of Plant Science, Bharathidasan University (BDUT 1001).

2.4. Extraction of B. monosperma flowers

Air dried *B. monosperma* flowers were finely powdered and extracted with methanol in soxhlet apparatus for 24 h. Then the extract was concentrated under reduced pressure in a rotator evaporator, which was partitioned with water and ethyl acetate (EtOAc). After partition, the persistent water phase was treated with n-butanol (3 times) and dried with sodium sulphate. Subsequently, the NBE was concentrated by rotator evaporator and stored at 4 °C [21].

2.5. Isolation of active compounds from B.monosperma flowers by column chromatography

The dried NBE of *B. monosperma* flowers was subjected to Silica-100 macroporous adsorption resin column. The column was rinsed with EtOAc (discarded), then EtOAc with various concentrations of MeOH (5%, 15%, 30%, 50%, 70%, and 95%, v/v) was used as mobile phase to acquire three fractions successively.

2.5.1. High performance thin layer chromatography (HPTLC)
The collected fractions were dissolved in HPLC grade methanol (1 mg/mL) and subjected to HPTLC. The sample was spotted on pre-coated silica gel F254 TLC plates using CAMAG Linomat V automatic sample Spotter and the plates were developed in EtOAc methanol (30:70, v/v) solvent system. The plates were scanned in TLC scanner 3 (CAMAG) at 254 nm. The Rf values, spectra, Lambda max and peak areas of the resolved bands were recorded and relative percentage area of each band was calculated from peak areas.

2.5.2. High pressure liquid chromatography (HPLC)
Among the three collected fractions, fraction II was analyzed on a Waters 2545 HPLC-photodiode array system (Quaternary gradient module) with symmetry C_{18} column (250 mm × 4.6 mm, 5 µm) at a column temperature of 28 °C. Two mobile phases, A and B, were used. Mobile phase A was 0.1% (v/v) phosphoric acid in water, while mobile phase B was acetonitrile. A ratio of 70% A and 30% B was applied in the first 15 min. After 15 min, a ratio of 50% A and 50% B was used for the next 25 min. Finally, 5% A and 95% B were used after 30 min for an additional 15 min. The solvent flow rate was 1 mL/min and the sample injection volume was 20 µL. The UV–visible spectra were recorded in the wavelength range of 200–800 nm.

2.5.3. Spectrometric identification
2.5.3.1. Isocoreopsin (2-(3, 4-Dihydroxy-phenyl)-7-(3, 4, 5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-chroman-4-one (1)).
UV: 217, 348, 367, 408 nm. ESI–MS: Isocoreopsin compound displays a peak at m/z (M + H)$^+$ 435.36 (calcd m/z 434.39). ^{1}H and ^{13}C NMR data (400 and 100 MHz, DMSO- d_6) (Table 1).
2.5.3.2. Butrin (2-[4-Hydroxy-3-(3, 4, 5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-phenyl]-7-(3, 4, 5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-chroman-4-one (1)).
UV: 217, 329, 368 nm. ESI–MS: Butrin compound displays a peak at m/z (M +Na)$^+$ 619.2 (calcd m/z 619.16). ^{1}H and ^{13}C NMR data (400 and 100 MHz, DMSO- d_6) (Table 1).
2.5.3.3. Isobutrin (3-[4-Hydroxy-3-(3, 4, 5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-phenyl]-1-[2-hydroxy-4-(3, 4, 5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-phenyl]-propenone).
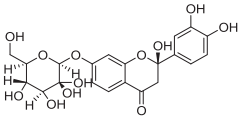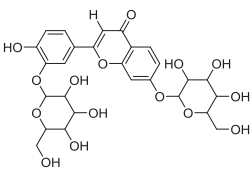UV: 217, 348, 367, 408 nm. ESI–MS: Isobutrin compound displays a peak at m/z (M+H)$^+$ 597.1 (calcd m/z 596.17). ^{1}H and ^{13}C NMR data (400 and 100 MHz, DMSO- d_6) (Table 1).

2.6. Quantification of bioactive compounds

2.6.1. Determination of total flavonoids content
The total flavonoids content of different extracted compounds of *B. monosperma* flowers were determined according to $AlCl_3$ method. Different concentrations of each compound (2000 µg/mL) were separately mixed with 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M potassium acetate to a final volume of 3 mL with distilled water, followed by incubation for 30 min at room temperature. The

Table 1.
^{13}C and ^{1}H NMR spectroscopic data for compounds 1,2,3 with ESI–MS fragmentation ions.

Compound	$\delta\,^{1}$H (J, Hz)	$\delta\,^{13}$C	Structure
Isocoreopsin	7.720 (2.0)	190.08	
	7.681 (1.0)	164.61	
	6.898 (4.0)	163.09	
	5.427 (6.0)	145.13	
	3.112 (2.0)	128.36	
	0.979 (7.0)	115.30	
		113.49	
		102.57	
		77.10	
		75.94	
		73.26	
		73.04	Molecular Weight : 434.39338
		60.73	Molecular formula : $C_{21}H_{22}O_{10}$
		39.88	
		38.73	
		33.63	
		31.23	
		28.96	
		28.49	
		22.04	
		13.87	
Butrin	7.225 (3.0)	190.08	
	6.979 (3.0)	192.00	
	6.796 (3.0)	165.01	
	6.776 (3.0)	164.61	
	4.945 (5.0)	163.05	
	4.668 (5.0)	149.09	
	3.655 (7.0)	145.06	
	2.644 (3.0)	145.01	
		132.44	
		132.03	
		128.36	
		115.30	
		113.49	
		102.57	
		77.10	
		75.94	
		73.26	Molecular Weight : 596.537
		73.04	Molecular formula : $C_{27}H_{32}O_{15}$
		60.73	
		33.63	
		31.23	
		29.00	
		28.96	
		28.64	
		28.49	
		22.04	
		13.87	
Isobutrin	7.680 (3.0)	192.00	
	6.983 (3.0)	190.05	
	6.796 (3.0)	165.01	
	6.760 (3.0)	163.05	
	4.980 (5.0)	149.09	
	4.810 (3.0)	147.08	
	4.668 (2.0)	145.08	
	3.655 (7.0)	145.06	
	2.644 (3.0)	145.01	
		137.04	
		132.03	
		129.69	
		128.36	Molecular Weight : 596.53398
		127.88	Molecular formula : $C_{27}H_{30}O_{15}$
		121.69	
		115.75	
		110.90	
		103.52	
		99.69	
		79.23	
		77.17	
		76.36	
		73.30	
		69.88	
		60.49	
		60.05	
		42.97	

absorbance was recorded at 415 nm. The amount of distilled water was substituted by the same amount of fraction in the blank. Naringenin (100, 200, 300, 400, 500 and 1000 µg/mL) as a standard was used to make a calibration curve. The total flavonoids content of the compound was expressed as milligram acid equivalents (Naringenin) per gram extract [22].

2.6.2. Evaluation of DPPH radical scavenging activity

The reaction mixture was prepared by dissolving dried flower extracts of 3 mg in 50% aqueous ethanol. About 100–500 µL of the mixture was taken and made up to 750 µL with distilled water; 0.1 mM ethanolic DPPH solution was added to achieve a final volume of 1.5 mL. Then the mixture was incubated in the dark at room temperature for 30 min, and reduction of purple color was read at 517 nm. Ascorbic acid present in solution A was used as a standard and absolute ethanol as a blank. Radical scavenging activity of isolated phenolic compounds was expressed as the percentage of inhibition of free radical and calculated using the following formula [23].

$$\text{DPPH scavenged}\,(\%) = \left[\left(\text{Abs control–Abs test}\right)/\text{Abs control}\right] \times 100\%$$

Where the Abs control is the absorbance of the control reaction, whereas Abs test is the absorbance of the compound/ standard.

2.7. Experimental animals

Five-week-old male Wistar albino rats were obtained from Indian Institute of Science (IISC), Bangalore, India, and maintained in our animal house in a ventilated, temperature-controlled room at 25 °C with 12/12 h light/dark cycle for 1–2 weeks prior to the actual commencements of the experiment. They were provided with standard food pellets and drinking water (ad libitum). The protocol for the study was approved by Institutional Animal Ethics Committee (IAEC), Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.

2.7.1. In vivo experimental protocol

Two protocols were designed for the evaluation of potential chemo preventive agents against AOM induced colon cancer in wistar albino rats [24]. In the first protocol, wistar albino rats were injected (subcutaneously) twice with AOM at the dosage of 15 mg/kg during the first two weeks of a five-week experimental period, and the NBEs were administered (intraperitonial) during the fourth week (two weeks after the last injection of AOM). This protocol was used to evaluate the effects of the test compound during the initiation phase in order to understand the carcinogen metabolism. The second protocol was similar to the first in most respects except that the animals would not be exposed to the test compounds until aberrant crypt foci (ACF) has grown in the colon of the rats until the end of fifth week. During the second two-week period, the NBE was administered intraperitoneally to evaluate the effects on outgrowth and progression of ACFs. Six groups of Wistar albino rats (6 rats/group) were classified for treatment regimen (Table 2). After the treatment period, the experimental animals were sacrificed by intramuscular administration of ketamine (90 mg/kg).

2.7.2. Histological observation of ACF formation

After treatment, 10–15 pieces of colonic tissues containing the nodule shape of ACF were dissected from the mid-distal part of each colon. They were placed in a petri dish containing saline solution and were washed and dried to be embedded in paraffin wax. These samples were sectioned parallel using a microtome and stained with hematoxylin and eosin (HE). Histopathological identification of ACF was based on the criteria of multiplicity of aberrant crypts. Multiplicity was determined as the number of crypts in each focus and categorized as containing up to two, three or more aberrant crypts/focuses. Fixed colorectal tumors were processed and stained, and crypts were identified as described.

2.8. Cell viability assay

The human liver cancer cell line HepG2 and the human colon

adenocarcinoma cell line HT 29 were purchased from National Centre for Cell Science (NCCS), Pune, India and cultured in DMEM medium with supplementation of 10% FBS, penicillin, streptomycin and fungizone. After 24 h of incubation in serum-free medium, the cells were exposed to isolated pure compounds at the indicated concentrations for 48 h for MTT assay. The cell viability of different test compounds was determined using the equation: (OD_{570} of the treated sample/OD_{570} of the untreated sample) $\times$ 100. All test samples mentioned above were dissolved in DMSO. The final concentration of DMSO was $< 0.1\%$ and 0.1% DMSO was used as control.

2.9. Data analysis

All the experiments were conducted in triplicates. The results were depicted as mean $\pm$ SD (standard deviation). One way ANOVA was done by using SPSS version 16 and graph pad prism software through Duncan's multiple range tests for comparing means with significance levels of $p < 0.05$ and $p < 0.01$.

3. Results and discussion

3.1. NBE inhibiting property on ACF formation in AOM induced animal model

To explicate the overall performance of isocoreopsin, butrin and isobutrin, a well-established short- and long-term protocol was used to determine NBE efficacy on inhibiting ACF formation in AOM induced rats. In terms of body weight, NBE did not show any considerable changes among the various groups. During necropsy, no pathologic alterations were found in any organs including the liver, lungs, and kidneys. Moreover, the colonic ACF formation due to AOM treatment was confirmed under the observation of light microscope after HE staining (Fig. 1). The inhibitory effect of different doses of NBE on AOM-induced colonic ACF formation is shown in Fig. 2. Male Wistar albino rats injected with 50, 100 and 150 mg/kg NBE showed significant decrease in the number of total mean ACF/colon (70%–80%; $p < 0.01$) compared with the rats injected with AOM alone (Fig. 2). Whereas AOM induced rats contained three or more aberrant crypts, which were reduced significantly after the treatment with NBE at 150 mg/kg when compared to silibinin (positive control) treated group. These studies implied that NBE exhibited a protective effect on AOM induced CRC.

3.2. Identification of isocoreopsin, butrin and isobutrin from NBE

The NBE was subjected to 60–120 mesh size silica gel column chromatogram to furnish two fractions and their purity was confirmed by HPTLC. The fraction I obtained from column chromatography contained only one compound (Compound 1). The HPTLC results clearly indicated that the fraction II was confirmed to be isomerized compounds, butrin and isobutrin, with the Rf values of 0.68 and 0.65 min, respectively, when developed with methanol:

Table 2.
Grouping of animals and dose of test agents against AOM induced colon cancer animals.

Groups	Test agent	Dose (mg/kg)
Group 1	Saline only	0
Group 2	AOM only	15
Group 3	AOM+Silibinin	100
Group 4	AOM+(n-butanol) extracts	50
Group 5	AOM+(n-butanol) extracts	100
Group 6	AOM+(n-butanol) extracts	150

EtoAC (30:70, v/v). Further, the fraction II was subjected to reverse-phase HPLC C_{18} column with acetonitrile as mobile phase. Initially, it was shown that two inseparable peaks were very close to each other at a retention time of 16 min, and then were separated by using 0.1% phosphoric acid in mobile phase solution with the retention time of 19.9 and 26.2 min. Surprisingly, these two purified compounds were later found to be isomers (Compound 2 and 3) (Fig. 3).

Compound 1 (Fig. 4) was obtained as a pale yellow amorphous solid and had a molecular formula of $C_{21}H_{22}O_{10}$ based on ESI–MS. The IR spectrum of compound 1 indicated the presence of hydroxyl (3400 cm^{-1}) and carbonyl (1645 cm^{-1}) groups. The ^{1}H NMR spectrum evidenced signals assignable to seven multiple protons at ä 0.70 and 1.05, a methylene group adjacent to ketone at ä 2.05 and 2.25, all six D2O-exchangeable phenolic protons at ä 4.76 and 5.53, five aromatic protons appeared as multiplets at ä 6.34 and 7.46 and another aromatic proton adjacent to ketone appeared as multiplet at ä 7.69 and 7.77. Similarly, the ^{13}C NMR spectrum of compound 1 contained 21 signals indicating one methylene (ä 28.9), one acyclic oxymethine (ä 60.7), four cyclic oxymethines (ä 73.0 and 77.1), one oxygen-attached tertiary carbon and two aromatic olefinic methines (ä 102.5), six aromatic methines (ä 113.4, 115.3, 128.3 and 145.1), two aromatic tertiary carbons (ä 163.0 and 164.6) and a ketone carbonyl carbon (ä 190.0). In addition, the ESI–MS fragmentation of compound 1 also yielded the structural information. A fragment ion at m/z 273.07 (M – 162.05) occurred from loss of 2-(hyroxymethyl) tetrahydro-2 h-pyran-3, 4, 5-triol group in compound 1. Our results indicated that ^{1}H and ^{13}C NMR data of compound 1 significantly resembled those in Table 1. Thus, the structure of compound 1 was identified as isocoreopsin (2-(3, 4-Dihydroxy-phenyl)-7-(3, 4, 5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-chroman-4-one (1)).

Compounds 2 and 3 were obtained as pale yellow thin film and the molecular formula were assigned to be $C_{27}H_{32}O_{15}$ based on the ion peak at 597 (M+Na)$^+$ in the ESI–MS spectrum. The ESI–MS spectrum of compound 2 gave two fragment ions as those of compounds 1 and 3. The ^{1}H and ^{13}C NMR data of compound 2 significantly resembled those in Table 1. Moreover, these results coincided with the early report by Gupta et al. [25]. Thus, the structure of compound 2 was identified as butrin (2-[4-Hydroxy-3-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-phenyl]-7-(3, 4, 5-trihydroxy-6 hydroxymethyl-tetrahydro-pyran-2-yloxy)-chroman-4-one (1)). Further, the yellow amorphous solid of compound 3 was obtained and the ion peak at 597 (M+H)$^+$ in the ESI–MS spectrum indicated that this molecular formula could be $C_{27}H_{32}O_{15}$. Also, the ESI–MS spectrum of compound 3 imparted two fragment ions. Interestingly, the ^{1}H and ^{13}C NMR data of compound 3 considerably resembled those of the compounds in Table 1. On the basis of the above results, the structure of compound 3 was identified as isobutrin (3-[4-Hydroxy-3-(3, 4, 5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-phenyl]-1-[2-hydroxy-4-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-phenyl]-propenone).

Our results demonstrated that three compounds in NBE were identified and the most prominent among them was compound 1. It would be interesting to know that the greater yield of compound 1 from NBE may be a better source than the earlier report of EtoAC extract. However, significant amounts of compounds 2 and 3 were isolated as mentioned previously by Gupta et al. [25]. Surprisingly, isocoreopsin closely resembles silybin, silymarin and quercetin, which show promising effects in treatment of various diseases. This indicates that the full elucidation of extracted compounds of B. monosperma flowers focuses better insight into its nutritional and medicinal properties.
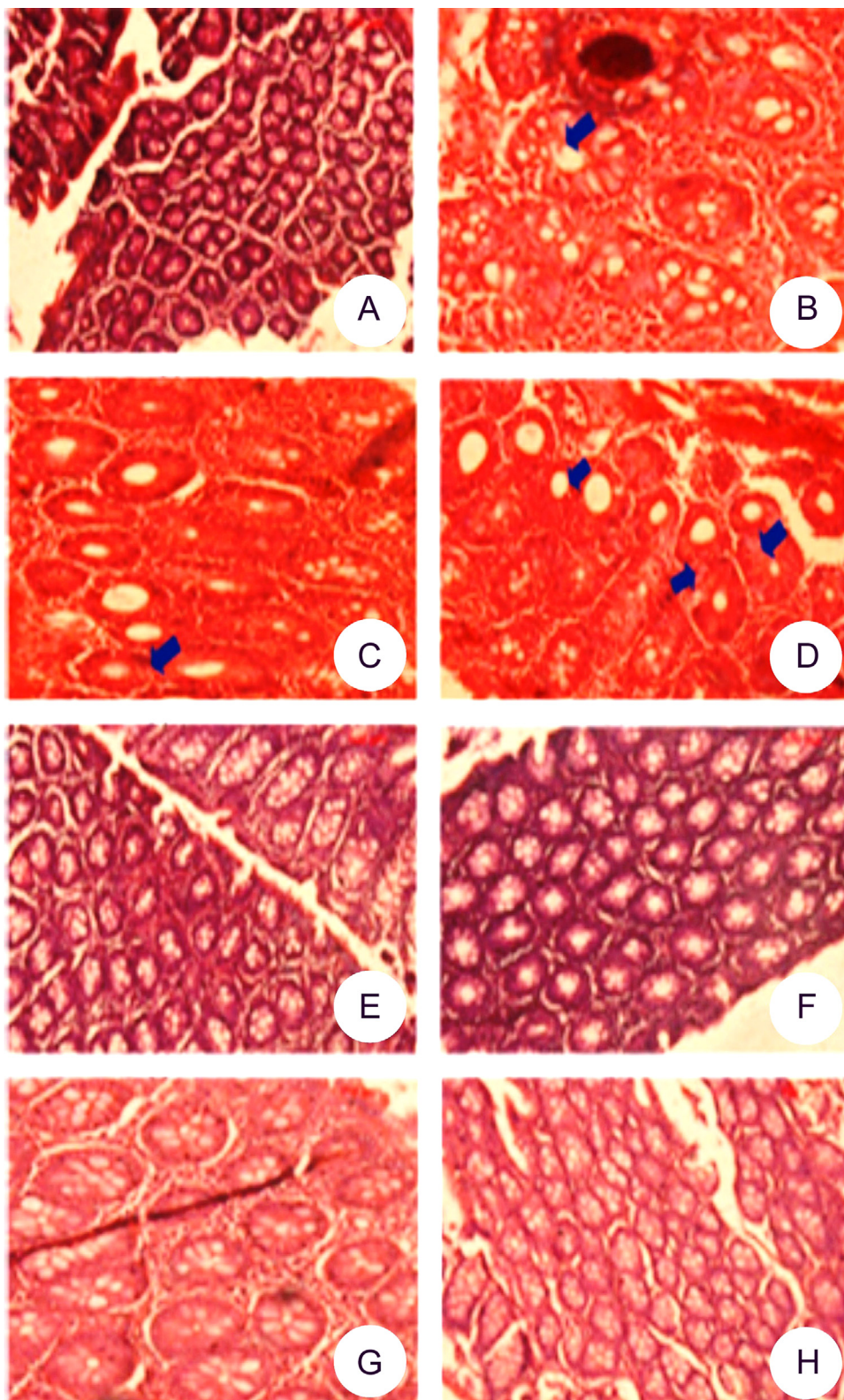
Fig. 1. Histopathological examination of ACF formation in experimental groups (hematoxylin-eosin staining). (A) Normal colon, (B) ACF with single crypt, (C) ACF with dual crypts, (D) ACF with three crypts, (E) Silibinin treated group (short term), (F) Silibinin treated group (long term), (G) NBE treated group (short term) and (H) NBE treated group (long term).

3.3. Characterization of bioactive contents from NBE

Previous studies have reported that the maximum concentration of flavonoid (25.29%) is present in NBE rather than in the absolute ethanol, methanol and other aqueous extracts [26,27]. In this study, for the first time, we have shown the flavonoid contents of all three compounds from NBE to access their bioactivity of each individual. The flavonoid contents of three different compounds of
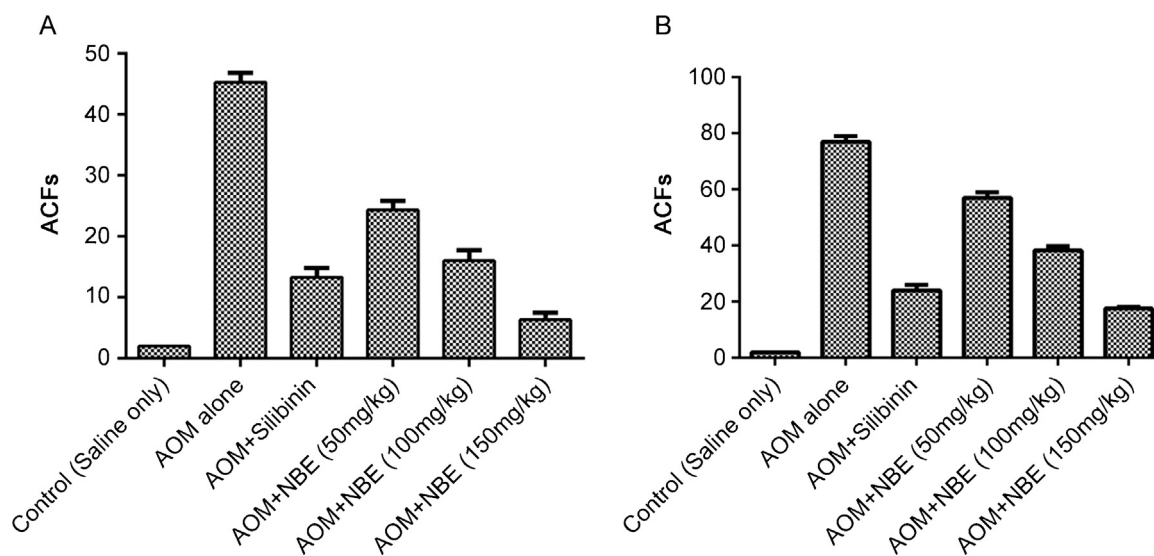
Fig. 2. Effects of n-butanol extract (NBE) on aberrant crypt formation: (A) NBE effects on AOM induced short-term and (B) NBE effects on AOM induced long-term experiment.
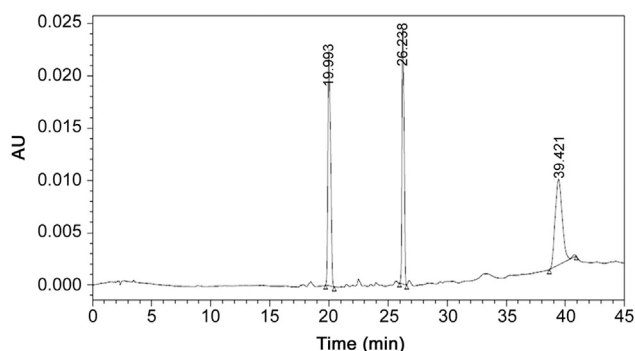

Fig. 3. HPLC results of butrin and isobutrin with retention time of 19.9 and 26.2 min, respectively.
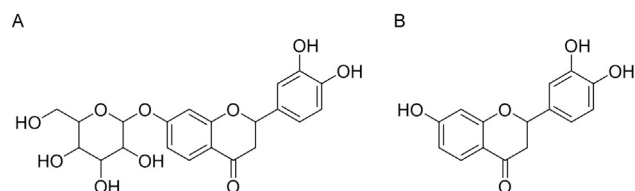

Fig. 4. (A) Structure of isocoreopsin and (B) its fragmentation ion at m/z 272.

NBE (isobutrin > isocoreopsin > butrin) are shown in Table 3. The HPLC fraction of isobutrin showed higher flavonoid content (96.00 ± 0.130) than those of other two compounds in NBE.

3.4. Free radical scavenging activity of NBE

Fig. 5 depicts the dose-dependent DPPH radical scavenging activity of isolated compounds from NBE. Antioxidant substance inhibited the spread of free radicals in biological systems by undergoing a redox reaction with the phenolic compounds, which might restrain the polymerization of free radicals and other oxidizing reactions [28]. Based on the concentration, the phenolic compounds established a dual mode of mechanism in antioxidant (low concentration) and pro-oxidant (high concentration) effects [29]. The EC50 values of extracts ranged from 10 to 50 µg/mL and isocoreopsin of flower extracts furnished the highest DPPH radical

scavenging activity with EC50 values of 10 µg/mL. The earlier results interpreted that butein had a strong antioxidant activity (87% at 1 mg/mL) with EC50 value of 0.36 mg/mL when compared to butrin (22%) [30]. These findings clearly indicate that the isolated compounds from n-butanol have significant activity at the minimum dose. Since it has been suggested that flavonoids are potential antimutagenic, antiallergenic, antiviral, anti-inflammatory, antioxidant, anticarcinogenic agents and are reported to induce apoptosis, inhibit cell proliferation and angiogenesis [31]. From these studies, it implies that these three active compounds may have broader medical values, especially chemopreventive efficacy against human cancer.

Table 3.
Flavonoid contents of *B. monosperma* flower extract compounds.

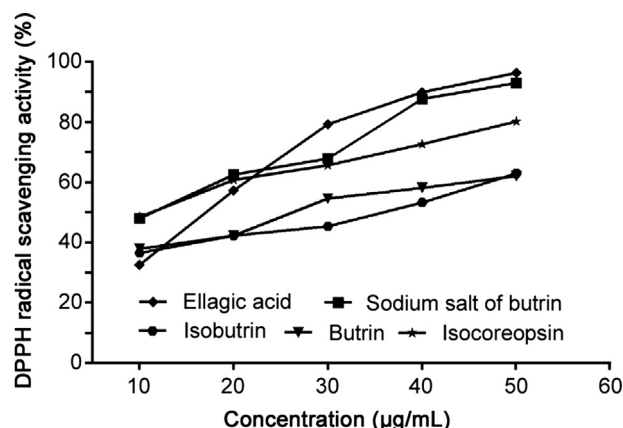| Extract of *B. monosperma* flowers	Total flavonoid (µg naringenin equiv/mg extracts)
Isocoreopsin	79.82 ± 0.295[a]
Butrin	5.68 ± 0.286[b]
Isobutrin	96 ± 0.130[c]

[a,b,c]$p < 0.05$ between the values.

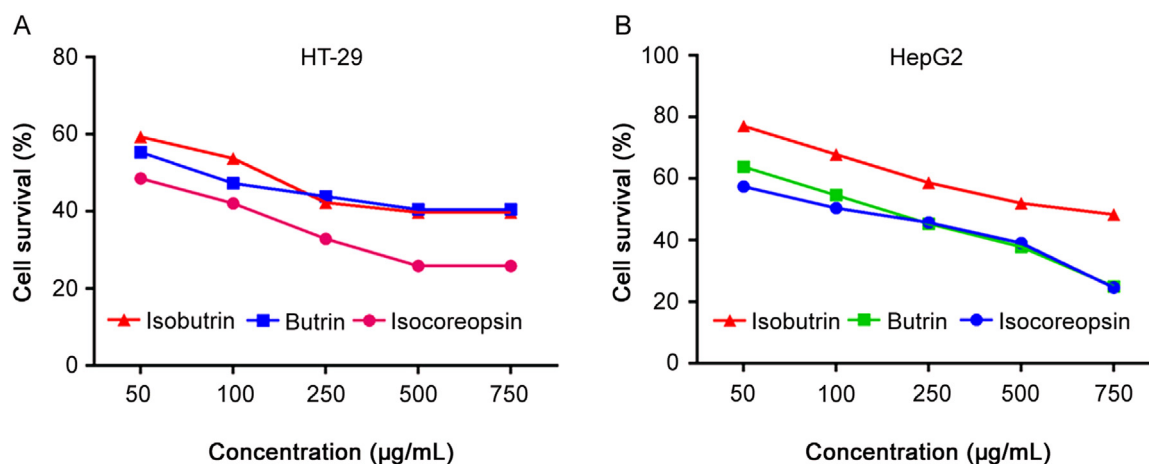**Fig. 5.** Dose-dependent DPPH free radical scavenging activity of compounds in NBE.

Fig. 6. Anticancer activity of three compounds in NBE on (A) human colon cancer and (B) liver cancer cell lines at 48 h.

3.5. Anticancer activity

All three compounds isolated in this study showed antiproliferative activity against HT-29 and HepG2 cell lines (Fig. 6). However, significant response was achieved for isocoreopsin with the concentration of 50 μg/mL, followed by butrin and isobutrin (100 μg/mL and 500 μg/mL) at 48 h. Our results also indicated that isocoreopsin against HT-29 cell line revealed interesting differential antiproliferative activities with more than two fold effects when compared to that for HepG2 cell line. Whereas, the butrin (150 mg/mL) produced approximately similar results in both cell lines and isobutrin (500 mg/mL) was significantly less effective in HepG2 cell line. Nevertheless, these results revealed that the compound isocoreopsin has potential cytotoxicity in suppressing colon and hepatocarcinoma cancer cell lines.

Wnt signaling gene is responsible for malignancies in colon, rectum, skin, liver, brain and prostate cells, due to the activation of β-Catenin [32]. Moreover, abnormal expression of GSK-3β (Glycogen synthase kinase-3β), APC (Adenomatous polyposis coli) and β-Catenin genes leads to down regulate the downstream targeted genes of tumor suppressor genes and it has been observed in >90% of colorectal cancer development. This implies that it has a possibility to inhibit Wnt/β-Catenin (HT-29) and NF-kB (HepG2) signaling pathways. Interestingly, our results clearly indicated that the individual compound exhibited cell-growth inhibition, when compared to the earlier anticancer property of butein, butrin and aqueous extracts of *B. monosperma* with different cancer cell lines [33]. Our previous studies on silico docking demonstrated that the active compounds, isobutrin, butrin and isocoreopsin, isolated from *B. monosperma* flowers have strong binding interaction with Wnt β-catenin protein which is associated with CRC. Moreover, the isocoreopsin obeyed the Lipinski rule of five parameters and fulfilled the ADMET predictions. This proposes that it may have potential anticancer activity against CRC [34,35].

4. Conclusion

In the present study, the 150 mg/kg of NBE treated group showed the strongest inhibitory effect and decreased the total number of ACF formation (per rat) by 92% (short-term) and 78% (long-term), respectively. These results suggest that the n-butanol extracts may have potential chemopreventive efficacy by inhibiting ACF formation in colon and mainly contain three bioactive compounds, namely isocoreopsin, butrin and isobutrin. The novel glucoside isocoreopsin of NBE showed a better free radical scavenging and anticancer activity. However, the flavonoid contents for these three different compounds differed significantly. Our results clearly illustrate the significant antiproliferative effect of isocoreopsin on colorectal cancer cell line and that it may be facilitated to target the subtype specific cancer treatments in future. Further investigation on molecular pathways of cancer targeted genes will be a worthy endeavor for better understanding of the bioactive compounds from *B. monosperma*.

References

[1] Y. Jun, Z. Wen, M. Paul, et al., Adenomatous polyposis coli (APC) differentially regulates β-Catenin phosphorylation and ubiquitination in colon cancer cells, J. Biol. Chem. 28 (2006) 17751–17757.
[2] C.S. Hung, H.H. Liu, M.T. Huang, et al., Knockdown survivin expression reduces the efficacy of curcumin treatment in hepatocellular carcinoma cells, Ann. Surg. Oncol. 19 (2012) 3547–3555.
[3] R. Cabrera, D.R. Nelson, Review article: the management of hepatocellular carcinoma, Aliment. Pharmacol. Ther. 31 (2010) 431–476.
[4] Y.J. Chang, C.J. Tai, L.J. Kuo, et al., Glucose-regulated protein 78 (GRP78) mediated the efficacy to curcumin treatment on hepatocellular carcinoma, Ann. Surg. Oncol. 18 (2011) 2395–2403.
[5] R.P. Bird, Role of aberrant crypt foci in understanding the pathogenesis of colon cancer, Cancer Lett. 93 (1995) 55–71.
[6] A.P. Fields, S.R. Calcagno, M. Krishna, et al., Protein kinase C is an effective target for chemoprevention of colon cancer, Cancer Res. 69 (2009) 1643–1650.
[7] T. Mainardi, S. Kapoor, L. Bielory, Complementary and alternative medicine: herbs, phytochemicals and vitamins and their immunologic effects, J. Allergy Clin. Immunol. 123 (2009) 283–294.
[8] A. Ghasemzadeh, N. Ghasemzadeh, Flavonoids and phenolic acids: role and biochemical activity in plants and human, J. Med. Plants Res. 5 (2011) 6697–6703.
[9] K. Avula Muralidhar, K. Sudhakar Babu, T. Ravi sankar, et al., Evaluations of wound healing properties of bioactive fractions from the extract of *Butea monosperma* (lam) stem bark, Int. J. Phytomed. 3 (2011) 41–49.
[10] K. Sharma Ashish, M. Chaudhary, R. Kumar, et al., Evaluation of antiinflammatory and analgesic activity of *Butea monosperma* gum, Nov. Sci. Int. J. Pharm. Sci. 1 (2012) 60–64.
[11] B.P. Singh, S. Swati, Antimicrobial activity and spectral characterization of flower of *Butea monosperma*, Res. J. Pharm. Bio. Chemi. Sci. 3 (2012) 598–606.
[12] B.T. Divya, S. Mini, *In vitro* radical scavenging activity of different extracts of *Butea monosperma* bark, Int. J. Curr. Pharma. Res. 3 (2011) 3.
[13] Y.L. Ramachandra, C. Ashajyothi, S. Padmalatha, Evaluation of antidiabetic and

antipyretic activity of *Butea monosperma* (lam) extracts on rats, RaiInt. J. Pre-clin. Pharm. Res. 3 (2012) 73.

[14] J. Banu Rekha, B. Jayakar, Anti cancer activity of ethanolic extract of Leaves of *Butea monosperma* (Lam) Taub, Curr. Pharm. Res. 1 (2011) 106–110.

[15] G. Mathan, G. Fatima, K.A. Saxena, Gupta, et al., Chemoprevention with Aqu-eous extract of *Butea monosperma* flower results in normalization of nucleas morphometry and inhibition of a proliferation marker in liver tumors, Phyt-other. Res. 25 (2011) 324–328.

[16] C. Tenzin, S. Surendra Kumar, K. Vijay, Chemopreventive and anti-cancer properties of the aqueous extract of flowers of *Butea monosperma*, J. Ethno-pharmacol. 129 (2010) 208–213.

[17] A. Sehrawat, V. Kumar, Butein imparts free radical scavenging, anti-oxidative and proapoptotic properties in the flower extracts of *Butea monosperma*, Biocell 36 (2012) 63–71.

[18] J.M. Wong, K. Mafune, H. Yow, et al., Ubiquitin-ribosomal protein S27a gene overexpressed in human colorectal carcinoma is an early growth response gene, Cancer Res. 53 (1993) 1916–1920.

[19] M. Bienz, H. Clevers, Linking colorectal cancer to Wnt signaling, Cell 103 (2000) 311–320.

[20] A. Hata, R.S. Lo, D. Wotton, et al., Mutations increasing auto inhibition in-activate tumour suppressors Smad2 and Smad4, Nature 388 (1997) 82–87.

[21] H. Wagner, B. Geyer, M. Fiebig, et al., Isobutrin and butrin, the antihepatotoxic principles of *Butea monosperma* flowers, Planta Med. 52 (1986) 77–79.

[22] M. Mack, R. Ashwell, Ndhlala, F. Jeffrey, et al., Phenolic composition, anti-oxidant & acetylcholinesterase inhibitory activities of *Sclerocarya birrea* and *Harpephyllum caffrum* (Anacardiaceae) extracts, Food Chem. 123 (2010) 67–76.

[23] A. Hegedus, R. Engel, L. Abranko, et al., Antioxidant and antiradical capacities in apricot (*Prunus armeniaca L.*) fruits: variations from genotypes, years, and analytical methods, J. Food Sci. 75 (2010) C722–C730.

[24] M.J. Michal, Colon cancer chemoprevention with ginseng and other botanicals, J. Korean Med. Sci. 16 (2001) 81–86.

[25] S.R. Gupta, B. Ravindranath, T.R. Seshadri, The glucosides of *Butea mono-sperma*, J. Phytochem. 9 (1970) 2231–2235.

[26] S. Manish, Lavhale, S.H. Mishra, Evaluation of free radical scavenging activity of *Butea monosperma* Lam, Ind. J. Exp. Bio. 45 (2007) 376–384.

[27] G. Piyush, B. Bibhilesh, S. Mendhe, et al., Antioxidant activity of flavonoidal content of *Butea monosperma*, Int. J. Pharm. Sci. Res. 1 (2010) 4.

[28] A. Lilia, Conde-Hernandez, A. Jose, et al., Total phenolic and antioxidant ac-tivity of *Piper auritum* and *Porophyllum ruderale*, Food Chem. 142 (2014) 455–460.

[29] S. Chung, M. Janelle, T. Mou, et al., Inhibition of carcinogenesis by dietary polyphenolic compounds, Annu. Rev. Nutr. 21 (2001) 381–406.

[30] R. Zafar, A. Nahid, K. Abubakar, et al., Butrin, isobutrin, and butein from medicinal plant *Butea monosperma* selectively inhibit nuclear factor-kb in activated human mast cells: suppression of tumor necrosis factor-α, inter-leukin (IL)-6 and IL, J. Pharm. Exp. Ther. 333 (2010) 354–363.

[31] E.C. Maria, F. Marta, S. Pilar, et al., Phenolic compounds in *Brassica* vegetable, Mol 16 (2011) 251–280.

[32] Y. Takigawa, A.M. Brown, Wnt signaling in liver cancer, Curr. Drug. Targets 9 (2008) 1013–1024.

[33] P.V. Prasad, P.K. Subhaktha, A. Narayana, et al., Palasa (*Butea monosperma* (Lamk.) Taub.) and its medico-historical study, Bull. Ind. Inst. Hist. Med. 36 (2006) 117–128.

[34] P. Navaneethakrishnan, C.N. Prashantha, S. Boopathi, et al., In silico design of *Butea monosperma* floral derived compounds and its inhibitory effect on β-Catenin, GSK-3β and APC complex proteins in colorectal cancer, Int. J. Drug. Dis. 5 (2013) 191–197.

[35] P. Navaneethakrishnan, C.N. Prashantha, S. Boopathi, et al., Antiproliferative activity of N-butanol floral extract from Butea monosperma against hct 116 colon cancer cells; drug likeness properties and in silico evaluation of their active compounds toward glycogen synthase kinase-3β/axin and β-catenin/T-cell factor-4 protein complex, Asian J. Pharm. Clin. Res. 8 (2015) 134–141.