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RESEARCH ARTICLE

Biological effects of *Byrsocarpus coccineus in vitro*

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Abstract

Context: *Byrsocarpus coccineus* Schum. and Thonn. (Connaraceae) is a scandent shrub widely employed as a medicinal remedy for various disease conditions in West Africa.

Objective: This study evaluated fractions of *B. coccineus* for modulation of cytochrome P450 (CYP) enzyme activity, cytokine production, and proliferation.

Materials and methods: The BROD (benzyloxyresorufin *O*-debenzylase) and BFCOD (benzyloxy-4-[trifluoromethyl]-coumarin *O*-debenzyloxylase) assays were used to evaluate effect on CYP2B1/2 and CYP3A4 enzyme activity. Effects on cytokine production and proliferation of HT29 cells were investigated using interferon expression assay and MTT (3-3[4,5-dimethyl-2-thiazolyl]-2-5-diphenyl-2H-tetrazolium bromide) assay, respectively.

Results: Fractions derived from the organic solvent extraction of *B. coccineus* produced significant ($p < 0.05$) stimulation of human hepatic CYP2B1/2 activity in the BROD assay. The greatest effects were elicited at 1 ng/mL corresponding to ~3-fold stimulation of enzyme activity. Enhancement of CYP3A4 enzyme activity was also observed in the BFCOD assay. Other fractions from the organic extract showed significant antiproliferative effects on HT29 cells at 100 µg/mL. Fractions obtained from the aqueous extract of *B. coccineus* (1 µg/µL) significantly stimulated the expression of IFNα2a and IFNβ in peripheral blood mononuclear cells (PBMC), causing a maximum 26-fold increase of IFNα2a-transcript.

Discussion and conclusion: The effect on CYP suggests that *B. coccineus* may reduce the therapeutic efficacy of co-administered drugs. This justifies the need for proper education of patients by healthcare practitioners on the outcomes of drug–herb interactions. This study identifies several *in vitro* activities that could underlie the attributed uses of this plant in traditional African medicine (TAM).

Keywords: *Byrsocarpus coccineus*, cytochrome P450 stimulation, interferon α2a, interferon β, antiproliferative effect, traditional African medicine

Introduction

Byrsocarpus coccineus Schum. and Thonn. (Connaraceae) is a plant commonly found across west and tropical Africa. It is a scandent shrub of savanna thickets and secondary jungle with delicate pink-tinged foliage and sweet-scented flowers (Burkill, 1985). Local names in Nigeria include "tsaamiyar-kasa" (Hausa, north), "oke abolo" (Igbo, East), and "orikoteni" (Yoruba, southwest) (Burkill, 1985). In addition to its popular use as an ornamental plant, various preparations of the plant using leaves, roots (scraped bark and sap) and whole plant have been used to treat diverse ailments. The decoction or infusion of the leaf of the plant has been used for skin and mouth disorders, German measles, jaundice, gonorrhea, urinary problems, impotence, anemia, primary and secondary sterility, blennorrhagia, tachycardia, and as an abortifacient (Neuwinger, 1996). The plant has also been used for swellings and tumors, hemorrhage and as an emetic (Burkill, 1985; Adjanohoun et al., 1986; Neuwinger, 1996). In previous studies, the *in vivo* analgesic (Akindele

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& Adeyemi, 2006a), antidiarrhea (Akindele & Adeyemi, 2006b), antipyretic (Akindele & Adeyemi, 2007) and anxiolytic/sedative (Akindele & Adeyemi, 2010) activities of the leaf aqueous extract of the plant have been investigated and reported.

In the present study, we investigate the effects of *B. coccineus* on human immune cells and a human cancer cell line in order to rationalize the reported use of this plant for tumors. Interferons are cytokines produced by some immune system cells. They possess antiviral, antiseptic, antiproliferative and immunostimulatory activities (Khan et al., 1998; Uematsu & Akira, 2007). Both type I interferons (IFNα and β) and interferon γ have been shown to play a role in the host immune response to cancer (Dunn et al., 2006). Interferon therapy is used in conjunction with chemotherapy and radiation in the treatment of many cancers (Gilewski & Golomb, 1990; Clark & Weiner, 1995; Uematsu & Akira, 2007).

In African countries and developing countries of other continents of the world, self-medication and combination therapy using western medicines and traditional herbal medicines are frequent (Agbonon et al., 2010), based on personal conviction of synergistic or additive pharmacological effect (Pekthong et al., 2008). Patients often do not report herbal and alternative medicine use to physicians (Shakeel et al., 2010), but there are a number of examples where interactions between herbal medicines and drugs can be clinically significant. Many case reports exist on irregular bleeding and unwanted pregnancies when St. John's wort (*Hypericum perforatum* L. (Clusiaceae); an antidepressant herb) is taken concurrently with ethynylestradiol (Schwarz et al., 2003). Clinically significant interactions have also been reported from the use of grapefruit juices with calcium antagonists, antihistamine and benzodiazepine treatment (Ameer & Weintraub, 1997; Bailey et al., 1998; Kupferschmidt et al., 1998). Based on its potential for use along with conventional drugs and in view of the many examples of herbal remedies altering expression or activity of hepatic CYP450 enzymes, thus affecting beneficially or adversely the impact of co-administered drugs, an aim of this study was to investigate the effects of extracts and derived fractions of *B. coccineus* on cytochrome P450 enzyme activity. The hepatic microsomal cytochrome P450 group of enzymes is responsible for the metabolism of many xenobiotics and its modulation is the most common cause of drug–drug and food–drug interactions (Girennavar et al., 2007).

Materials and methods

Plant material

Fresh *B. coccineus* plant was collected from Iju-Ogundimu a town in Ifako-Ijaiye local government area of Lagos State, Nigeria, in the month of June 2007. Botanical identification and authentication was carried out by J.D. Olowokudejo Professor of the Department of Botany, Faculty of Science, University of Lagos, and T.K. Odewo, Senior Superintendent of the Forestry Research Institute of Nigeria (FRIN), Ibadan. The voucher specimen (FHI 106623) was deposited in the herbarium of the Forestry Research Institute.

Extraction and fractionation

Fresh leaves of *B. coccineus* were air-dried until a constant weight was obtained. The dried material (200 g) was macerated with EtOAc–MeOH (1:1) for 48 h with mechanical stirring. The filtered supernatant was dried down to produce extract B1. The remaining solid residue was macerated with EtOH–H_2O (1:1), also for 48 h with mechanical stirring. The filtered supernatant was dried down to produce extract B2 (see Figure 1). Another portion of the dried material (200 g) was macerated with H_2O for 48 h with mechanical stirring (extract BW). Extract B1 was partitioned between hexane (fraction BX) and MeOH–H_2O (80:20). The 80% MeOH fraction was then partitioned between BuOH (fraction B1Y) and H_2O (fraction B1Z). Extract B2 was partitioned directly between BuOH (fraction B2Y) and H_2O (fraction B2Z). B1Y and B2Y were then combined (8.22 g) and subjected to normal phase silica gel chromatography using a gradient system of increasing iPrOH in CH_2Cl_2 (0, 2, 4, 6, 8, 10, 20, 50, and 100% iPrOH) to obtain fractions F1–F9. The column was washed with MeOH to obtain fraction F10. Based on bioactivity obtained, fraction F6 (0.14 g) was separated to give six subfractions (F6(1)–F6(6)) through reversed phase C-18 column chromatography using a gradient system of increasing MeOH in H_2O (20, 40, 60, 80, and 100% MeOH). The extracts and fractions were used in the cytochrome P450 activity study (BFCOD and BROD assays) and the MTT cell viability assay.

BW (0.66 g) was subjected to HP-20 column chromatography with H_2O, and 20, 40, 60, 80, and 100% MeOH to obtain six fractions (BW1-BW6, see Figure 2). Fractions BW7 and BW8 were obtained with acetonitrile (ACN) and H_2O–MeOH backward wash, respectively. Fraction BW5 was further fractionated by high performance liquid chromatography (HPLC) (Discovery C18 column, 5 μm particle size, 250 × 4.6 mm, Supelco; flow rate, 1.0 mL/min; detection by UV at 300 nm) using a linear MeOH–H_2O gradient (20–100% MeOH over 60 min) to obtain 19 fractions (BW5(1)–BW5(19)) (see Figure 2). The extracts and fractions were used in the interferon expression study.

The choice of assays carried out on fractions was based on the bioactivity of the crude extracts.

Cytochrome P450 enzyme activity assay

The fluorogenic substrates benzyloxyresorufin (BR) and 7-benzyloxy-4-trifluoromethylcoumarin (BFC), selective for CYP2B1/2 and CYP3A4, respectively (Pekthong et al., 2008; Huber et al., 2008; Stresser et al., 2000), were synthesized from the reactions of resorufin or trifluoromethylcoumarin with benzyl iodide (Prough et al., 1978) and their identities were verified by ^{1}H NMR. BR

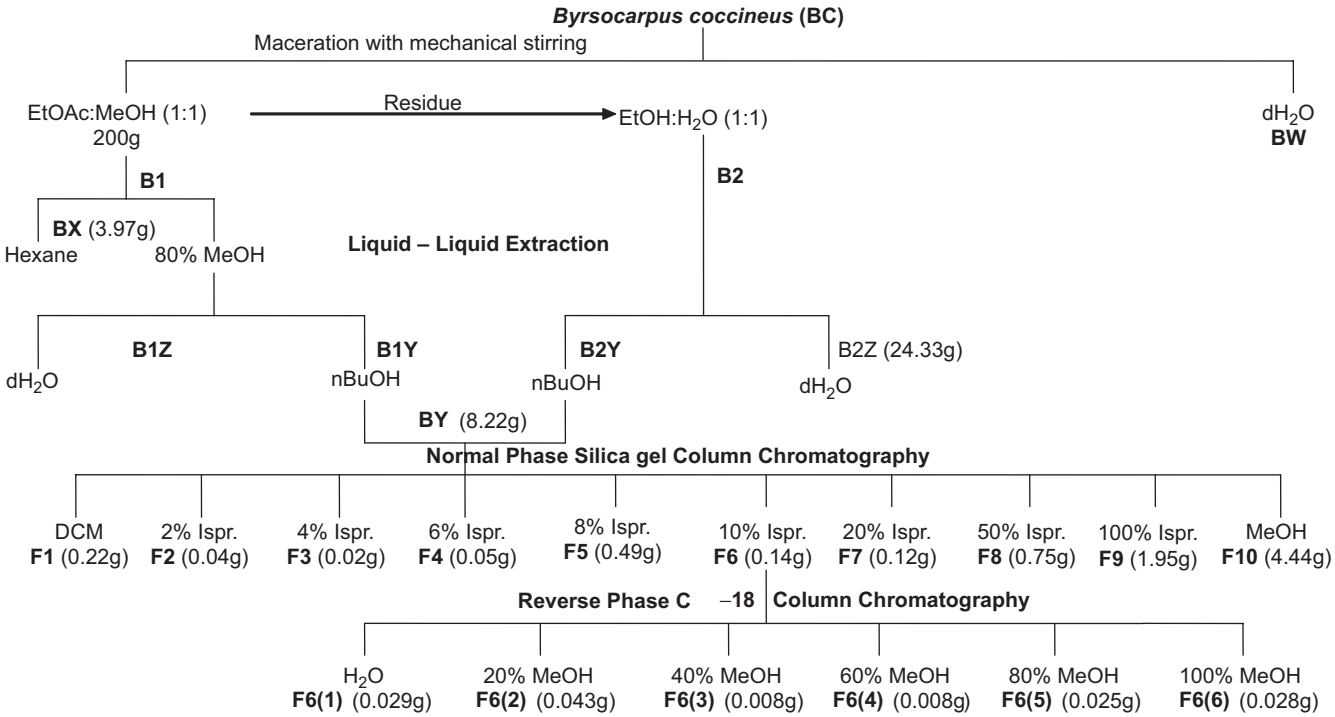

Figure 1

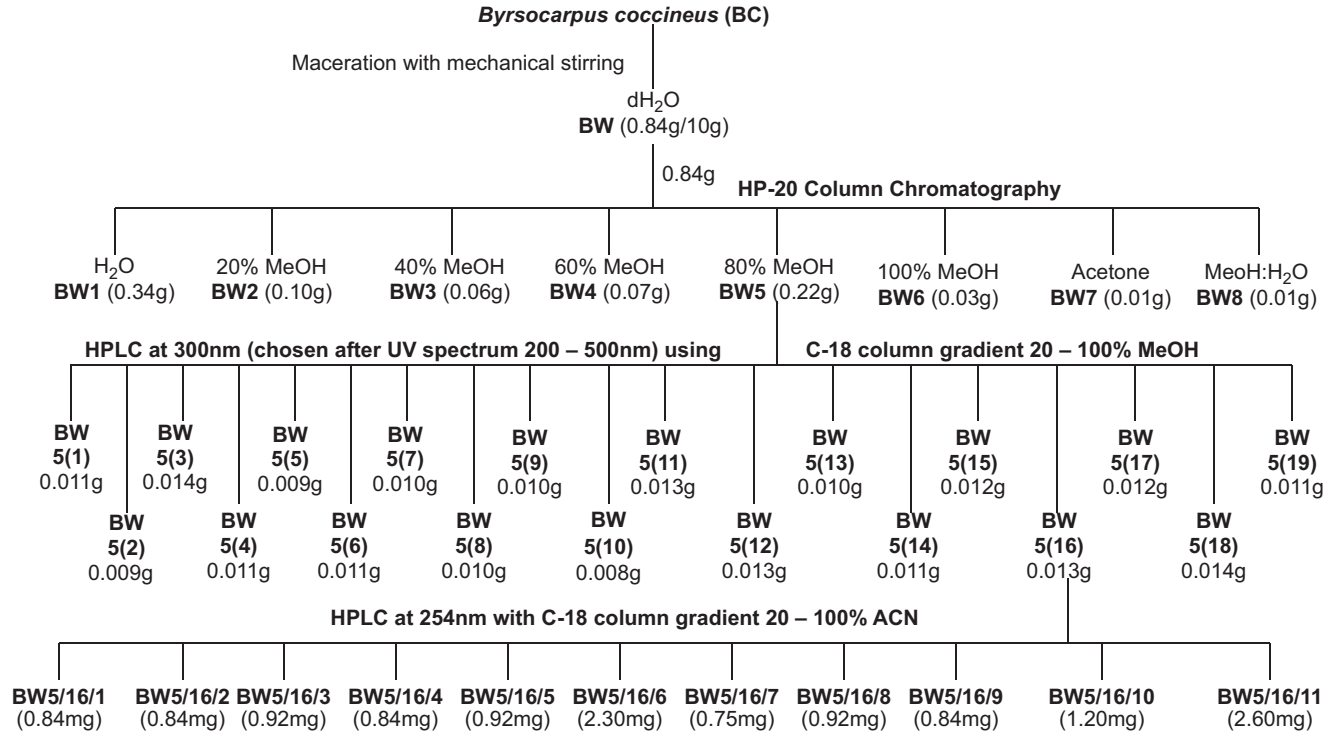

Figure 2

and BFC were purified to >99% pure as evaluated by HPLC. NADPH and other reagents were purchased from Sigma-Aldrich, St. Louis, MO. Human liver microsomes were obtained from de-identified transplant-quality liver obtained from the Department of Surgery, University of Florida, under a protocol approved by the Institutional Review Board.

The BROD assay measured the conversion of BR to resorufin (Prough et al., 1978; Pohl & Fouts, 1980), while the BFCOD assay measured the conversion of BFC to 7-hydroxy-4-trifluoromethylcoumarin (HFC, Renwick et al., 2000; Stresser et al., 2000). In a volume of 1 mL, reactions contained 100 µL test sample dissolved in methanol (evaporated under N_2), 5 µM BR or 100 µM BFC, 0.1 M

HEPES, pH 7.6, 2% BSA, 2 mM NADPH, and 0.25 mg (BROD) or 2 mg (BFCOD) human liver microsomes. All components except for NADPH were equilibrated at 37°C for 1 min before NADPH addition. Reactions were incubated for 10 min at 37°C before they were stopped by addition of 2.5 mL (BROD) or 3 mL (BFCOD) ice cold MeOH. The mixtures were left to stand for 20 min while protein flocculation occurred, then centrifuged for 15 min at 600 *g*. Reaction products were measured by fluorescence ($\lambda_{ex}/\lambda_{em}$ 550/585 nm for resorufin in the case of the BROD assay; 410/530 nm for HFC in the case of the BFCOD assay). Standard curves of known concentrations of the products were used to convert fluorescence readings to product concentrations. Specific activities were calculated as shown below. EC_{50} values were calculated by non-linear curve-fitting using GraphPad Prism 4 (GraphPad Software, CA).

$$\text{Specific activity} = \frac{[\text{pmol} - \text{blank}]}{[\text{protein(mg)} \times \text{incubation time(min)}]}$$

Immunomodulation assay

Cell culture

Human peripheral blood mononuclear cells (PBMC) were isolated from the peripheral blood of healthy donors by Ficoll-Hypaque density gradient centrifugation (leukopack, peripheral blood leukocyte (PBL)) obtained from Lifesouth Community Blood Center (Gainesville, FL) using Lymphoprep (Axis-Shield, Oslo, Norway). Briefly, the contents of the leukopac were diluted three times its volume in sterile 1× phosphate buffered saline (PBS) pH 7.4 (Gibco, Carlsbad, CA). A portion of this (15 mL) was layered onto 10 mL of Lymphoprep®. The sample was then centrifuged for 25 min at 22°C and 1200 rpm. The PBMC were collected at the interface and washed twice with PBS and centrifuged each time for 10 min at 4°C and 1200 rpm. Cell viability was assessed before the last wash in a standard hematocytometer using Trypan blue exclusion. These cells were then cultured at a concentration of 1×10^6 cells/mL and maintained in DMEM medium (Sigma) supplemented with 2 mM L-glutamine (Life Technologies, Paisley, UK), 5000 U/mL penicillin (Sigma), 5000 U/mL streptomycin sulfate (Sigma), and 10% v/v fetal bovine serum (Gibco). The PBMC were cultured with nothing (control), 1 µL of 70% EtOH, or with different extracts of the plant at a final concentration of 1 µg/µL for 48 h. After culture, the cells were centrifuged, and lyzed with Trizol (Invitrogen, Carlsbad, CA) for RNA isolation.

Table 1. Primers used for quantitative PCR.

IFNβ	5′-CAGCCGATTCATCGAGCACTCGC[FAM]G-3′
	5′-TTCCAGGACTGTCTTCAGATGG-3′
IFNα2A	5′-GACTCCCTCACAGCCAGAAATGGAG[FAM]C-3′
	5′-ATGACCTGGAAGCCTGTGTGat-3′

Reverse transcription and real time polymerase chain reaction

Total cellular RNA was isolated from cells using Trizol. Final RNA concentration was measured at a 1:100 dilution in DEPC water in a Bioware DNA spectrophotometer (Biochrome, Cambridge, UK). Reverse transcription was performed using Superscript II first-strand synthesis for RT-PCR kit (Invitrogen) primed with oligo (dT) (Invitrogen), using 1 µg RNA per sample, according to the manufacturer's instructions. Subsequently,

Table 2. Effects of *B. coccineus* organic solvent extract fractions on cytochrome P450 activity in the BROD and BFCOD assays.

Treatment	BROD assay Concentration (ng/mL)	BROD assay Specific activity (pmol/min/mg)	BFCOD assay Concentration (ng/mL)	BFCOD assay Specific activity (pmol/min/mg)
Control	-	12.12 ± 0.51	-	160.4 ± 3.36
F6(2)	10^{-4}	12.64 ± 0.75	10^{-3}	257.3 ± 15.29[b]
	10^{-3}	16.51 ± 1.05	10^{-2}	269.6 ± 7.78[b]
	10^{-2}	23.6 ± 3.65[b]	10^{-1}	287.7 ± 5.62[b]
	10^{-1}	30.51 ± 5.06[b]	1	283.0 ± 6.7[b]
	1	31.84 ± 1.98[b]	10	299.2 ± 5.72[b]
F6(3)	10^{-4}	9.7 ± 0.9	10^{-3}	233.5 ± 2.4[b]
	10^{-3}	11.71 ± 0.86	10^{-2}	262.6 ± 5.14[b]
	10^{-2}	20.32 ± 3.87	10^{-1}	257.2 ± 5.37[b]
	10^{-1}	24.07 ± 2.17[b]	1	301.4 ± 3.96[b]
	1	26.41 ± 4.28[b]	10	303.2 ± 15.0[b]
F(6)4	10^{-4}	10.48 ± 1.22	10^{-3}	317.9 ± 18.89[b]
	10^{-3}	12.97 ± 1.32	10^{-2}	304.2 ± 3.45[b]
	10^{-2}	21.02 ± 1.66	10^{-1}	265.9 ± 3.34[b]
	10^{-1}	24.58 ± 1.6[b]	1	257.6 ± 5.48[b]
	1	26.91 ± 0.73[b]	10	249.3 ± 4.4[b]
Ketoconazole	(0.01 mM)	2.69 ± 0.31[a]	(0.01 mM)	148.5 ± 1.29
	(0.1 mM)	-	(0.1 mM)	61.14 ± 6.34[b]

Fractions F6(2), F6(3) and F6(4) were derived from organic solvent extraction of *B. coccineus* after the processes of maceration, liquid/ liquid extraction, silica gel and C-18 column chromatography as shown in Figure 1. Data are presented as mean ± SEM (n = 4). [a]p <0.05, [b]p <0.01 versus control (one way ANOVA followed by Dunnett's post hoc test).

quantitative analysis of IFNβ and IFNα2a was carried out by real-time PCR with fluorophore-labeled LUX primers (see Table 1) and their unlabeled counterparts (Invitrogen). Type I IFNs were selected for our studies because they are the first cytokines normally produced in response to immune stimuli and possess an immunoregulatory capacity for which they are also commonly used in the therapy of many diseases (Hilkens et al., 2003).

As previously published, the primers and PCR conditions were: 50°C, 2 min; 95°C, 2 min then (95°C, 15 s; 60°C, 30 s (IFNs) and 72°C, 1 min) for 45 cycles (Eksioglu et al., 2009). Reactions were conducted in a spectrofluorometric thermal cycler (MJ Research DNA Engine Opticon® 2 thermal cycler, BIORAD). Fluorescence was monitored during every PCR cycle at the annealing step. Results were analyzed with MJ Opticon Monitor 3.1 software (BIORAD). Results presented for all experiments represent triplicate determinations from separate healthy blood donors, represented as mean ± SEM.

Antiproliferative effect

HT29 cells (ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) containing 10% fetal bovine serum (Hyclone, Logan, UT), in a humidified atmosphere containing 5% CO_2 at 37°C. Cells were seeded into 96-well plates at a density of 10,000 cells/well (100 μL medium/well). After 24 h, fractions were added to wells at varying concentrations (as 1 μL stock solutions in EtOH–H_2O, 1:1). EtOH–H_2O was used as a negative control. After another 48 h, the plate was developed with MTT dye according to the manufacturer's protocol (G4000, Promega, Madison, WI). Data are expressed as percentage viability relative to negative controls, and an extract was deemed to have a significant effect on proliferation if this value was < 50%. Results obtained were expressed as mean ± SEM. The data were analyzed using one-way ANOVA followed by Dunnett's post hoc test. Results were considered significant when $p < 0.05$.

Table 3. Effects of *B. coccineu*s aqueous extract fractions on IFN expression.

PBMC	IFNβ	SEM	IFNα2a	SEM
control	1.0	0.2406	1.0	0.0787
BW 1	0.4961	0.0909	0.3807	0.0266
BW 2	1.3031	0.1923	0.2714	0.023
BW 3	0.4955	0.082	0.6425	0.0544
BW 4	0.6935	0.1396	0.6567	0.0593
BW 5	**6.8148**	**0.9612**	**4.1267**	**0.3731**
BW 6	4.5326	0.7374	1.6003	0.1515
BW 7	3.8798	0.4375	0.3639	0.0353
BW 8	4.8635	1.1508	0.2517	0.0194

Fractions BW1–BW8 were derived from the aqueous extract of *B. coccineus* after the processes of maceration and HP-20 column chromatography as shown in Figure 2. Data are presented as mean ± SEM. Bold type denotes fraction with the greatest effect selected for continued studies.

Results

Cytochrome P450 enzyme activity assay

BROD assay

Fractions F6(2), F6(3), and F6(4) (see Figure 1) produced significant ($p < 0.05$) concentration dependent stimulation of cytochrome P450 enzyme activity (see Table 2). At the highest concentration (1 ng/mL), F6(2), F6(3), and F6(4) elicited specific activity values of 32, 26, and 27 pmol/min/mg (compared to 12 pmol/min/mg for control) corresponding to approximately 3-, 2-, and 2-fold activity enhancements, respectively. Calculated EC_{50} values were 6.18×10^{-3}, 5.3×10^{-3}, and 4.83×10^{-3} ng/mL, respectively, for F6(2), F6(3), and F6(4). The order of potency is therefore F6(4) > F6(3) > F6(2). Ketoconazole (0.01 mM) was used as a positive control for inhibition.

BFCOD assay

Fractions F6(2), F6(3), and F6(4) (10^{-3} to 10 ng/mL) produced significant ($p < 0.05$) stimulation of cytochrome P450 enzyme activity (see Table 2). At the most efficacious concentrations [10 ng/mL for F6(2) and F6(3) and 10^{-3} ng/mL for F6(4)], F6(2), F6(3), and F6(4) elicited specific activity values of 299, 303, and 318 pmol/min/mg (compared to 160 pmol/min/mg for control), respectively, corresponding to approximately 2-fold activity enhancements. The functionality of the assay was confirmed with

Table 4. Effects of *B. coccineus* aqueous extract BW(5) fractions on IFNα expression.

PBMC	IFNα2a	SEM
control	1.0	0.0676
70% ETOH	0.3772	0.0404
BW CRUDE	2.8663	0.1572
BW(5)1	0.6225	0.0284
BW(5)2	4.9115	0.2681
BW(5)3	0.6206	0.0152
BW(5)4	**26.4311**	**5.194**
BW(5)5	2.1949	0.084
BW(5)6	1.2476	0.0862
BW(5)7	6.4382	0.4357
BW(5)8	0.8822	0.0508
BW(5)9	0.0715	0.0058
BW(5)10	0.0074	0.0013
BW(5)11	0.2907	0.0121
BW(5)12	0.2614	0.0113
BW(5)13	0.3507	0.0171
BW(5)14	0.4706	0.0229
BW(5)15	0.0201	0.0025
BW(5)16	3.6701	0.273
BW(5)17	0.0188	0.0025
BW(5)18	0.1127	0.0122
BW(5)19	0.0411	0.0072

Fractions BW(5)1-BW(5)19 were derived from the aqueous extract of *B. coccineus* after the processes of maceration, HP-20 column chromatography and HPLC at 300 nm using C-18 column gradient 20–100% MeOH as shown in Figure 2. Data are presented as mean ± SEM. Bold type denotes fraction with the greatest effect.

Table 5. Effects of *B. coccineus* extract fractions on HT29 cell viability.

Concentration (μg/mL)	% Cell viability											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F6(1)	F6(2)
1	112.2± 2.58	114.0± 1.0	113.3± 1.05	103.5± 3.13	102.4± 2.59	105.0± 4.09	106.8± 4.12	100.7± 4.59	95.82± 4.17	98.87± 1.28	107.5± 7.7	108.3± 6.04
10	67.38± 4.49	86.44± 3.05	70.36± 1.01	90.32± 5.97	93.39± 6.7	71.11± 12.38	85.55± 8.54	102.4± 2.41	102.4± 2.92	114.1± 14.98	101.5± 5.58	108.2± 7.76
100	34.7± 10.51	34.67± 5.53	36.69± 5.77	34.71± 14.03	20.56± 5.49	17.74± 5.27	22.24± 6.54	63.64± 8.63	109.5± 3.91	99.97± 6.52	103.6± 6.74	106.4± 4.7
Concentration (μg/mL)	% Cell viability											
	F6(3)	F6(4)	F6(5)	F6(6)	BW	BW1	BW2	BW3	BW4	BW5	BW6	BW7	BW8
1	105.0± 5.15	106.6± 6.84	102.7± 5.52	104.9± 3.38	108.9± 1.24	129.1± 1.11	136.7± 12.52	133.6± 2.06	131.8± 0.25	126.7± 0.55	110.6± 1.46	123.0± 5.92	120.1± 0.33
10	108.0± 5.09	104.3± 6.45	113.2± 5.46	102.7± 5.16	108.4± 2.54	128.9± 5.43	136.0± 3.7	138.8± 1.39	121.0± 0.16	119.7± 13.66	126.3± 0.53	126.5± 6.87	127.9± 2.04
100	103.0± 5.68	112.1± 2.28	56.94± 12.7	53.92± 5.37	102.1± 1.32	125.2± 1.86	121.5± 4.86	120.3± 0.83	106.2± 2.56	93.47± 2.11	98.14± 1.85	82.9± 5.32	125.4± 1.55

Fractions F1–F10 and F6(16) were derived from organic solvent extraction of *B. coccineus* after the processes of maceration, liquid-liquid extraction, silica gel and C-18 column chromatography as shown in Figure 1; Fractions BW(18) were derived from HP-20 column chromatography of the aqueous extract of *B. coccineus* (BW) as shown in Figure 2. Data are presented as mean ± SEM (n = 3).

ketoconazole, which inhibited enzyme activity to 149 and 61 pmol/min/mg for 0.01 and 1 mM, respectively.

Immunomodulation assay

In the preliminary study, the aqueous leaf extract of *B. coccineus* (BW, see Figure 2) produced the greatest effect in increasing IFNβ expression at the concentration of 0.1 μg/μL relative to the organic solvent extracts (B1 and B2, data not shown). Considering the fractions obtained from BW by HP-20 column chromatography, fraction BW5 produced the greatest effects on IFNβ and IFNα2a expression, causing 7- and 4-fold increases, respectively (Table 3). Fractionating BW5 further by HPLC, fractions BW5(1) to BW5(19) were obtained. Of these fractions, BW5(4) produced the greatest significant effect on IFNα2a expression eliciting a 26-fold increase (Table 4).

Antiproliferative effect

The fractions generated after normal phase column chromatography, F1 to F7 (see Figure 1), produced significant inhibitory effects (>50%) on HT29 cell viability only at the highest concentration of 100 μg/mL, giving values of 34.7%, 34.67%, 36.69%, 34.71%, 20.56%, 17.74%, and 22.24%, respectively (see Table 5). F8, F9, F10 and fractions subsequently obtained from F6 [F6(1) to F6(6)] (which produced the greatest inhibitory effect on cell viability) did not produce significant effects on HT29 cell viability (Table 5). The loss of activity of fractions derived from the active F6 can be explained by the fact that many phytomedicines exert their beneficial effects through the additive or synergistic action of several chemical compounds contrasting with synthetic pharmaceuticals based on single chemical entities (Briskin, 2000). As shown in Table 5, the aqueous extract of *B. coccineus* (BW) and fractions obtained from it (BW1 to BW8) did not produce any significant effect on HT29 cell viability in the antiproliferative assay.

Discussion

The cytochrome P450 enzyme family is involved in the metabolism and detoxification of environmental carcinogens, steroids, bile acids, fatty acids, eicosanoids, fat soluble vitamins, and drugs (Wrighton & Stevens, 1992; Guengerich et al., 2003). These enzymes transform lipophilic drugs into more polar compounds that can be excreted in urine (Girennavar et al., 2007). Depending on the nature of xenobiotics, the outcome may be activation of a prodrug, deactivation of a biologically active compound or conversion of an active drug into an active metabolite (Issa et al., 2006). According to Girennavar et al. (2007), induction and inhibition of drug-metabolizing enzymes are common mechanisms for drug interactions. Herbal medicines are commonly combined with modern drugs (Pekthong et al., 2008). Additionally, certain observations have increased interest in investigation of herb–drug interactions. St. John's wort, which has been found to induce cytochrome P450s (particularly CYP3A4), has been reported to decrease cyclosporine plasma concentrations resulting in kidney rejection (Pekthong et al., 2008). *Ginkgo biloba* L. (Ginkgoaceae), which has been shown to display beneficial effects on the vascular system, memory, cognition and gene regulation (Pekthong et al., 2008), was also found *in vitro* to strongly inhibit CYP2C9, and to a lesser extent, CYP1A2, CYP2E1 and CYP3A4 (Gaudineau et al., 2004).

In light of increasing reports of drug–herb interactions, the effects of *B. coccineus* on cytochrome P450 activity was investigated in this study. Fractions obtained from the plant extract stimulated CYP450 activity in both the BROD and BFCOD assays. The fact that the fractions were derived from the organic extracts of the plant makes the observed result relevant in situations where the leaves are macerated in alcohol or eaten, rather than when extraction is water based. The BROD assay is reported to be selective for human CYP2B6 and CYP3A4 (Niwat et al., 2003), while the BFCOD assay is linked with CYP3A4 activity (Stresser et al., 2000). Stimulation of activity, by heterotropic positive cooperativity, has been shown with CYP3A, but not to our knowledge with other P450 isoforms. For example, α-naphthoflavone was shown to stimulate CYP3A4 activity (Ueng et al., 1997). Stimulation of CYP2B1/2 and CYP3A4 activity by *B. coccineus* could possibly lead to reduced bioavailability and enhanced metabolism of co-administered drugs, most likely leading to a decrease in therapeutic efficacy. This finding gives further credence to the need for health care practitioners to ensure proper counseling of their patients about the possible implications of herb–drug interactions. However, it is possible that different results could be obtained *in vivo*. Zhou et al. (2007) reported that many known *in vitro* inhibitors of CYP proteins actually serve to induce those proteins when administered *in vivo,* with clotrimazole and other imidazoles, chlorpromazine, metyrapone, etc., as common examples. Therefore further *in vivo* studies are needed to gain a more complete understanding of the effects of this plant extract on CYP expression.

In this study, fractions from the aqueous extract of *B. coccineus* significantly stimulated the expression of IFNα2a and IFNβ in PBMC. The induction of interferon expression leads to diverse effects associated with antiviral, antiproliferative, and immunostimulatory activities (Weinstock-Guttman et al., 1995; Peters, 1996). The results obtained in this study provide a possible rationale for the anecdotal use of *B. coccineus* in TAM for the treatment of venereal diseases and tumors.

Significant reductions in the viability of a human colon cancer cell line (HT29) were only observed for some fractions at the highest concentration tested (100 μg/mL). It is therefore possible that the antiproliferative effect attributed to the plant results less from a direct effect on cell viability, and more from stimulation of interferon expression as evident from the results obtained from gene expression studies. Presumably *in*

vivo this would result in a heightened immune response to cancer cells.

Conclusions

The results obtained in this study show that fractions obtained from the organic extract of *B. coccineus* stimulated cytochrome P450 (CYP2B1/2 and CYP3A4) metabolizing enzyme activity. This suggests that the plant may reduce bioavailability and enhance the metabolism of co-administered drugs, possibly reducing their therapeutic efficacy. Furthermore, results obtained in this study also show that fractions obtained from the aqueous extract of *B. coccineus* stimulated the expression of interferons and reduced the viability of HT29 cells at high concentrations. The results obtained in this study may hint at the mechanism of action in the reported uses of *B. coccineus* in TAM (Neuwinger, 1996).

Declaration of interest

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