Effects of the extracts and derived fractions of four medicinal plants on hepatic microsomal enzymes: an *in-vitro* study

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ABSTRACT

Background: The use of medicinal plants for treatment of diverse ailments has continued to increase worldwide, especially in developing countries. The concurrent use of herbal medicines and conventional drugs is common. Herb-drug interaction through the inhibition/induction of cytochrome (CYP) P450 metabolizing enzymes could result in pharmacotoxicity/therapeutic failure.

Objective: This study investigated the extracts and fractions of four medicinal plants (*Crinum glaucum*, *Baphia nitida*, *Byrsocarpus coccineus*, and *Sanseviera liberica*) for possible effects on hepatic microsomal enzymes *in-vitro*.

Methods: The benzyloxy-4-[trifluoromethyl]-coumarin O-debenzyloxylase (BFCOD; CYP3A4) and benzyloxyresorufin O-debenzylase (BROD; CYP2B1/2) assays were used.

Results: The ethyl acetate:methanol and ethanol:water extracts of the plants demonstrated significant ($p < 0.05$) inhibitory effect on CYP activity, pronouncedly at the highest concentration (1 mg/ml) in the BROD assay. The aqueous extracts and fractions of all the plants did not significantly affect CYP activity in both assays. The same trend of observation was recorded in the BFCOD assay except that the ethyl acetate:methanol extract of *C. glaucum* did not significantly affect CYP activity. The ethyl acetate:methanol extract of *B. nitida* and *B. coccineus* elicited significant stimulatory effect at 0.01 and 0.1 mg/ml in the BROD assay. The hexane and n-butanol fractions of the extracts showed varying tendency to cause stimulation of CYP activity at lower doses and inhibition at the highest dose.

Conclusion: Findings suggest that the aqueous extracts/fractions of the plants investigated have little or no tendency to significantly affect CYP activity. Other organic solvent extracts/fractions possess the ability to inhibit CYP activity, especially at high doses.

Keywords: *Crinum glaucum*, *Baphia nitida*, *Byrsocarpus coccineus*, *Sanseviera liberica*, Herb-drug interaction, Cytochrome P450 enzymes

Effets des extraits et fractions dérivées de quatre plantes médicinales sur les enzymes microsomales hépatiques: étude in vitro

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RESUME

Contexte: L'utilisation de plantes médicinales pour le traitement de diverses affections est en pleine croissance dans le monde entier, surtout dans les pays en développement. L'utilisation concomitante de médicaments à base de plantes et de médicaments conventionnels est courante. L'interaction herbe-médicament par l'inhibition/l'induction des enzymes métabolisant le cytochrome (CYP) P450 pourrait entraîner un échec thérapeutique/pharmaco-toxicité.

Objectif: Cette étude a étudié les extraits et fractions de quatre plantes médicinales (*Crinum glaucum*, *Baphia nitida*, *Byrsocarpus coccineus* et *Sanseviera liberica*) pour les effets possibles sur les enzymes microsomales hépatiques *in vitro*.

Méthodes: Les essais de benzyloxy-4- [trifluorométhyl] -coumarine O-débenzyloxylase (BFCOD, CYP3A4) et benzyloxyresorufine O-débenzylase (BROD; CYP2B1 2) ont été utilisés.

Résultats: Les extraits d'acétate d'éthyle: méthanol et éthanol: eau des plantes ont montré un effet inhibiteur significatif ($p < 0,05$) sur l'activité du CYP, nettement à la concentration la plus élevée (1 mg/ml) dans l'essai BROD. Les extraits aqueux et les fractions de toutes les plantes n'ont pas eu d'effet significatif sur l'activité du CYP dans les deux dosages. La même tendance d'observation a été enregistrée dans le test BFCOD, sauf que l'extrait d'acétate d'éthyle et de méthanol de *C. glaucum* n'a pas eu d'effet significatif sur l'activité du CYP. L'extrait d'acétate d'éthyle et de méthanol de *B. nitida* et de *B. coccineus* a entraîné un effet stimulant significatif à 0,01 et 0,1 mg/ml dans l'essai BROD. Les fractions d'hexane et de n-butanol des extraits ont montré une tendance variable à provoquer une stimulation de l'activité du CYP à des doses plus faibles et une inhibition à la dose la plus élevée.

Conclusion: Les résultats suggèrent que les extraits/fractions aqueuses des plantes étudiées ont peu ou pas de tendance à affecter significativement l'activité du CYP. D'autres extraits/fractions de solvant organique possèdent la capacité d'inhiber l'activité du CYP, en particulier à des doses élevées.

Mots-clés: *Crinum glaucum, Baphia nitida, Byrsocarpus coccineus, Sanseviera liberica*, interaction herbe-médicament, enzymes du cytochrome P450

INTRODUCTION

The patronage of Traditional Medicine (TM) and Complementary and Alternative Medicine (CAM) has increased tremendously over the years. A strong component of both TM and CAM is the use of medicinal plants for the treatment of disease conditions. According to Leonti and Casu[1], herbal medicine and phytotherapy are used as therapeutic alternatives to biomedicine for the treatment of mild and chronic health problems. It has been reported that patients frequently combine conventional treatment and alternative approaches rather than exclusive use of either.[2] Duru et al.[3] reported a high prevalence of traditional medicine use and concurrent use of orthodox medicine. The general expectation is that of synergistic/summative effects. Examples of botanicals gaining popularity even in conventional medicine include Garlic (*Allium sativum*), Ginseng (*Panax ginseng*), Ginger, Ginkgo, St. John's Wort, Saw palmetto (*Serenoa repens*) etc.[4]

As is the case with drug-drug interaction, herb-drug interaction has received considerable attention in recent times. According to Fasinu et al.[5], mechanisms of pharmacokinetic herb-drug interaction include alteration in gastrointestinal functions which impact on drug absorption, induction/inhibition of metabolic enzymes and transport proteins, and alteration of renal excretion of drugs and their metabolites. Cho and Yoon[6] based on their *in-vitro* and *in-vivo* investigation of ten herbs and relevant phytochemicals reported that herbs can interact with cytochrome P450 and P-glycoprotein as inhibitors and/or inducers. It has been reported that numerous known drug interactions with prescription medications are mediated through CYP3A4[7] and the most common pharmacokinetic interactions usually involve either the inhibition or induction of the metabolism of drugs catalyzed by the important enzymes, cytochrome P450 (CYP).[8]

Crinum glaucum A. Chev (Amaryllidaceae), *Baphia nitida* Baill. (Papilionaceae), *Byrsocarpus coccineus* Schum. and Thonn. (Connaraceae), and *Sansevieria liberica* Gerome and Labroy (Agavaceae) are Nigerian medicinal plants with preparations used to treat diverse ailments and are likely to be used concurrently with orthodox medicines. *Crinum glaucum* is a bulbous plant widely used in Traditional African Medicine (TAM) for the treatment of diverse ailments. The plant is commonly called "Crinum lily" or "Spider lily" with the local names in Nigeria being "Isu meri" (Yoruba, South-west), "Ede chukwu" (Igbo, South-east) and "Albasarkwa adi" (Hausa, North). Preparations of the bulb and flower-stalks of the plant are used as antimicrobial, purgative, rubefacient, anti-cough, anti-allergic and female fertility enhancing remedies.[9] *C. glaucum* has also been reported to be used as antispasmodics and in the treatment of various ailments such as cough, asthma, convulsion[10], sores, sexually transmitted diseases and backache.[11] Various biological activities of *C. glaucum* extracts have been investigated and reported. These include smooth muscle relaxant[12], analgesic and anti-inflammatory[13], antianaphylactic[14], memory enhancing[10], and anticonvulsant[15] activities.

Baphia nitida is a shrub/tree commonly found in the interior and coastal regions of tropical Africa. Commonly called "Cam wood", the plant is locally known in Nigeria by various names, including "Irosun" (Yoruba, South-west), "Majigi" (Hausa, North), "Ufie" and "Aboshi" (Igbo, South-east). Preparations of the leaves, bark, roots, and twigs are used in the treatment of constipation, skin diseases, venereal diseases, ringworm, flatulence, smallpox, as enema[9], and as stimulant.[16] The plant is also used as anti-inflammatory agent against inflamed and infected umbilical cord[17], fungal infections of the skin[18], and treatment of breast cancer.[19] Extracts of the plant have been investigated and reported to possess haemostatic[20], negative chronotropic and inotropic[21], anti-inflammatory[17], anxiolytic/sedative, skeletal muscle relaxant[22], antidiarrhoeal[23], and airway relaxant[24] activities.

Byrsocarpus coccineus, commonly called "Crimson thyme" is a shrub with delicate pink-tinged foliage and white, sweet-scented flowers widely spread across tropical Africa.[25] Locally, it is known in Nigeria as "Tsaamiyar-kasa" (Hausa, North), "Oke abolo" (Igbo, South-east), and "Orikoteni" (Yoruba, South-west). Uses of preparations of the leaves, roots and whole plant in TAM include venereal disease, impotence, tumour, ulcer, jaundice, inflammation, diarrhoea etc.[9,25] In terms of pharmacological profiling, the plant has been reported to possess molluscidal[26], uterotonic[27], in-vitro antioxidant[28], antimicrobial[29,30], hepatoprotective and in-vivo antioxidant[31], anxiolytic and sedative[32], antihypertensive[33] and anti-diabetic[34] activities.

Sansevieria liberica is a perennial plant with thick woody rhizomes, widely distributed in the tropical, subtropical and temperate zones of the world. The plant is locally known as "Mooda" (Hausa, North), "Ebube-agu" (Igbo, South-east), and "Oja ikoko" (Yoruba, South-west), and its common names include "Mother in-law tongue", "African bowstring" and "Leopard lily". Preparations of the leaves and rhizomes of the plant are used in the

treatment of parasitic infections,[35] ear and eye infections, pain, inflammation, fever, and diarrhoea.[36] Various investigators have reported the CNS depressant and anticonvulsant[37], analgesic[38], antidiarrhoeal[39], in-vitro antitrypanosomal, antileishmanial, antiplasmodial[35,40], hepatoprotective[41], anti-inflammatory[42], and in-vitro and in-vivo anticancer[43] activities of extracts of the plant.

A number of studies have been conducted to investigate the effects of herbal remedies and derived constituents on hepatic microsomal enzymes activity *in-vitro*. He et al.[44] reported that gypenosides exhibited competitive inhibition of CYP2D6 and therefore cause herb-drug interactions via inhibition of this CYP isoform. Mekjarusku et al.[45] stated that *Kaempferia parviflora* extract modulated several CYP450 enzyme activities, hence its use with drugs or other herbs should raise concern for potential drug-herb interactions. It has also been reported that the ethanolic extract of *Hibiscus sabdariffa* caused inhibition of CYP isoforms *in-vitro*.[46]

The aim of this study was to investigate extracts and fractions derived from four Nigerian medicinal plants (*Crinum glaucum*, CG; *Baphia nitida*, BN; *Byrsocarpus coccineus*, BC; and *Sanseviera liberica*, SL) for possible effects (inhibition/induction) on hepatic microsomal enzymes *in-vitro*. The findings in this evaluation will help determine the likely effect of these herbs on prescription drugs based on the common practice of concurrent use with herbal remedies.

METHODS

Plant materials

Fresh plant materials (*C. glaucum*- bulb; *B. nitida* and *B. coccineus* – leaves; and *S. liberica* – rhizomes) were obtained from domestic environments in Alimosho and Ifako/Ijaiye Local Government Areas *(B. nitida* and *B. coccineus)* and a local herb market in Mushin Local Government Area, all of Lagos State, Nigeria. The botanical identification and authentication of the different plant materials were done in the Department of Botany and Microbiology, Faculty of Science, University of Lagos, Lagos, Nigeria and the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The voucher specimen of the plant materials were deposited at the herbarium of the Institutes with assigned reference numbers (*C. glaucum*- LUH 5082; *B. nitida*- FHI 106544; *B. coccineus*- FHI 106623; and *S. liberica*- FHI 107621).

Extraction and fractionation

Leaf materials (BC and BN) were air-dried until a constant weight was obtained and pulverized, while bulbs (CG) and rhizomes (SL) were chopped into pieces. The leaves, bulbs and rhizomes were washed with clean water before processing. The respective plant materials (200 g) were macerated with ethyl acetate (EtOAc) and methanol (MeOH) (1:1) for 48 h with mechanical stirring (Extract 1). The residue from this extraction was macerated with ethanol (EtOH) and de-ionized water (H_2O) (1:1), also for 48 h with mechanical stirring (Extract 2). Another portion of the dried material (200 g) was macerated with H_2O for 48 h with mechanical stirring (Extract 3). Extract 1 for the respective plants were partitioned between hexane (Fraction 1X) and 80% MeOH. The 80% MeOH fractions were then partitioned between n-butanol (BuOH) (Fraction 1Y) and H_2O (Fraction 1Z). Extract 2 of the different plants were partitioned directly between BuOH (Fraction 2Y) and water (Fraction 2Z). The extracts (1, 2 and 3) and fractions derived from extracts 1 and 2 were used in the cytochrome P450 activity assays.

Investigation of effect on cytochrome P450 enzyme activity

Benzyloxyresorufin (BR) and benzyloxy-4-trifluoromethylcoumarin (BFC) are fluorogenic substrates selective for CYP2B1/2 and CYP3A4, respectively.[47-50] Ketoconazole is a known inhibitor of CYP3A4 as well as other P450s and was used as a positive control.

Chemicals and reagents

All chemicals, solvents and reagents were obtained from Sigma-Aldrich, St. Louis, MO, USA or Fisher Scientific, Orlando, FL, USA. The following solutions were used in assays: 5 mM 7-benzyloxy-4-trifluoromethylcoumarin (BFC) in acetonitrile, 1 M Hepes pH 7.6, bovine serum albumin (BSA, 10% in water), nicotinamide adenine dinucleotide phosphate (NADPH, 9 mg/ml freshly prepared), water, 0.5 M Tris base, methanol (in dispenser), 0.5 mM and 0.05 mM 7-hydroxy-4-trifluoromethylcoumarin (HFC) in acetonitrile, 1 M Hepes-NaOH buffer pH 7.6 (13.32 g Hepes + required amount of NaOH to give pH 7.6 per 50 ml final volume), TLC-pure benzyloxyresorufin (BR) in methanol (2.089×10^{-4} M), and TLC-pure resorufin in methanol (2×10^{-6} M).

Washed human hepatic microsomes were prepared by differential centrifugation as described by James et al.[51] Aliquots were stored at - 80 °C until use in assays. The human liver was a de-identified transplant-quality liver

obtained from the Department of Surgery, University of Florida, Gainesville, FL, US under a protocol approved by the Institutional Review Board.

Benzyloxy-4-[trifluoromethyl]-coumarin O-debenzyloxylase (BFCOD) assay

This assay is based on the monooxygenation of BFC to HFC which was measured by a modification of published methods.[49,52] The water bath was set at 37 °C and the number of tubes needed for duplicate incubations was determined with a blank tube assigned to each set. Tubes were numbered and BFC added (0.02 ml of 5 mM stock). The solvent was evaporated under nitrogen and Hepes buffer (0.1 ml giving a final concentration of 0.1 M) was added. Appropriate volume of water was added to each tube to make a total final volume of 1 ml and vortex mixing was carried out. Microsomes were added (0.267 ml of 7.5 mg/ml stock giving a final bath concentration of 2 mg/ml) and sample tubes were placed in water bath for 1 min. Three millilitre methanol was added to the blank tubes. NADPH (0.1 ml of 9 mg/ml stock giving a final assay concentration of 2 mM) was added to all sample tubes at timed intervals (every 15 seconds). The sample tubes were incubated in the shaking water bath for precisely 10 min. and the reaction was then stopped by adding 3 ml methanol and vortex-mixing. The tubes were allowed to stand for 20 min. after which they were centrifuged at 600 *g* for 15 min. Three millilitre of the clear supernatant in each case was transferred to a clean tube and 0.75 ml of 0.5 M Tris base was added. Fluorescence was then read at excitation 410 nm and emission 530 nm using a fluorimeter. The standard curve was prepared with HFC (0, 250, 500, 1250, 2500 and 5000 pmol). The pmol HFC formed in sample tubes was determined from the standard curve. The specific activity was calculated as given below:

Specific activity = [pmol – blank] ÷ [protein (mg) × incubation time (min.)]

For the BFCOD assay, the amount of protein was 2 mg and the incubation time was 10 min. Samples were run in quadruplicate.

Benzyloxyresorufin O-debenzylase (BROD) assay

The basic principle of this assay is the conversion of BR to resorufin.[53,54] The water bath was turned on at 37 °C and NADPH solution was prepared and placed on ice. The sample and standard tubes were prepared with buffer (0.1 ml), BSA solution (0.2 ml) and made up to 1 ml with water. BR (0.024 ml of 2.089×10^{-4} M giving a final concentration of 5 µM) was added to the samples and resorufin (0, 0.0025, 0.005, 0.01, 0.02, and 0.05 ml corresponding to 0, 5, 10, 20, 40, and 100 pmol) to the standard tubes. The sample tubes were then placed in incubation bath. At appropriate time intervals (20 seconds), microsomes (0.0333 ml of 7.5 mg/ml stock or 0.0226 ml of 11.07 mg/ml stock giving 0.25 mg protein) and NADPH were added to the sample tubes (0.1 ml of 9 mg/ml stock). The sample tubes were incubated for 5 min. and the reaction was thereafter stopped by adding 2.5 ml ice-cold methanol and vortex-mixing thoroughly. Methanol was added to blank tubes before NADPH addition. The tubes were left standing for 20 min. to flocculate protein after which they were centrifuged for 15 min. at full speed. To the standard tubes, 2.5 ml methanol was added as for samples. The fluorescence of the supernatant from standards and samples were measured at excitation 550 nm and emission 585 nm. The fluorescence reading was converted to pmol resorufin from the standard curve.

Specific activity = [pmol – blank] ÷ [protein (mg) × incubation time (min.)]

For the BROD assay, the amount of protein was 0.25 mg and the incubation time was 10 min. Samples were run in quadruplicate.

Statistical analysis

Results (specific activity in pmol/min/mg of hepatic microsomal enzymes) obtained were expressed as mean ± S.E.M. (n = 4). The data were analyzed using one-way ANOVA followed by Dunnett's post-hoc test (GraphPad Prism 5 Software; GraphPad Software, Inc., La Jolla, CA, USA). Results were considered significant at $p < 0.05$.

RESULTS

Effect of extracts on cytochrome P450 enzyme activity

In the BFCOD assay, the ethyl acetate:methanol extract of *B. nitida* caused significant reduction in CYP activity, compared to control, with peak inhibitory effect (92.1%; $p < 0.0001$) produced at the highest concentration of 1 mg/ml. This effect was comparable to that elicited by ketoconazole (65.75%). *C. glaucum* extract at the different concentrations used did not significantly change the metabolizing enzyme activity relative to control. *S. liberica* significantly reduced ($p < 0.001$) CYP activity only at the highest concentration of 1 mg/ml, with effect (57.5%) being comparable to that of ketoconazole. Significant inhibitory effect was elicited by *B. coccineus* at concentrations of 0.1 ($p < 0.01$) and 1 mg/ml ($p < 0.0001$) with the later effect (96%) being comparable to that of ketoconazole (Figure 1(A)). In respect of the BROD assay, the ethyl acetate:methanol extract of *B. nitida* (1 mg/ml; 48.7%; $p < 0.05$), *C.*

glaucum (1 mg/ml; 51.5%; p < 0.05), *S. liberica* (0.01, 0.1 and 1 mg/ml; 45.1, 44.5 and 68.2% respectively; p < 0.05, 0.001) and *B. coccineus* (1 mg/ml; 48.9%; p < 0.05) significantly inhibited CYP activity with these effects being comparable to that of ketoconazole (65.1%). However, *B. nitida* (0.01 and 0.1 mg/ml; 50.4 and 47.7% respectively; p < 0.05) and *B. coccineus* (0.01 mg/ml; 66.5%; p < 0.001) significantly increased the enzyme activity compared to control and ketoconazole (p < 0.0001) (Figure 1(B)).

Considering the ethanol:water extracts in respect of the BFCOD assay, *B. nitida* (0.1 and 1 mg/ml; 48.9 and 91.7% respectively; p < 0.01, 0.0001), *C. glaucum* (1 mg/ml; 37.4%; p < 0.05), *S. liberica* (0.1 and 1 mg/ml; 35.4 and 73.0% respectively; p < 0.05, 0.0001), and *B. coccineus* (0.1 and 1 mg/ml; 49.4 and 93.7% respectively; p < 0.001, 0.0001) significantly inhibited CYP activity, with peak effects at the highest concentration used. These effects were comparable to that elicited by ketoconazole, 65.8% (Figure 2(A)). The same trend of result was observed in the BROD assay in respect of *B. nitida* (0.01-1 mg/ml; 51.8, 73.9 and 85.5% respectively; p < 0.001, 0.0001), *C. glaucum* (1 mg/ml; 51.4%; p < 0.001), *S. liberica* (0.1 and 1 mg/ml; 42.8 and 81.7% respectively; p < 0.01, 0.0001), and *B. coccineus* (1 mg/ml; 71.5%; p < 0.0001) (Figure 2(B)). The water extracts of all the plants investigated did not cause significant change in CYP activity, in both the BFCOD ((Figure 3(A)) and BROD (Figure 3(B)) assays relative to control.

Effect of fractions on cytochrome P450 enzyme activity

In respect of the fractions generated from the ethyl acetate: methanol and ethanol: water extracts, BN-1X (1 mg/ml; 75.8%; p < 0.0001), BN-1Y (1 mg/ml; 70.6%; p < 0.0001), BN-2Y (0.1 and 1 mg/ml; 39.3 and 82.7% respectively; p < 0.05, 0.0001), and BN-2Z (1 mg/ml; 77.6%; p < 0.0001) significantly inhibited the metabolizing enzyme activity relative to control with effects being comparable to that of ketoconazole (56.6%). The BN-1Z fraction (0.01-1 mg/ml) did not significantly change CYP activity relative to control. However, BN-1X, BN-1Y (0.01 mg/ml; 87.85 and 80.4% respectively; p < 0.0001) and BN-2Y (0.01 mg/ml; 42.1%; p < 0.05) significantly increased CYP activity compared to control and ketoconazole (Figure 4(A)). *C. glaucum* fractions did not generally significantly change the metabolizing enzyme activity compared to control except in the case of CG-1X which significantly increased (112.5%; p < 0.05) CYP activity relative to control at the concentration of 0.1 mg/ml (Figure 4(B)).

In respect of *S. liberica* fractions, metabolizing enzyme inhibitory effect was only observed with SL-1Y (1 mg/ml; 90.7%; p < 0.0001) compared to control. This effect was comparable to that of ketoconazole (54.0%). The SL-1Z and SL-2Z fractions (0.01-1 mg/ml) did not significantly change CYP activity compared to control. However, SL-1X, SL-1Y (0.01 and 0.1 mg/ml; 80.4, 125.3, 82.9 and 85.3% respectively; p < 0.0001), and SL-2Y (0.01 and 0.1 mg/ml; 71.1 and 53.1% respectively; p < 0.001, 0.05) significantly increased CYP activity relative to control and ketoconazole (p < 0.0001) (Figure 5(A)). *B. coccineus* fractions (0.01-1 mg/ml) did not significantly change CYP activity compared to control (Figure 5(B)).

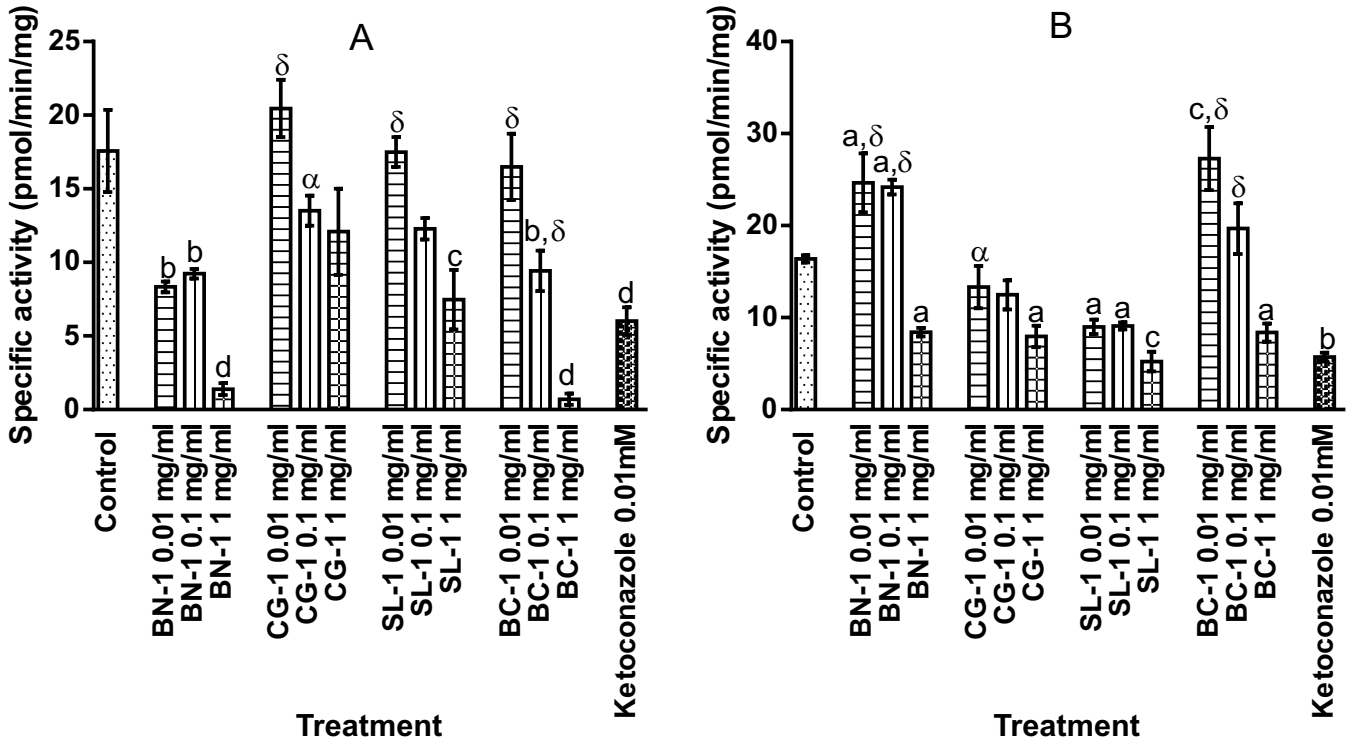

Figure 1: Effect of ethyl acetate:methanol extracts in the BFCOD (A) and BROD (B) assays. Data are presented as mean ± S.E.M. (n = 4). $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, $^{d}p < 0.0001$ vs. control; $^{\alpha}p < 0.05$, $^{\delta}p < 0.0001$ vs. ketoconazole (One way ANOVA followed by Dunnett's post-hoc test).

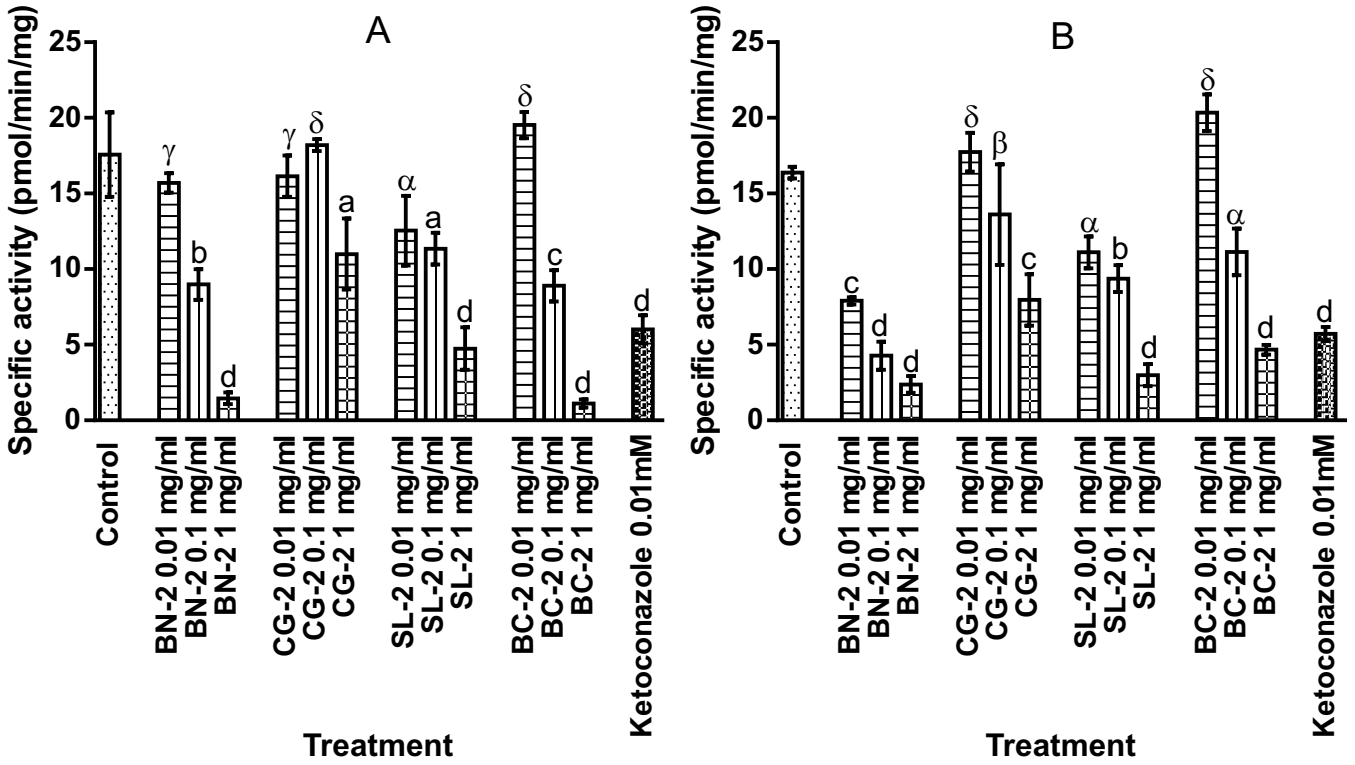

Figure 2: Effect of ethanol:water extracts in the BFCOD (A) and BROD (B) assays. Data are presented as mean ± S.E.M. (n = 4). $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, $^{d}p < 0.0001$ vs. control; $^{\alpha}p < 0.05$, $^{\beta}p < 0.01$, $^{\gamma}p < 0.001$, $^{\delta}p < 0.0001$ vs. ketoconazole (One way ANOVA followed by Dunnett's post-hoc test).

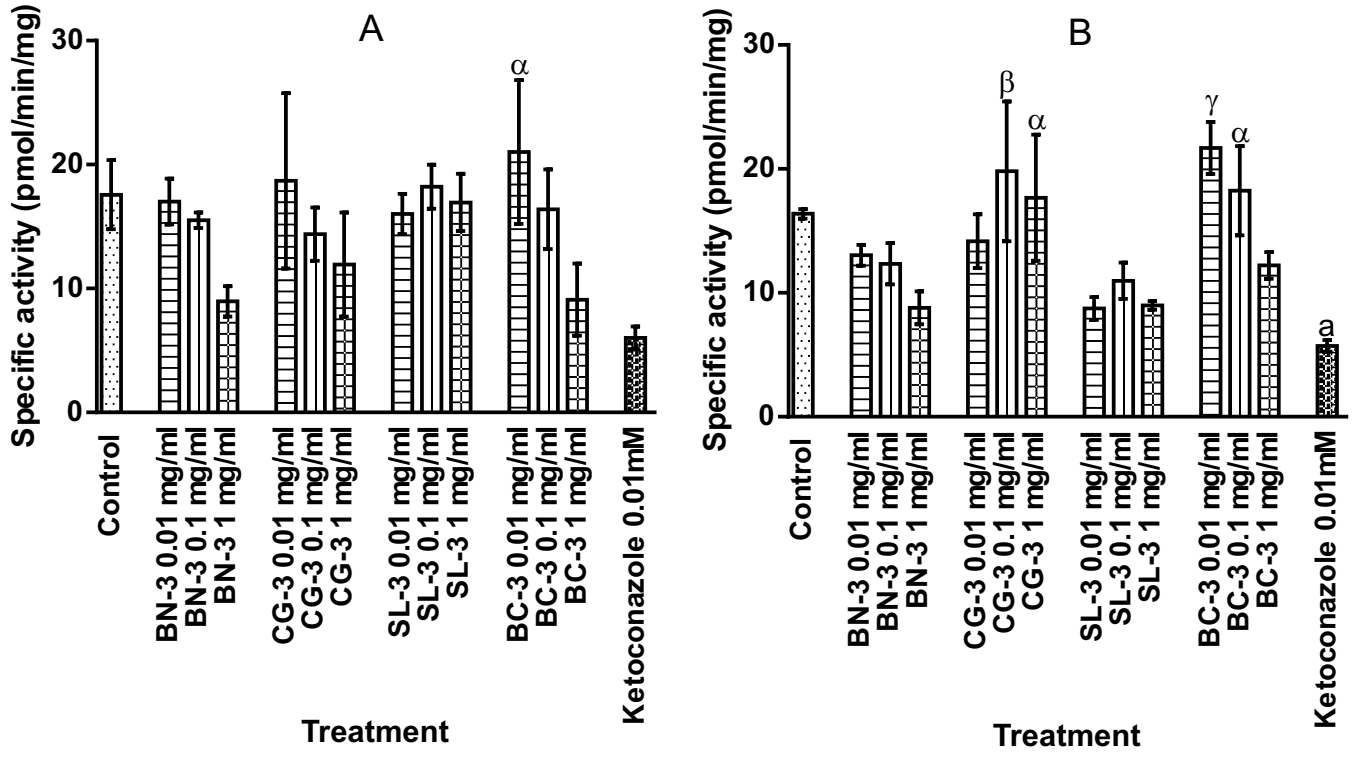

Figure 3: Effect of water extracts in the BFCOD (A) and BROD (B) assays. Data are presented as mean ± S.E.M. (n = 4). $^{a}p < 0.05$ vs. control; $^{\alpha}p < 0.05$, $^{\beta}p < 0.01$, $^{\gamma}p < 0.001$ vs. ketoconazole (One way ANOVA followed by Dunnett's post-hoc test).

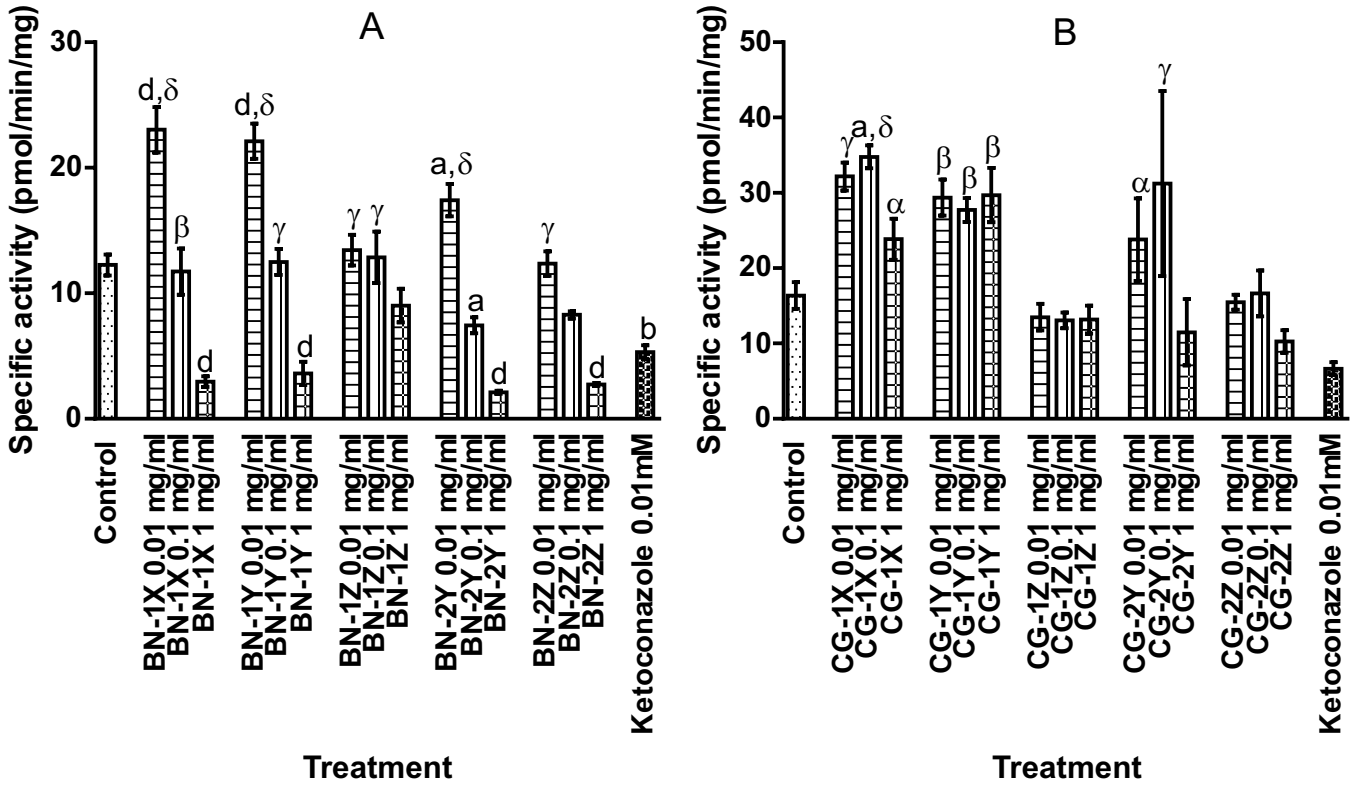

Figure 4: Effect of BN (A) and CG (B) fractions in the BROD assay. Data are presented as mean ± S.E.M. (n = 4). $^{a}p < 0.05$, $^{b}p < 0.01$, $^{d}p < 0.0001$ vs. control; $^{\alpha}p < 0.05$, $^{\beta}p < 0.01$, $^{\gamma}p < 0.001$, $^{\delta}p < 0.0001$ vs. ketoconazole (One way ANOVA followed by Dunnett's post-hoc test).

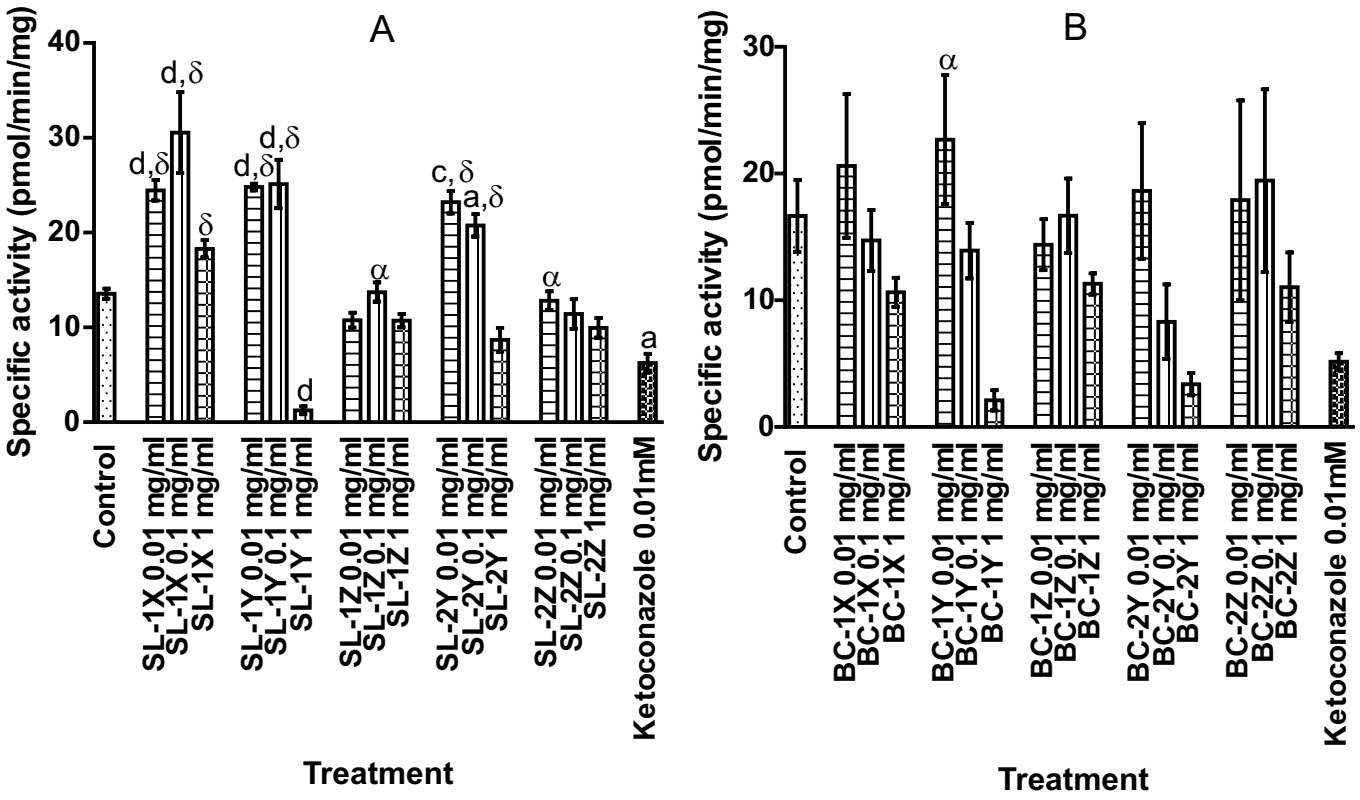

Figure 5: Effect of SL (A) and BC (B) fractions in the BROD assay. Data are presented as mean ± S.E.M. (n = 4). $^{a}p < 0.05$, $^{c}p < 0.001$, $^{d}p < 0.0001$ vs. control; $^{\alpha}p < 0.05$, $^{\delta}p < 0.0001$ vs. ketoconazole (One way ANOVA followed by Dunnett's post-hoc test).

DISCUSSION

The induction and inhibition of drug-metabolizing enzymes are the most common causes of altered drug biotransformation during drug-drug and food-drug interactions.[50,55] This study was conducted to investigate the potential of extracts and fractions of 4 documented Nigerian medicinal plants *(Crinum glaucum, Baphia nitida, Byrsocarpus coccineus and Sanseviera liberica)* to inhibit or induce hepatic microsomal enzymes using the BROD and BFCOD assays. As mentioned earlier, BR and BFC are fluorogenic substrates selective for CYP2B1/2 and CYP3A4, respectively.

CYP2B1 and CYP2B2 are structurally related isoenzymes with very similar substrate specificities and CYP2B enzymes metabolize a diverse group of compounds, including pesticides, chemotherapeutics such as cyclophosphamide, tobacco-specific nitrosamines and drugs of abuse such as cocaine, nicotine, and antidepressants.[56] CYP3A (3A4, 3A5 and 3A7) is the most abundant subfamily of cytochromes P450, representing about 30% of the entire CYP450 enzymes in human liver and being responsible for processing more than 50% of therapeutic drugs.[57] It has been reported that practically all the medicinal plants that demonstrated activity in the Phase 1 metabolism are substrates for the CYP3A family; macrolide antibiotics, anti-arrythmics, benzodiazepines, immune modulators, HIV antivirals, antihistamines, calcium channel blockers and HMG CoA reductase inhibitors are examples of classes of medications metabolized by the CYP3A subfamily; and that the probability of herb-drug interactions with this isoform is high.[57,58]

In this study, the ethyl acetate:methanol and ethanol:water extracts of *C. glaucum, B. coccineus, B. nitida and S. liberica* demonstrated significant inhibitory effect on CYP activity, most especially at the highest concentration used, in the BROD assay. However, the aqueous extracts of all the plants did not significantly affect the metabolizing enzyme activity. The same trend of observation was recorded in the BFCOD assay except that the ethyl acetate:methanol extract of *C. glaucum* did not significantly affect CYP activity. An important observation is the finding that the aqueous extracts of all the plants investigated did not significantly affect CYP activity in the BFCOD assay, as also observed in the BROD assay. Also, the aqueous fractions of all the plant extracts generally did not significantly affect the metabolizing enzyme activity.

Unlike in the BFCOD assay and other extracts, the ethyl acetate: methanol extract of *B. nitida and B. coccineus* elicited significant stimulatory effect at the lower concentrations used in this study. The hexane and n-butanol fractions of the extracts showed varying tendencies to cause stimulation of CYP activity at lower doses and inhibition at the highest dose. In previous *in-vitro* studies, it was reported that gypenosides exhibited competitive inhibition of CYP2D6[44]; *Kaempferia parviflora* extract modulated several CYP450 enzyme activities [45]; and the ethanolic extract of *Hibiscus sabdariffa* caused inhibition of CYP isoforms *in-vitro*.[46] These are valid reasons to suspect potential for herb-drug interaction on the basis of modulation of CYP450 enzymes.

The findings in this study suggest that the aqueous extracts and fractions of all the plants investigated, unlike those derived from other organic solvents, had no significant effect on CYP (CYP2B1/2 and CYP3A4) activity. This observation is significant in view of the fact that water is almost universally the solvent used to extract activity of medicinal plants and herbs.[59] However, it has been reported that ethanol is the most commonly used organic solvent by herbal medicine manufacturers because the finished products can be safely used internally by consumers of herbal extracts.[60,61] In contrast to water extracts, the plant extracts and fractions derived from other organic solvents demonstrated a high propensity to inhibit CYP activity, especially at the highest concentration, in this study. This finding is significant based on the fact that dosage appropriation is one of the major criticisms of TM and use of medicinal plant remedies, and there is a high tendency that herbal medicines are likely to be taken in excess owing to the false belief of direct proportionality between dose and efficacy.

According to Fasinu et al.[5], the risk of pharmacokinetic drug interaction may result in either pharmacotoxicity or treatment failure. The CYP inhibitory effect of the extracts investigated in this study suggest predisposition to pharmacotoxicity of co-administered drugs, and this may be very problematic in respect of drugs with narrow therapeutic index e.g. warfarin lithium, digoxin, phenytoin, gentamycin, amphotericin B, 5-fluorouracil, zidovudine etc.).

CONCLUSION

The results obtained in this study suggest that the aqueous extracts and fractions of the Nigerian medicinal plants investigated (*Crinum glaucum, Baphia nitida, Byrsocarpus coccineus and Sanseviera liberica*) have little or no tendency to significantly affect CYP (CYP2B1/2 and CYP3A4) metabolizing enzyme activity. Other organic solvent extracts and fractions however, possess the ability to considerably inhibit the activity of the metabolizing enzymes especially at high doses, hence caution should be exercised in the co-administration of these herbal medicines with prescribed orthodox medications, especially those with narrow therapeutic window. This also calls for improved education of physicians on natural product medicines and herb-drug interaction, and the proper counseling of patients on the possible effects and dangers of concurrent intake of herbal medicines with conventional drugs.

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