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**Original Article**

# Microbial transformation of some simple β-carboline alkaloids

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## ABSTRACT

Microbial transformation of some simple β-carboline alkaloids e.g.: harmaline, harmalol, harman and harmine was carried out. The study resulted in isolation of ten metabolites. The structures of these metabolites were established using physical and spectroscopic techniques including: melting points, UV, IR, $^{1}$H NMR, $^{13}$C NMR and mass spectrometry. The metabolites were identified as: **1** (2-acetyl-3-(2-acetamidoethyl)-7-methoxyindole), **2** (acetamide), **3** (harmalol), **4** (2-acetyl-3-(2-acetamidoethyl)-7-hydroxyindole), **5** (harman-2-oxide), **6** (6-hydroxy-harman), **7** (acetamide), **8** (6-hydroxy harmine), **9** (harmine-2-oxide), and **10** (harmol). The antioxidant and cytotoxic activities of the substrates and their metabolites were investigated. The results revealed that harman-2-oxide presented strong free radical scavenging activity, which was much more potent than the positive control ($IC_{50}$= 18±1.2 µg/ml). Harmine, 6-hydroxy-harmine, harman-2-oxide, harmine-2-oxide, harmalol and 6-hydroxy harman presented the highest cytotoxic activity comparable to doxorubicin ($IC_{50}$= 0.60 µg/ml).

**Key words:** N-oxidation, O-Demethylation, Hydroxylation, Tryptamines, DPPH assay, SRB assay.

## INTRODUCTION

β-Carboline alkaloids are a large group of natural and synthetic indole alkaloids with different degrees of aromaticity, some of which are widely distributed in nature, including various plants, foodstuffs, marine creatures, insects, mammalians as well as human tissues and body fluids. These compounds are of great interest due to their diverse biological activities as they have been shown to intercalate into DNA, to inhibit CDK, topoisomerase, and monoamine oxidase, and to interact with benzodiazepine receptors and 5-hydroxy serotonin receptors [1]. Furthermore, they have several pharmacological properties including sedative, anxiolytic, hypnotic, anticonvulsant, antitumor, antiviral, antiparasitic as well as antimicrobial activities [2].

Harmaline (1-methyl-7-methoxy-4,9-dihydro β-carboline), a fluorescent psychoactive compound, was first isolated from the seeds of *Peganum harmala* (L.) in the family *Zygophyllaceae* [3]. Harmalol (3,4-dihydro-1-methyl-9H-pyrido[3,4-b]indol-7-ol) was also isolated from seeds of *Peganum harmala* L., and from demethylation of harmaline [4]. Harmalol was found to inhibit enzymatically and non-enzymatically induced lipid peroxidation of liver microsomes, and to attenuate oxidative damage of hyaluronic acid, cartilage collagen, and IgG [5]. It also inhibited the carcinogen-activating enzymes such as cytochrome P450 1A1 enzymes (CYP1A1) [6].

Harman (1-methyl -9 H-pyrido[3,4-b]indole), which was isolated from *Passiflora incarnata* (L.) family *Passifloraceae* [3], has been demonstrated to play a role in the processes of drug abuse and dependence. Harman is a natural inhibitor of monoamine oxidase type A, as it binds reversibly to the active site of this enzyme *in vivo* [7]. Dietary sources for mammalian harman play probably only a minor role, because the concentrations in beer and wine, as well as other foodstuffs are too low to contribute substantially to endogenous levels of harman [8].

Harmine (7-methoxy-1-methyl-9 H-pyrido[3,4-b] indole), which was isolated from seeds of *Peganum harmala* L. [9], is a very important natural product due to its interesting chemistry, pharmacological importance, and therapeutic potentials [10]. Harmine possesses antidepressant activity by interacting with MAO A and several cell-surface receptors, including serotonin receptor 2A (5-hydroxytrytamine receptor 2A, 5-HT2A) [11]. Several studies were conducted regarding mammalians and microbial metabolism of simple

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β-carboline alkaloids. They were oxidized to several ring-hydroxylated, N-oxidation and demethylated products [12]. This study significantly describes the use of microbial transformation systems as tool to introduce new biologically active drug from natural products.

## MATERIAL AND METHODS

***General experimental procedures***: UV spectra were measured on a Shimadzu UV- 1800 spectrophotometer. IR spectra were recorded with a Jasco FT/IR-6100 spectrophotometer. Electron ionization mass spectra were performed with a Thermo Scientific ISQ Single Quadrupole Mass Spectrometer. $^{1}H$ NMR and $^{13}C$ NMR spectra were recorded with Bruker High Performance Digital FT-NMR Spectrometer Avance III operating at 400 MHz for proton and 100 MHz for carbon. Chemical shifts were given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. Melting point determination was carried out using Gallenkamp melting point apparatus. TLC was performed using precoated TLC sheets silica gel G 254 F (E. Merck, Germany). For column chromatography, silica gel (E. Merck, 70-230 mesh) and sephadex LH-20 (Sigma- Aldrich chemical Co.) were used. Harmine was obtained from Fluka Company, harmaline and harmalol were obtained from Infodine Company, and harman was obtained from Sigma Aldrich, they were used as substrates. All the reagents and solvents used for separation and purification were of analytical grade.

***Microorganisms and culture condition:*** Microorganisms were obtained from the American Type Culture collection (ATCC, Rockville, Maryland), Northern Regional Research Laboratories (NRRL, Peoria, Illinois) and Assiut University Mycological Center (AUMC).

***Component of culture media:***All fermentation experiments utilize a liquid media of the following composition: 10 g dextrose, 5 g yeast extract, 5 g $K_2HPO_4$, 5 g NaCl and 10 ml glycerin in one liter of distilled water, which was sterilized at 121°C for 15 min [13].

***Cultivation of microorganisms:*** Sabouraud dextrose agar (Oxoid) slants were used to subculture these microorganisms and kept at 4°C. Cells from two-week old slants were inoculated to autoclaved culture medium and kept on a gyratory shaker (Labline, 200 rpm) for 72 hours to give the stage I culture. An inoculum of 5 ml culture was inoculated with sterilized culture media per (50 ml) and kept on a gyratory shaker for an additional 24 hours to give the stage II culture. Each substrate was added to stage II culture media as a solution for determination of the maximum conversion of the substrates. Fermentation broth was analyzed periodically by extraction with an equal volume of EtOAc after alkalinization with ammonium hydroxide solution. After evaporation of the solvent, the residue was chromatographed on silica gel plates using solvent systems chloroform: acetone: $NH_3$ (5:4:1) for harmaline, harmalol and harman, and benzene: ethyl acetate (4:1) for harmine. The plates were detected under UV light at 254 and 365 nm, and sprayed with Dragendorff's and P-anisaldehyde reagents afterwards [13].

***Microbial transformation screening of alkaloid substrates:*** Forty strains of fungi of different classes were used in the initial screening of simple β-carboline substrates (harmaline and harmalol), thirty strains for harman biotransformation and six strains of fungi for harmine biotransformation. Standard two-stage fermentation technique was used (Table 1) [13]. Large scale biotransformation was performed using *Rhodotorula rubra* NRRL y1592 and *Cunninghamella blackesleeana* NRRL 1369 for harmaline, *Rhodotorula rubra* NRRL y1592 for harmalol, *Cunninghamella elegans* NRRL 2310 and *Penicillium chrysogeneum* ATCC 10002 for harman and *Cunninghamella blackesleeana* NRRL 1369 for harmine.

***Large scale fermentations:*** Harmaline (200 mg) was dissolved in 10 ml DMF and equally divided among ten flasks each containing 100 ml of stage II culture. After maximum conversion, the fermentation broth of harmaline was exhaustively extracted with an equal volume of ethyl acetate after rendered alkaline with concentrated ammonium hydroxide (1 ml/30 ml culture). The extracts were combined and evaporated to dryness under reduced pressure to give 0.5 and 1.2 g semi-solid residues, respectively. The same procedures for fermentation and extraction were adopted using 2% substrates in DMF, for all the tested substrates except harmine. Harmine was prepared as a 4% solution in DMF-acetone mixture (9:1). After harmalol transformation by *Rhodotorula rubra* NRRL y1592, the yield was 1g residue. Harman transformation by *Cunninghamella elegans* NRRL 2310 and *Penicillium chrysogeneum* ATCC 10002 yielded 800 and 750 mg residue, respectively. Harmine transformation by *Cunninghamella blackesleeana* NRRL 1369 yielded 300 mg residue.

***Chromatographic isolation of the metabolites:*** Metabolite (**1**) was isolated from harmaline transformation by *Rhodotorula rubra* NRRL y1592. The residue containing the metabolite was dissolved in chloroform-methanol mixture (1:1), loaded on silica gel column and gradiently eluted

with chloroform, then chloroform-methanol mixture till 10%. It was obtained from fractions (26-34) in the form of white powder (25mg, 6% yield). The metabolite was obtained as white crystals, m.p. 162°C, $R_f$ = 0.60 using solvent system chloroform:acetone:$NH_3$ (5:4:1). MS: *m/z* (%): 274 (100), 216 (30), 184 (66). UV $\lambda_{max}$ (MeOH) nm: 218, 258, 336 nm. IR (KBr) $cm^{-1}$: 3288, 3092, 2933, 2792, 1640, 1529.$^1$H NMR ($CDCl_3$): δ11.91 (1 H, s, H-1), 7.48 ( 1 H, d, *J* = 8.7 Hz,H-5), 6.71 (1 H, dd, *J* = 8.7, 1.9 Hz, H-6), 6.74 (1 H, d, *J* = 1.9 Hz, H-8), 3.21 (2 H, t, *J*= 6.8 Hz, H-1'), 3.47 ( 2 H, t, *J* = 6.8 Hz, H-2'), 3.74 (3 H, s, $OCH_3$), 2.53 (3 H, s, H-11), 1.72 (3 H, s, H-13), 8.03 (1 H, s, N-H).$^{13}$C NMR ($CDCl_3$): δ132.6 (C-2), 122.1 (C-3), 138.2 (C-4), 122.1 (C-5), 112.7 (C-6), 160.1(C-7), 94.6 (C-8), 138.2 (C-9), 25.2 (C-1'), 41.4 (C-2'), 55.8 ($OCH_3$), 28.3 (C-11), 191.0 (C-10), 23.7 (C-12), 171.0 (C-13).

Metabolite **(2)** was obtained from fractions (36-40) further purified using sephadex LH-20 column. The metabolite was obtained as white crystals (10 mg, 4% yield), m.p. 80°C, $R_f$ = 0.4 using solvent system Benzene:ethyl acetate (4:1). MS: m/z (%): 59 (100). IR ($cm^{-1}$): 3300, 3350, 1690.$^1$H NMR (DMSO-$d_6$): δ 1.77 (3H, s, H-1), 6.69 (1H, s, N-H), 7.29 (1H, s, N-H').$^{13}$C NMR (DMSO-$d_6$): δ 22.8 (C-1), 172.3 (C-2).

Metabolite **(3)** was isolated from harmaline transformation by *Cunninghamella blackesleeana* NRRL 1369. The residue containing the metabolite was dissolved in chloroform-methanol mixture (2:1) and loaded on silica gel column. Isocratic elution was carried out using chloroform:acetone: $NH_3$ (5:4:1). Metabolite (**3**) was obtained from fractions (25-30) after evaporation and further purification using sephadex LH-20 column. The metabolite was obtained as an orange yellow crystals (30 mg, 15% yield), m.p. 212° C, $R_f$ = 0.15 using solvent system chloroform:acetone: $NH_3$ (5:4:1). MS; *m/z* (%); 200 (100), 185 (65), 159 (55), 132 (45). UV $\lambda_{max}$ (MeOH) nm: 207, 257, 371. IR (KBr) $cm^{-1}$: 3490, 3145, 1873, 1614, 1590, 677.
$^1$H NMR (DMSO-$d_6$): δ3.95 (2H, t, *J*=7 Hz, H-3), 2.97 (2H, t, *J*=7 Hz, H-4), 7.25 ( 1 H, d, *J* = 8.9 Hz, H-5), 6.71 (1 H, dd, *J* = 8.9, 1.9 Hz, H-6), 6.85 ( 1 H, d, *J* = 1.9 Hz, H-8), 11.35 (1H, s, H-9), 2.26 (3H, s, H-10), 5.45 ( 1 H, s, OH). $^{13}$C NMR (DMSO-$d_6$): δ 142.9 (C-1), 45.6 (C-3), 21.2 (C-4), 122.6 (C-5), 115.9 (C-6), 157.4 (C-7), 94.8 (C-8), 19.1 (C-10), 119.1 (C-4a), 125.7 (C-4b), 144.1 (C-8a), 125.6 (C-9a).

Metabolite (**4**) was isolated from harmalol transformation by *Rhodotorula rubra* NRRL y1592. The residue containing the metabolite was dissolved in chloroform-methanol mixture (1:1), loaded on silica gel column and gradiently eluted with chloroform then chloroform-methanol mixture till 40%. Metabolite **(4)** was obtained from fractions (29-31) after evaporation. It was obtained as an off-white crystals (10 mg, 3 % yield); m.p.150°C, $R_f$ = 0.45 using solvent system chloroform: acetone: $NH_3$ (5:4:1). MS: *m/z* (%); 260 (100), 202 (27), 184 (70), 159 (34). UV λmax (MeOH) nm: 220, 258, 336. IR (KBr) $cm^{-1}$: 3292, 3217, 2732, 1924, 1690, 1220. $^1$H NMR (DMSO-$d_6$): δ 7.47 ( 1H, d, *J* = 8.8 Hz, H-5), 6.69 (1 H, dd, *J* = 8.8, 1.8 Hz, H-6), 6.74 (1 H, d, *J* = 1.8 Hz, H-8), 3.24 (2 H, t, *J*= 6.9 Hz, H-1'), 3.45 (2 H, t, *J* = 6.9 Hz, H-2'), 2.57(3H, s, H-11), 1.92 (3H, s, H-13), 11.9 (1 H, s, $N_9$H), 8.03 (1 H, s, NHCO), 5.36 (1 H, s, OH). $^{13}$C NMR (DMSO-$d_6$): δ 131.6 (C-2), 122.6 (C-3), 138.8 (C-4), 122.1 (C-5), 112.1 (C-6), 157.4 (C-7), 96.6 (C-8), 138.7 (C-9), 25.1 (C-1'), 41.3 (C-2'), 28.1 (C-11), 191.9 (C-10), 22.5 (C-13), 172.3 (C-12).

Metabolite (**5**) was isolated from harman transformation by *Cunninghamella elegans* NRRL 2310. The residue containing the metabolite was dissolved in chloroform-methanol mixture (2:1), loaded on silica gel column and gradiently eluted with chloroform then chloroform-methanol till 90 %. Metabolite (**5**) was obtained from fractions eluted with $CHCl_3$:methanol (8.5:1.5) after evaporation and purification using sephadex LH-20 column**.** This metabolite was obtained in the form of yellowish white crystals (9 mg, 3 % yield); m.p. 240°C, $R_f$ = 0.1 using solvent systems benzene: methanol: $NH_3$ (4:1:1). MS; *m/z* (%): 198 (60), 182 (100), 167 (20), 154 (60). UV $\lambda_{max}$ (MeOH) nm: 256, 316, 344, 358. IR (KBr) $cm^{-1}$: 3353, 3195, 1926, 1289, 1010. $^1$H NMR ($CDCl_3$): δ 8.07 (1 H, *d*, *J*= 6.4 Hz, H-3), 7.78 (1 H, *d*, *J*= 6.4 Hz, H-4), 7.93(1 H, *d* , *J*= 6.7 Hz, H-5), 7.21( 1H, *dd*, *J*= 6.7, 7.2 Hz, H-6), 7.49 (1 H, *dd*, *J*= 7.2, 7.5 Hz, H-7), 7.45 (1 H, d, *J*= 7.5 Hz, H-8), 11.61 (1 H, *s*, NH), 2.71(3H, *s*, H-10).$^{13}$C NMR ($CDCl_3$): δ 134.6 (C-1), 130.6 (C-3), 114.2 (C-4), 121.0 (C-5), 120.7 (C-6), 128.3 (C-7), 111.8 (C-8), 11.7 (C-10), 123.6 (C-4a), 121.3 (C-4b), 142.6 (C-8a), 136.0 (C-9a).

***Preparation of harman-2-oxide:*** A mixture of harman (100 mg) and *m*-chloroperbenzoic acid (100 mg) was dissolved in 2 ml $CHCl_3$ and allowed to stand at room temperature for 24 hours. The mixture was then washed with 5% $NaHCO_3$ solution in distilled water. The organic layer was evaporated and the residue was purified using sephadex LH-20 column, to yield harman-2-oxide as white crystals (15 mg, 15%). The physical data was identical to those of the product obtained from microbial transformation of harman by *Cunninghamella elegans* NRRL 2310 [14].

Metabolite (**6**) was isolated from harman transformation by *Penicillium chrysogeneum* ATCC 10002. The residue containing the metabolite was dissolved in chloroform-methanol mixture (2:1), loaded on silica gel column and gradiently eluted with chloroform then chloroform-methanol mixture till 80 %. Metabolite (**6**) was obtained from fractions eluted with $CHCl_3$-methanol (7:3) after evaporation and purification using sephadex LH-20 column. It was obtained in the form of yellowish white crystals (40 mg, 8.9 % yield), m.p. 319° C, $R_f$= 0.4 using solvent systems chloroform: acetone: $NH_3$ (5:4:1).

MS; *m/z*(%):198 (100), 184 (65), 170 (20), 156 (60). UV $\lambda_{max}$ (MeOH) nm: 234, 246, 258, 288. IR (KBr) $cm^{-1}$:3450, 1690, 1243, 1372. $^1$H NMR ($CDCl_3$): δ 8.07( 1H, d, *J*= 5.4 Hz, H-3), 7.76 ( 1H, d, *J*= 5.4 Hz, H-4), 7.48 (1 H, d, *J*= 2.2 Hz, H-5), 7.12 ( 1 H, dd, *J*= 8.7, 2.2 Hz, H-7 ), 7.42 ( 1 H, d, *J*= 8.7 Hz, H-8), 11.35 ( 1 H, s, NH), 2.71 (3 H, s, C-10), 6.42 (1H, s, OH).$^{13}$C NMR ($CDCl_3$): δ142.0 (C-1), 136.0 (C-3), 113.0 (C-4), 105.7 (C-5), 151.2 (C-6), 118.4 (C-7), 112.4 (C-8), 18.6 (C-10), 128.4 (C-4a), 135.9 (C-4b), 122.4 (C-8a), 142.2 (C-9a).

Metabolite (**7**) was isolated from harmine transformation by *Cunninghamella elegans* NRRL 1392. Harmine was used in large scale fermentation [15]. The metabolite was isolated using a combination of silica gel and sephadex LH-20 column chromatography. Fractions (18-21) of silica gel column yielded metabolite (**7**). This metabolite was obtained as white crystals (20 mg, 4 % yield), mp 80°C, $R_f$ = 0.4 using solvent system benzene:ethyl acetate (4:1).MS; *m/z* (%): 59 (100). IR (KBr) $cm^{-1}$: 3300, 3350, 1690.$^1$H NMR (DMSO-$d_6$): δ 1.79 (3H, *s*, H-1), 6.69 (1H, *s*, NH), 7.29 (1H, *s*, NH').$^{13}$C NMR (DMSO-$d_6$): δ 22.9 (C-1), 172.1 (C-2).

Metabolites (**8**-**10**) were isolated from harmine transformation by *Cunninghamella blackesleeana* NRRL 1369. The residue containing the metabolites was dissolved in benzene:methanol mixture (4:1) and loaded on silica gel column. Elution was done isocratically using benzene: methanol: $NH_3$ (4:1:1).

Metabolite (**8**) was obtained from fractions (5-10) after evaporation. It was obtained as yellow powder (25 mg, 12.5 % yield), m.p. 240° C, $R_f$= 0.33 using solvent system benzene: ethyl acetate (4:1). MS; *m/z* (%): 228 (55), 213 (100), 187 (33), 160 (40). UV $\lambda_{max}$ (MeOH) nm: 265, 303, 330. IR (KBr) $cm^{-1}$: 3505, 3176, 2782, 1636, 1474, 1205. $^1$H NMR (DMSO-$d_6$): δ 8.06 ( 1H, d, *J*= 4.5 Hz, H-3), 7.85 ( 1H, d, *J*= 4.5 Hz, H-4), 7.58 (1H, s, H-5), 3.83 (3H, s, C-7-OC<u>H</u>$_3$), 6.91 ( 1 H, s, H-8), 11.53 ( 1 H, s, NH), 2.66 (3 H, s, H-10), 6.33 (1H, s, OH). $^{13}$C NMR (DMSO-$d_6$): δ142.7 (C-1), 135.0 (C-3), 113.5 (C-4), 108.8 (C-5), 147.7 (C-6), 149.2 (C-7), 97.4 (C-8), 19.3 (C-10), 120.1 (C-4a), 133.5 (C-4b), 121.9 (C-8a), 141.4 (C-9a), 54.0 (C-7-O<u>C</u>H$_3$).

Metabolite (**9**) was obtained from fractions (13-19) after evaporation. It was obtained as white crystals (15 mg, 7.5 % yield), mp 226° C, $R_f$ = 0.2 using solvent system benzene: ethyl acetate (4:1). MS; *m/z* (%): 228 (60), 211 (100), 196 (25), 169 (40). UV $\lambda_{max}$(MeOH) nm: 213, 250, 331. IR (KBr) $cm^{-1}$: 3404, 3059, 2604, 1724, 1615. $^1$H NMR (DMSO-$d_6$): 8.06 ( 1H, d, *J*= 6.6 Hz, H-3), 7.86 (1H, d, *J*= 6.6 Hz, H-4), 8.02 (1H, d, *J*= 8.5 Hz, H-5), 6.88 (1H, dd, *J*= 8.5, 2 Hz, H-6), 3.88 (3H, s, C-7-OC<u>H</u>$_3$), 6.99 (1H, d, *J*= 2 Hz, H-8), 11.59 (1H, s, NH), 2.63 (3H, s, H-10). $^{13}$C NMR (DMSO-$d_6$): δ 132.3 (C-1), 131.2 (C-3), 113.4 (C-4), 122.9 (C-5), 109.8 (C-6), 159.2 (C-7), 95.0 (C-8), 12.9 (C-10), 115.4 (C-4a), 119.9 (C-4b), 143.2 (C-8a), 137.0 (C-9a), 55.8 (C-7-O<u>C</u>H$_3$).

Metabolite (**10**) was obtained from fractions (21-30) after evaporation. The compound was obtained as yellowish crystals (15 mg, 7.5 % yield), $R_f$= 0.1 using solvent systems benzene: ethyl acetate (4:1). Yellowish crystals (15 mg, 7.5 % yield), $R_f$= 0.1 using solvent systems benzene:ethyl acetate (4:1). MS: *m/z* (%): 198 (100), 170 (35), 155 (12), 99 (30). UV $\lambda_{max}$ (MeOH) nm: 241, 303, 337. IR (KBr) $cm^{-1}$:3050, 3077, 2450, 2039, 1022, 883.

$^1$H NMR (DMSO-$d_6$): δ 8.13 (1H, d, *J*= 5.3 Hz, H-3), 7.74 (1H, d, *J*= 5.3 Hz, H-4), 7.94 (1 H, d, *J*= 8.4 Hz, H-5), 6.76 (1H, d, *J*= 8.4 Hz , H-6), 6.98 (1 H, s, H-8), 11.42 (1 H, s, NH), 2.74 (3 H, s, H-10).

$^{13}$C NMR (DMSO-$d_6$): δ143.0 (C-1), 137.9 (C-3), 111.9 (C-4), 122.9 (C-5), 110.5 (C-6), 158.7 (C-7), 97.1 (C-8), 20.7 (C-10), 114.1 (C-4a), 128.4 (C-4b), 135.0 (C-8a), 141.1 (C-9a).

**Biological activity:**

***A-Antioxidant activity***

The antioxidant activity of different simple β-carbolines and their isolated pure metabolites was determined by the DPPH radical scavenging method. The procedures were carried out according to the method reported by Kadri A. *et al* (2011) [16]. The antioxidant activity was expressed as $IC_{50}$ (Table 2 and Figure 2). Quercetin was used as a positive control.

***B-Cytotoxic activity***

The cytotoxicity of simple β-carboline alkaloid substrates and the isolated metabolites was

determined according to the method reported by Skehan *et al* (1990) [17]. The cytotoxicity of each sample was expressed as $IC_{50}$ (Table 3 and Figure 3).

## RESULTS AND DISCUSSION

Microbial transformation of some simple β-carboline alkaloids e.g.: harmaline, harmalol, harman and harmine were carried out. The study resulted in the isolation of ten metabolites. The structures of these metabolites were established using physical and spectroscopic techniques including: melting points, UV, IR, $^1$H NMR, $^{13}$C NMR and mass spectrometry**.** Identification of the isolated metabolites (Figure 1) was based on comparison of our data with those reported in published literatures. The mass spectrum of metabolite (**1**) showed the presence of molecular ion peak at m/z 274, presumably corresponding to a molecular formula of $C_{15}H_{16}N_2O_3$. The fragment at m/z 216 (m-58) comes from cleavage at the α-carbon on the alkyl chain, which is a preferential cleavage site of tryptamines [18]. The $^1$H NMR data of metabolite (**1**) showed that the metabolite exhibited three sharp proton singlets at 2.52 ppm of the acetyl moiety, and 1.70 ppm of the N- acetyl moiety. $^{13}$C NMR of metabolite (**1**) showed fifteen peaks which corresponded to fifteen different carbon atoms. Two carbonyl signals at 191.9 ppm (ArCO) and 171.0 (-NCO), and two high field methyl resonances at 28.3 ppm (Ar-CO-$CH_3$) and 23.7 ppm (–$NCOCH_3$) were observed. All spectral data of metabolite (**1**) were identical to that of 2-acetyl-3-(2-acetamidoethyl)-7-methoxyindole [14]. Mass spectrum of metabolite (**2**) showed a molecular ion peak at m/z 59, presumably corresponding to a molecular formula of $C_2H_5NO$. IR spectrum showed characteristics of two peaks of $NH_2$ at 3300 and 3350 cm$^{-1}$, and a carbonyl (C=O) sharp peak at 1690 cm$^{-1}$, consistent with the amide carbonyl group. It has a superimposed IR spectrum with acetamide. Based on $^1$H and $^{13}$C NMR data of metabolite (**2**), the compound is identified as acetamide [19].

Biotransformation of harmaline using the fungus *Cunninghamella blackesleeana* NRRL 1369 resulted in production of metabolite (**3**). The compound was isolated using a combination of silica gel and sephadex LH-20 column chromatography. Mass spectrum showed the presence of a molecular ion peak at m/z 200, presumably corresponding to a molecular formula of $C_{12}H_{12}N_2O$ which is less than harmaline by 15 (possibly $CH_3$ group). IR spectrum of this metabolite showed the appearance of an OH group signal at 3490 cm$^{-1}$, which is not present in harmaline. The $^1$H NMR data of metabolite (**3**) showed a lack of singlet at 3.73 ppm integrating for three protons (methyl group) which are present in harmaline, and an appearance of another broad singlet integrating for one proton at 5.45 ppm, due to OH group resulting from the demethylation reaction. $^{13}$C NMR of metabolite (**3**) showed a lack of methyl group signals at 55.2 ppm present in harmaline and C-6 signal deshielded by 4 ppm due to the produced hydroxyl group. All data were consistent with that reported for the O-demethylated product and identical to those reported for harmalol [20]. This metabolite was obtained for the first time via microbial transformation.

Biotransformation of harmalol using the fungus *Rhodotorula rubra* NRRL y1592 resulted in production of metabolite (**4**). The compound was isolated using a combination of silica gel and sephadex LH-20 column chromatography. Mass spectrum showed the presence of molecular ion peak at m/z 260, presumably corresponding to a molecular formula of $C_{14}H_{16}N_2O_3$. The fragment at m/z 202 (M-58) comes from cleavage at the α-carbon on the alkyl chain, which is a preferential cleavage site of tryptamines [18]. The $^1$H NMR data of metabolite (**4**) revealed the presence of two sharp three proton singlet at 2.57 ppm of the acetyl moiety and 1.90 ppm of the N- acetyl moiety. $^{13}$C NMR of metabolite (**4**) showed two carbonyl signals at 191.91 ppm (ArCO) and 172.32 ppm (NCO) and two up field methyl resonances at 28.05 ppm ($ArCOCH_3$) and δ 22.50 (–$NCOCH_3$). All spectral data of metabolite (**4**) were identical to that reported for 2-acetyl-3-(2-acetamidoethyl)-7-hydroxyindole [14].

Biotransformation of harman using the fungus *Cunninghamella elegans* NRRL 2310 resulted in production of metabolite (**5**). The metabolite was isolated using a combination of silica gel and sephadex LH-20 column chromatography. Mass spectrum showed the presence of molecular ion peak at m/z 198 presuming to be corresponding to a molecular formula of $C_{12}H_{10}N_2O$. The presence of peak at m/z 182 (M-16) suggests the presence of N-oxide function. The existence of the fragment ion of m/z 115 precisely revealed that the oxidation happened on ring B or ring A, not ring C [21]. The presence of free hydroxyl group was ruled out because of the lack of hydroxyl bands in the IR spectrum and the absence of exchangeable protons other than NH protons in the $^1$H NMR spectrum. The $^1$H NMR data of metabolite (**5**). showed that the metabolite exhibited the same number and type of protons as the substrate (harman) with the exception of slight shielding of H-3 and H-10 due to the N-Oxide group. $^{13}$C NMR of metabolite (**5**) showed a significant shielding of C-1 (10 ppm), C-

10 (9 ppm), C-9a (5 ppm), C-3 (7 ppm), C-4b (8 ppm) and deshielding of C-8a (6 ppm). Based on the spectral data, metabolite (**5**) was identical to harman -2-oxide. This metabolite was prepared chemically and identified using the superimposed IR spectrum with harman-2-oxide, produced from microbial transformation of harman by *Cunninghamella elegans* NRRL 2310 [14].

Biotransformation of harman using the fungus *Penicillium chrysogeneum* ATCC 10002 resulted in production of metabolite (**6).** The metabolite was isolated using a combination of silica gel and sephadex LH-20 column chromatography. Mass spectrum showed the presence of a molecular ion peak at m/z 198, presumably corresponding to a molecular formula of $C_{12}H_{10}N_2O$. The fragment ion at m/z 131 suggested that the new hydroxyl group had been substituted on ring C [21]. The IR spectrum showed a broad absorption band at 3450 $cm^{-1}$, owing to an introduced free hydroxyl group and C-O stretching vibration band at 1243 $cm^{-1}$ not encountered in the substrate. $^1H$ NMR spectrum showed the presence of new exchangeable proton peak at 6.42 ppm other than NH proton. This information provided initial evidence which suggested hydroxylation of harman at C-6. The $^1H$ NMR data of this metabolite also showed disappearance of the doublet signal of C-6 of harman, due to its hydroxylation, and slight shielding of H-5 and H-7, due to hydroxylation at C-6. $^{13}C$ NMR of the metabolite (**6**) showed significant deshielding of C-6 by 30 ppm to 151 ppm and shielding of C-5 by 15 ppm and C-7 by 12 ppm due to hydroxylation. Based on these spectral data, metabolite (**6**) was identified as 6-hydroxy-harman [14]. This metabolite was obtained for the first time via microbial transformation using *Penicillium chrysogeneum* ATCC 10002.

Biotransformation of harmine using the fungus *Cunninghamella elegans* NRRL 1392 resulted in production of metabolite (**7**). The compound has a weak UV florescence at 254 nm and showed yellow spots in a dark background when sprayed with an anisaldehyde/sulfuric acid reagent. It gave a negative result with Dragendorff's reagent. Based on different spectroscopic methods including IR, mass spectroscopy, $^1H$ and $^{13}C$ NMR spectroscopy, this metabolite was identical to acetamide [19].

Biotransformation of harmine using the fungus *Cunninghamella blackesleeana* NRRL 1369 resulted in formation of three metabolites (**8**-**10**):
Mass spectrum of metabolite (**8**) showed the presence of a molecular ion peak at m/z 228, presumably corresponding to a molecular formula of $C_{13}H_{12}N_2O_2$, which is more than harmine by 16 (possibly OH group). The fragment ion at m/z 213 may be due to loss of $CH_3$ group. The fragment ion at m/z 131 suggested that the new hydroxyl group has been substituted on ring C [21]. The IR spectrum of metabolite (**8**) showed a broad absorption band at 3505 $cm^{-1}$, owing to an introduced free hydroxyl group not encountered in the substrate. The presence of new exchangeable proton peak at δH 6.33 ppm other than NH proton in the $^1H$ NMR spectrum, provided initial evidence which suggested hydroxylation of harmine at C-6. $^{13}C$ NMR of metabolite (**8**) showed a significant deshielding of C-6 by 29 ppm to 138 ppm, and shielding of C-5 by 14 ppm and C-7 by11 ppm due to hydroxylation at C-6. Based on these spectral data, metabolite (**8**) was identified as 6-hydroxy-harmine. This metabolite was obtained for the first time via microbial transformation.

Mass spectrum of metabolite (**9**) showed the presence of a molecular ion peak at m/z 228, presumably corresponding to a molecular formula of $C_{13}H_{12}N_2O_2$. The peak at m/z 211 (M-16) suggests the presence of N-oxide function. The $^1H$ NMR data of metabolite (**9**) showed that the resonances of proton signals were very close to harmine except slight shielding of H-3 and H-10 due to the N-Oxide group. $^{13}C$ NMR of metabolite (**9**) showed a significant shielding of C-1 by 10 ppm, C-10 by 8 ppm, C-9a by 4 ppm, C-3 by 8 ppm, C-4b by 7 ppm and deshielding of C-8a by 10 ppm. Based on these spectral data, metabolite (**9**) was identical to harmine-2-oxide [15].

Mass spectrum of metabolite (**10**) showed the presence of a molecular ion peak at m/z 198, presumably corresponding to a molecular formula of $C_{12}H_{10}N_2O_2$, which is less than harmine by 15 (possibly $CH_3$ group). The fragment ion peak at m/z 155 is due to the loss of CO and $CH_3$ groups. IR spectrum showed the appearance of OH group signal at 3429 $cm^{-1}$, which is not present in the substrate harmine. The $^1H$ NMR data of metabolite (**10**) showed a lack of singlets at 3.88 ppm integrating for three protons (methyl group) which are present in harmine, and appearance of another broad singlet integrating for one proton at 6.34 ppm due to OH group resulting from the demethylation reaction. All these data are consistent with an O-demethylated product and identical to those reported for harmol [15]. Metabolites (**9- 10)** were obtained for the first time via microbial biotransformation using *Cunninghamella blackesleeana* NRRL 1369.

## BIOLOGICAL ACTIVITY

***A-Antioxidant activity:*** Pro- or antioxidant properties of β-carboline derivatives may influence the antioxidant balance. It is therefore necessary to determine if the β-carboline derivatives influence

the number of free radicals or reactive oxygen species [22]. In the present study, the antioxidant activity of the substrates and the different isolated metabolites were investigated using the DPPH radical scavenging method. The ability of β-carboline derivatives to sweep off free radicals was expressed as $IC_{50}$. The results of antioxidant activity (Table 2 and Figure 2) revealed that harman-2-oxide presented strong free radical scavenging activity, which is much potent than quercetin. Harmalol, 6-hydroxyl harmine and 6-hydroxy harman, also showed strong free radical scavenging activity more potent than the positive control ($IC_{50}$= 18±1.2 µg/ml). These results are consistent with previous reports that antioxidant activity is increased by oxidation and hydroxylation reactions [23]. This is the first report concerning the antioxidant activities of the isolated metabolites.

***B- Cytotoxic activity:*** Investigation of the cytotoxic activity of simple β-carboline substrates and their isolated metabolites was carried out. The cytotoxic activity was determined using the MCF7 cell line (breast cancer) and SRB assay method [17]. The results are illustrated in (Table 3 and Figure 3). Harmine, 6-hydroxy-harmine, harman-2-oxide, harmine -2-oxide, harmalol and 6-hydroxy harman showed the highest cytotoxic activity compared to doxorubicin ($IC_{50}$= 0.60 µg/ml), indicating their high efficacy against the tested cell line. However, 2-acetyl-3-(2-acetamidoethyl)-7-methoxyindole and 2-acetyl-3-(2-acetamidoethyl)-7-hydroxyindole presented weak activity. Most of the β-carboline derivatives are endowed with antitumor, and DNA intercalating properties and their high DNA binding affinity is thought to be partially responsible for their pharmacological action. β-carboline structure seems to be an important basis for the design and synthesis of new antitumor drugs [1].

## CONCLUSION

Microbial transformation of some β-carboline alkaloids, using forty three microorganisms, resulted in the isolation and identification of several metabolites, namely: 2-acetyl-3-(2-acetamidoethyl)-7-methoxyindole, acetamide, harmalol, 2-acetyl-3-(2-acetamidoethyl)-7-hydroxyindole, harman-2-oxide, 6-hydroxy harman, 6-hydroxy-harmine, harmine-2-oxide and harmol. Harmalol and 6-hydroxy-harmine were obtained for the first time via microbial transformation in this study, while 6-hydroxy-harman, harmine-2-oxide, and harmol, were obtained for the first time via microbial biotransformation, using *Penicillium chrysogeneum* ATCC 10002 and *Cunninghamella blackesleeana* NRRL 1369. The antioxidant activity of the β-carboline substrates and their isolated metabolites was investigated and the results showed that harmane-2-oxide presented strong free radical scavenging activity, which is much more potent than quercetin. Harmalol, 6-hydroxyl-harmine and 6-hydroxy-harman also showed strong free radical scavenging activity more potent than the positive control. The cytotoxic activity of β-carboline substrates and their isolated metabolites were tested and the results revealed that harmine, 6-hydroxy-harmine, harman-2-oxide, harmine-2-oxide, harmalol and 6-hydroxy-harman showed the highest cytotoxic activity which is comparable to doxorubicin.

Table (1): Screening of harmaline, harmalol, harman and harmine biotransformation with microbial cultures.

| Microorganisms | Screening results | | | |
|---|---|---|---|---|
| | Harmaline | Harmalol | Harman | Harmine |
| *Aspergillus flavipes* ATCC 11013 | — | — | + | |
| *Aspergillus niger* NRRL 328 | — | + | — | |
| *Aspergillus versicolor* | + | + | — | |
| *Cunninghamella blackesleeana* MR 398 | + | + | — | +++ |
| *Cunninghamella blackesleeana* NRRL 1369 | +++ | — | — | |
| *Cunninghamella elegans* NRRL 1392 | — | — | — | ++ |
| *Cunninghamella elegans* NRRL 2310 | + | — | +++ | — |
| *Penicillium chrysogeneum* ATCC 10002 | + | — | +++ | |
| *Penicillium chrysogeneum* ATCC 10002 | + | — | | + |
| *Penicillium vermiculatum* NRRL 1009 | + | — | + | |
| *Rhodotorula rubra* NRRL y1592 | +++ | +++ | — | _ |

- No metabolites formed; + Trace conversion of substrates into metabolites; ++ Moderate conversion of substrates into metabolites; +++ Maximum conversion of substrates into metabolites.

Table (2): DPPH radical scavenging (%)* activities of simple β-carboline substrates and their isolated metabolites.

| Compound \ Conc.(μg/ml) | 100 | 50 | 25 | 12.5 | 6.25 | $IC_{50}$ (μg/ml) |
|---|---|---|---|---|---|---|
| Quercetin | 76.2±2.0 | 76±2.2 | 65.7±1.3 | 30±0.85 | 10±2.23 | 18 |
| Harmaline | 71.01±2.8 | 70.5±2.3 | 64.4±1.3 | 54.2±2.3 | 45.3±1.3 | 10 |
| Harmalol | 74.01±1.9 | 73.3±0.9 | 70.4±2.3 | 52.5±2.2 | 47.0±2.0 | 9 |
| Harman | 61.2±1.2 | 60.3±1.1 | 55.5±0.2 | 51.5±1.1 | 32.1±0.2 | 15 |
| Harmine | 60.00 ±0.9 | 59±1.1 | 55±2.6 | 49±2.0 | 30±1.8 | 14 |
| 2-acetyl-3-(2-acetamidoethyl)-7-methoxyindole | 54±2.0 | 53±1.2 | 45±0.8 | 30.5±1.3 | 26.3±1.5 | 45 |
| 2-acetyl-3-(2-acetamidoethyl)-7-hydroxyindole | 60.5±1.3 | 60.1±1.3 | 59±0.6 | 33.4±0.8 | 18.5±2.1 | 22 |
| Harman-2-oxide | 62.01±2.1 | 61.8±2.2 | 60.4±1.1 | 53.2±1.2 | 42.6±1.9 | 8 |
| 6-hydroxy harman | 64.50±2.3 | 64±1.6 | 63.1±2.4 | 45.3±1.5 | 33.4±2.1 | 12 |
| Acetamide | 61.87±1.9 | 60±0.7 | 58±1.0 | 43.1±2.7 | 40±0.8 | 20 |
| 6-hydroxy Harmine | 63.8±1.3 | 63.9±1.9 | 63±2.2 | 51±2.1 | 33±1.7 | 10 |
| Harmine-2-oxide | 65.9±1.2 | 61.5±0.1 | 61.1±2.5 | 46.9±2.8 | 44.7±0.8 | 20 |
| Harmol | 66.7±2.2 | 63.8±0.9 | 58.8±1.5 | 50.7±2.2 | 46.5±1.5 | 16 |

* Data obtained from triplicate determination (n=3) and shown as mean ± standard deviation (SD).$IC_{50}$ value is defined as the concentration of 50% scavenging of DPPH , it was calculated by linear regression analysis and expressed as Mean ± SD (n=3).

Table (3): Surviving Fraction (%)* of the cytotoxic activities of simple β-carboline substrates and their metabolites against MCF7 cell line**.**

| Compound \ Conc.(μg /ml) | 1 | 2.5 | 5 | 10 | $IC_{50}$ (μg/ml) |
|---|---|---|---|---|---|
| Doxorubicin | 0.35 | 0.35 | 0.33 | 0.34 | 0.60 |
| Harmaline | 0.68 | 0.51 | 0.50 | 0.30 | 2.48 |
| Harmalol | 0.80 | 0.65 | 0.40 | 0.28 | 3.05 |
| Harman | 0.54 | 0.25 | 0.25 | 0.22 | 1.03 |
| Harmine | 0.40 | 0.36 | 0.36 | 0.21 | 0.66 |
| 2-acetyl-3-(2-acetamidoethyl)-7-methoxyindole | 0.99 | 0.60 | 0.38 | 0.28 | 3.30 |
| 2-acetyl-3-(2-acetamidoethyl)-7-hydroxyindole | 0.93 | 0.74 | 0.55 | 0.46 | 5.60 |
| Harman-2-oxide | 0.45 | 0.37 | 0.37 | 0.36 | 0.70 |
| 6-hydroxy harman | 0.40 | 0.42 | 0.42 | 0.40 | 0.80 |
| 6-hydroxy Harmine | 0.40 | 0.34 | 0.34 | 0.33 | 0.64 |
| Harmine-2-oxide | 0.41 | 0.38 | 0.38 | 0.37 | 0.71 |
| Harmol | 0.43 | 0.41 | 0.41 | 0.40 | 0.74 |

*Data obtained from triplicate determinations (n=3) and shown as mean, $IC_{50}$ value is defined as the concentration at 50% Survival of MCF7 cell.

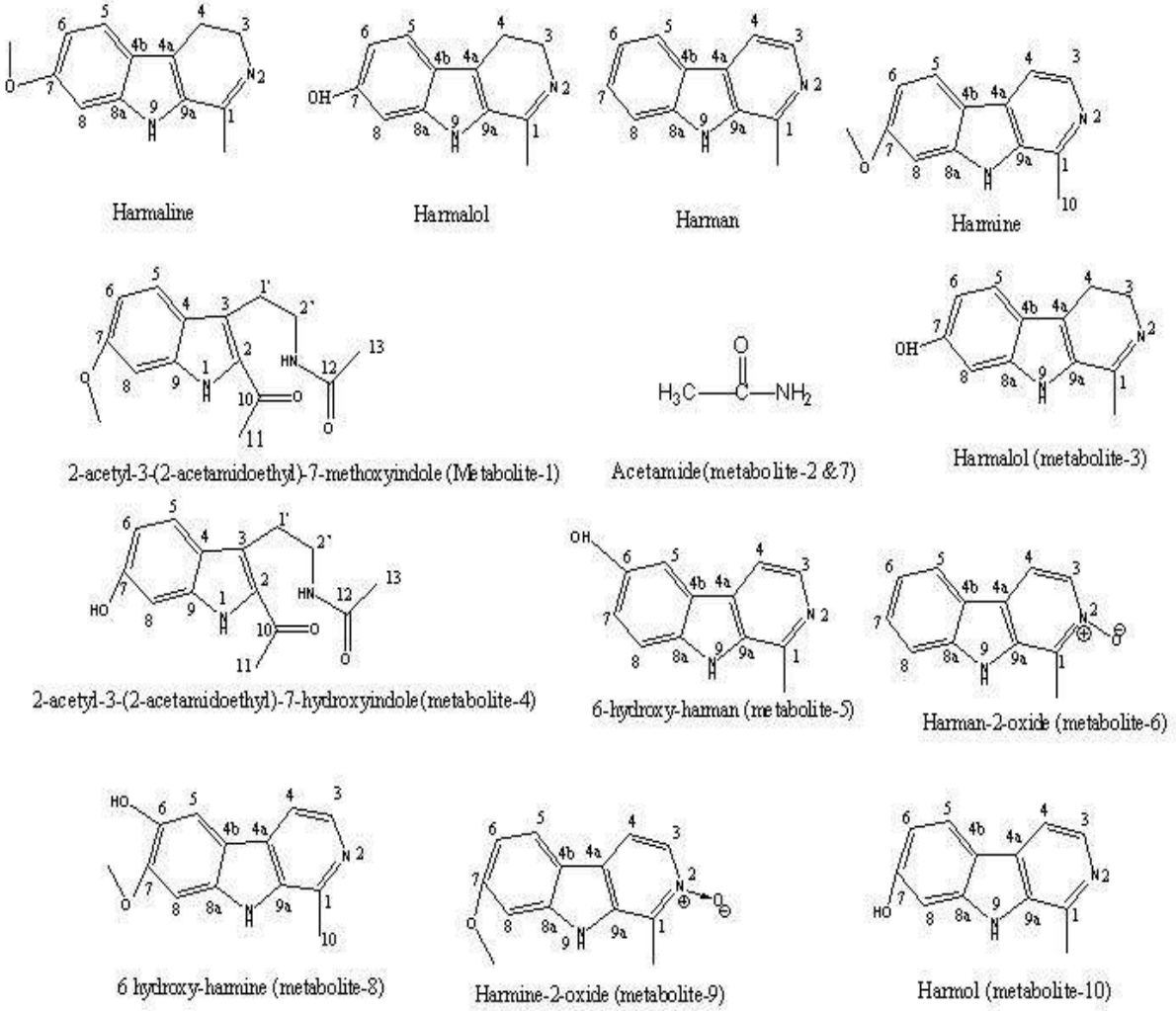


Figure 1: Chemical structures of substrates and major metabolites of β-carboline alkaloid under investigation.

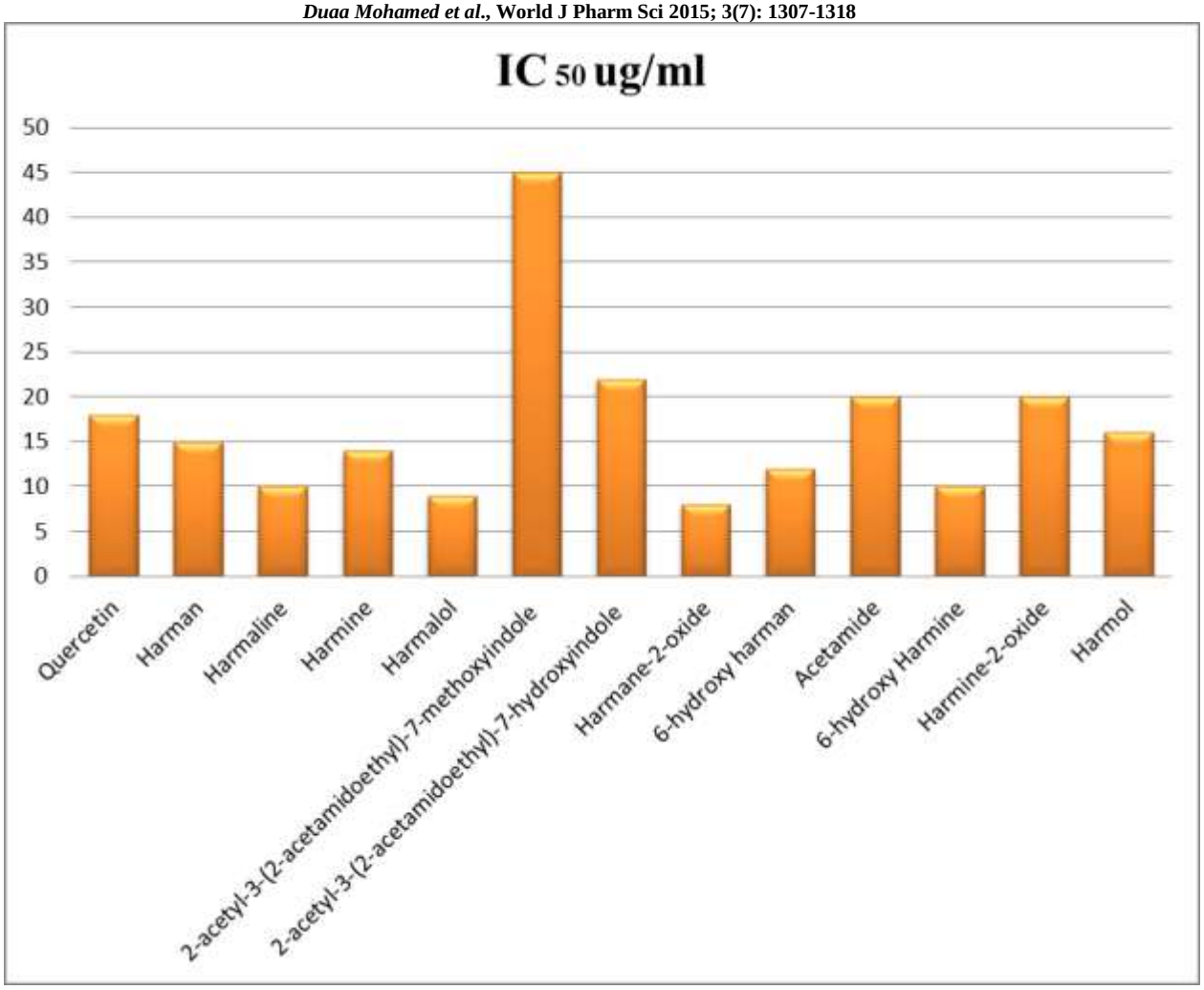


Figure 2: Antioxidant activity of β-carboline substrates and their metabolites.

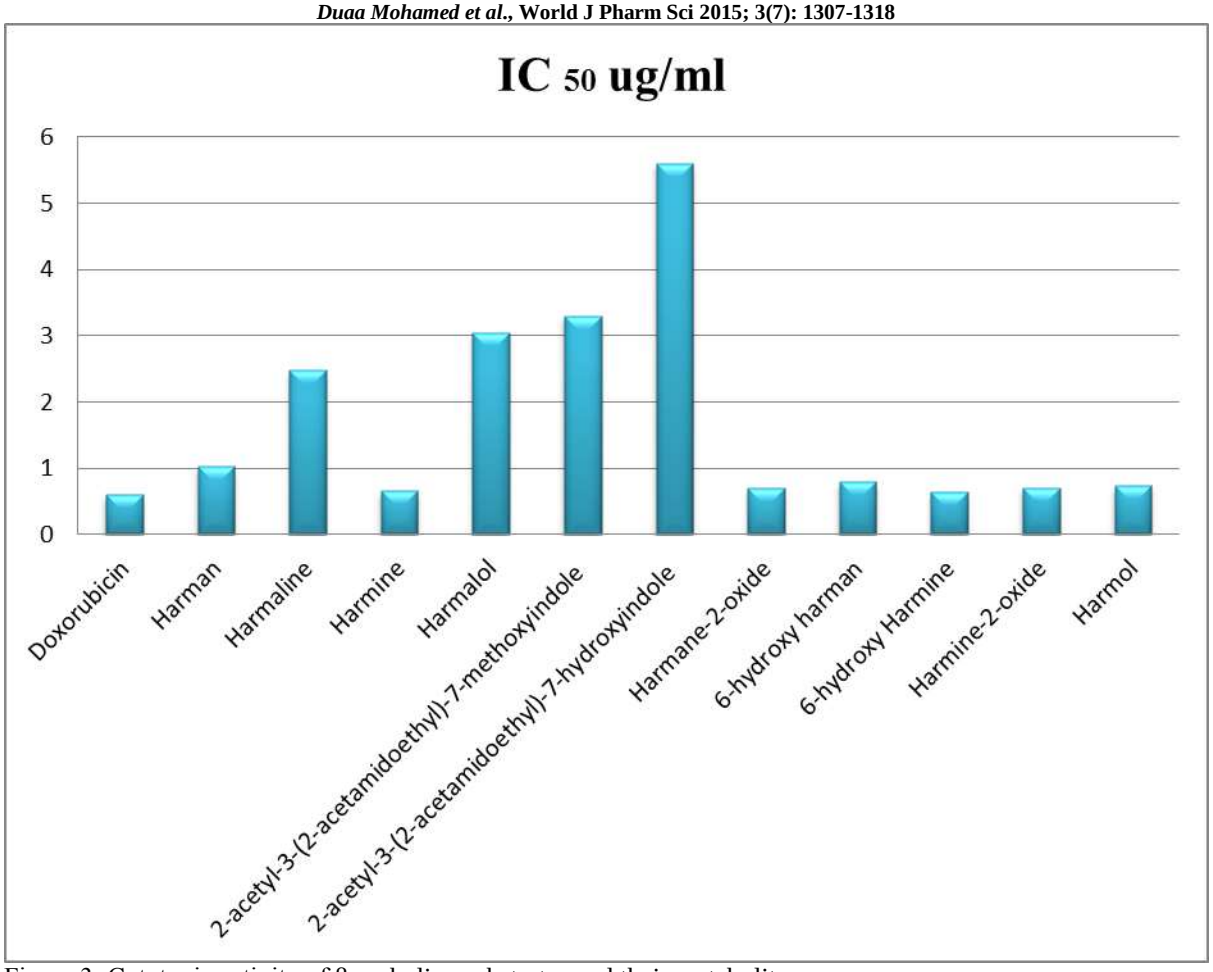


Figure 3: Cytotoxic activity of β-carboline substrates and their metabolites.

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