Ethiopian Journal of Science and Sustainable Development

e-ISSN 2663-3205 *Volume 7 (2), 2020*

Journal Home Page: www.ejssd.astu.edu.et

Research Paper

Molecular Docking Analysis of Antibacterial Indole Alkaloids from Roots of *Brucea Antidysentrica*

Tewabech Alemu, Kebede Shenkute, Rajalakshanan Eswaramoorthy, Solomon Girmay, Yadessa Melaku, Milkyas Endale*

Department of Applied Chemistry, School of Applied Natural Science, Adama Science and Technology University, P. O. Box 1888, Adama, Ethiopia

Article Info

Article History:
Received 8 January 2020
Received in revised form 25 February 2020
Accepted 13 March 2020

Keywords:
Antibacterial activity
Brucea antidysentrica
alkaloids
antioxidant activity
molecular docking

Abstract

Brucea antidysentrica is one of the medicinal plants used traditionally to treat various diseases such as leprosy, wound, diarrhea, fever, eye disease, rabies and tumor/cancer. Phytochemical screening test of dichloromethane/methanol (1:1) and methanol extracts revealed the presence of alkaloids, tannins, flavonoids, steroids and saponins, terpenoids, and phytosterols. Silica gel column chromatographic separation of dichloromethane/methanol (1:1) and acid-base extracts afforded compound **1**, canthin-6-one (**2**) and 1,11-dimethoxycanthin-6-one (**3**). The crude extracts and isolated compounds were screened for *in vitro* antibacterial activity against strains of *Salmonella thphimurium, Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. Canthin-6-one (**2**) and 1,11-dimethoxycanthin-6-one (**3**) exhibited promising antibacterial activity against *E. coli* and *S. thphimurium* (12.6±0.6 and 12.5±0.87 mm zone of inhibition, respectively) compared to ciprofloxacin (27.3±2.52 and 29±1.00 mm, respectively), at 0.5 mg/mL. The radical scavenging activity of dichloromethane/methanol (1:1) and acid-base extract were 83.3 % and 80 %, respectively, whereas alkaloids **1**-**3** displayed activity of 85.3 %, 87.5 % and 78.4 % at 100 µg/mL, respectively, suggesting that canthin-6-one (**2**) displayed promising radical scavenging activity. The molecular docking analysis showed compound **1** and 1,11-dimethoxycanthin-6-one (**3**) were found to show hydrogen bond interaction with active site amino acid residue PHE196 and ALA 51 at a distance of 1.5 A^o and 1.5 A^o, respectively. Hydrophobic interactions were observed between **2** and **3** with VAL201, LYS57, GLY200, ASN198, ALA51, LEU52 and ASN198, LEU52, VAL201, LYS57, respectively, suggesting the compounds may act as potential inhibitors of DNA gyrase enzyme.

1. Introduction

Plants have nutritional value, in the eye of local people, medicinal and ritual or magical values (Abbink, 1995; Pearce and Puroshothaman, 1992; Schippman et al., 2002; Cragg and Newman, 2005). Over the years research on traditional medicine contributed to discovery of several lead drugs (Adhikari et al., 2010; Saiprasana et al., 2012; Ugochukwu et al., 2013). *Brucea antidysentrica* (Simaroubaceae, Figure 1) is a species of flowering shrub/tree growing up to 7 m tall growing widely in tropical Africa-Guinea and Nigeria, East to Ethiopia and south to Angola, Malawi and Zambia, usually at the edge of semi-humid forests at relatively

*Corresponding author, e-mail: milkyas.endale@astu.edu.et

https://doi.org/10.20372/ejssdastu:v7.i2.2020.184

high altitudes (Hedenberg and Edvans, 1989). In Ethiopia, the plant is known by local name Aballo (Amharic) and Qomonyo (Afan Oromo) (Endashaw, 2007). Preparation made from different parts of the plant is used to treat various conditions such as leprosy, wound, diarrhea, fever, eye disease, rabies and tumor/cancer, among others (Fullas, 2001). Previous reports revealed that the genus is rich in quassinoids having wide spectrum of biological activities such as antitumor, antiviral, antiparasitic, herbicidal and antioxidant (Viswanad et al., 2011; Alves et al., 2014). We hereby report isolation, spectroscopic identification, antibacterial, antioxidant evaluation and molecular docking analysis work done on the roots of *B. antidysentrica*.

Figure 1: Picture of *B. antidysentrica* (Picture taken by Tewabech Alemu on Feb. 28, 2019)

2. Materials and Methods

2.1. General

TLC was performed using precoated aluminum backed supported silica gel 60 F254 (0.2 mm thickness) and glass supported silica gel 60 F254 (1.0 mm thickness), respectively. Alkaloids were detected on TLC stained with the Mayer reagent. Column chromatography was carried out using silica gel 60-120 mesh ASTM. The ultraviolet and visible (UV-Vis) spectrum was taken on Spectroscopic Genesys™ 2PC UV-Vis scanning spectrometer. The infrared (IR) data were recorded on a Perkin Elmer model FTIR spectrometer as KBr disks. ^{1}H and ^{13}C NMR data were obtained in $CDCl_3$ on a Bruker Avance 400 MHz.

2.2. Plant material collection and identification

The roots of *B. antidysentrica* were collected from the Amhara region, East Gojam zone, Dejen Woreda 236 km from Addis Ababa on February 28, 2019. The plant was identified by the botanist Shamble Alemu, National Herbarium of Ethiopia, Addis Ababa University (voucher code TA-001/2011). The roots were cut into small pieces, air-dried, and ground into a fine powder.

2.3. Extraction and isolation

Air-dried root powder was weighed (350 g) and extracted exhaustively with dichloromethane/methanol (1:1) for 72 hr at room temperature. The marc left was further extracted with 2 L methanol soaked for 72 hr at room temperature. The mixture was filtered, and concentrated under reduced pressure at 50°C using rotary evaporator to afford 12.77 g (3.65%) and 9.4 g (2.68%) crude extracts, respectively. Roots powdered (350 g) of *B.antidysentrica* was extracted in *n*-hexane (3 L) separately; the solution was filtered and the residue was extracted with ethanol (2 L) at room temperature for 72 hr. Then solvent was evaporated by using a rotary evaporator at 50°C to yield crude ethanol extract. The defatted ethanolic crude extract was suspended in 5% HCl to pH 5, pre-saturate with water and partition by adding chloroform, the extract was separated in to two layers. Then aqua and organic layers were separated by using separatory funnel three times. The acidic aqueous phases was basified by adding 5% NH_3 to pH 11, and partitioned by chloroform. Aqueous and organic layers were separated by using separatory funnel three times. The chloroform extracts were combined, and concentrated under vacuum rotary evaporator to obtain brown crude extract (5.1 g, 1.46%). The crude dichloromethane/methanol (1:1) extract (10 g) was adsorbed on 10 g of silica gel and subjected to silica gel (150 g) column chromatographic separation. Elution was carried out with increasing gradient of ethyl acetate in *n*-hexane. A total of 221 fractions were collected each concentrated under reduced pressure to dryness. Fractions that showed similar R_f values and the same characteristic color on TLC were combined. Fractions 29-31 afforded single spot (compound **1**) on TLC (EtOAc/*n*-hexane, 1:1, R_f value of 0.44, 30 mg), fractions 51-54 which showed single spot (canthin-6-one, **2**) on TLC (EtOAc/*n*-hexane 9:1, R_f value 0.57, 25 mg) and fractions 84-87 afforded the same single spot (1,11-dimethoxycanthin-6-one, **3**) on TLC (EtOAc/*n*-hexane 8:2, R_f value of 0.41, 30 mg).

2.4. Phytochemical screening test

2.4.1. Test for flavonoids: To DCM:MeOH (1:1) and MeOH crude extracts (0.5 g each), 10 mL of ethyl acetate was added and heated for 3 min using steam bath. The mixture was filtered, and the filtrate was mixed with 1 mL of dilute ammonia solution. Formation of intense yellow color ratifies the presence of flavanoids (Sofowora and Debiyi, 1978).

2.4.2. Test for saponins: To DCM/MeOH (1:1) and MeOH crude extracts (0.5 g), 5 mL of distilled water was added and shaken while heating to boil. Frothing showed the presence of saponins (Evans and Trease, 1989).

2.4.3. Test for phenols: To DCM/MeOH (1:1) and MeOH crude extracts (0.5 g), 5 drops of 2 % of $FeCl_3$ were added and formation of bluish green to black color indicates the presence of phenols (Roopashree et al., 2008).

2.4.4. Test for tannins: The crude extracts (DCM: MeOH (1:1) and MeOH extract) (0.5 g each) were boiled in 10 mL of water in a test tube and filtered. To the filtrate, 5 drops of 0.1 % $FeCl_3$ were added to give a brownish green or a blue-black color which confirms the presence of tannins (Ayoola et al., 2008).

2.4.5. Test for terpenoids (Salkowski test): DCM/MeOH (1:1) and MeOH crude extracts (0.5 g) were mixed with 2 mL of chloroform and 3 mL concentrated H_2SO_4 carefully to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids (Ugochukwu et al., 2013).

2.4.6. Test for steroids: To DCM/MeOH (1:1) and MeOH crude extracts (0.5 g), 10 mL of chloroform and 10 mL of concentrated H_2SO_4 were added by sides of the test tube. The upper layer turns red and H_2SO_4 layer showed yellow with green fluorescence (Alhadi et al., 2015) indicating the presence of steroids.

2.4.7. Detection of phytosterols (Salkowski's test): Crude extracts (DCM: MeOH (1:1), MeOH, 0.5 g each) were treated with a few drops of chloroform and filtered. To the filtrate, few drops of concentrated H_2SO_4 was added, shaken, and allowed to stand appearance of the golden yellow color indicates the presence of triterpenes (Roopashree et al., 2008).

2.4.8. Test for glycosides (modified Borntrager's test): Crude extracts (DCM: MeOH (1:1), MeOH, 0.5 g each) were treated with $FeCl_3$ solution and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution and formation of the rose-pink color in the ammonical layer confirms the presence of anthranol glycosides (Roopashree et al., 2008).

2.5. Antibacterial testing

2.5.1. Preparation of discs containing extracts

The same concentrations of 0.5 mg/mL were prepared from the extract, isolated pure compounds and the standard. The sample was applied into sterile agar-disc and dried at 37°C. The agar disc was weighed carefully to confirm the exact amount of the extract and isolated pure compounds being incorporated (compared to preweighed blank discs).

2.5.2. Bacterial culture

Escherichia coli was isolated from stool specimens in the clinic and identified according to routine cultural properties and biochemical tests. Four strains of each were included in the study. A few colonies from the overnight culture of Eosin Methylene Blue (EMB) agar was transferred into approximately 4-5 mL Tripticase soy broth (TSB) medium. The broth was incubated at 37°C for 3-4 hr, and the turbidity of suspension was adjusted to that of 0.5 McFarland barium sulfate standards. The standard suspension was used for both qualitative and quantitative antibacterial assays.

2.5.3. Bacterial susceptibility testing

Standardized inoculums (0.5 mg/mL) were introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums. Sterile agar-disc diffusion previously soaked in a known concentration of extract or pure compound (0.5 mg/mL per disc) was carefully placed at the center of the labeled seeded plate. The same procedure was used for all the MRSA strains used. The plates were incubated aerobically at 37°C and examined for zones of inhibition after 24 hr. The inhibition zones were measured with a ruler and compared with the control disc (disc containing only physiological saline). Strains of human pathogen microorganisms used in this study were as follows: two Gram-negative bacteria, *Escherichia coli*, *Salmonella thphimurium* and two Gram-positive bacteria *Staphylococcus aureus* and

Bacillus subtilis. The bacterial stock cultures were incubated for 24 hr at 37°C on nutrient agar medium (Adama Science and Technology University, Department of Applied Biology, Adama). The bacterial strains were grown in the Mueller–Hinton agar (MHA) plates at 37°C. The agar was melted (50°C), and the microorganism cultures were then added aseptically to the agar medium at 45°C in plates and poured into sterile petri dishes to give a solid plate. All these experiments were performed in triplicate. The plates were incubated for 24-48 hr at 37°C for bacteria. The inhibition zones produced by the plant extracts were compared with the inhibition zones produced by commercial standard antibiotics (ciprofloxacin). One dilution (0.5 mg/mL) of *B. antidysentrica* extract, pure compound, and standard drugs were prepared in DMSO using nutrient agar tubes. Mueller–Hinton sterile agar plates were seeded with indicator bacterial strains ($1.3 * 10^8$ cfu/mL) and allowed to stay at 37°C for 3 hr. Control experiments were carried out under similar conditions by using ciprofloxacin for antibacterial activity as a standard drug. The zones of growth inhibition around the disks were measured after 24 hr of incubation at 37°C for bacteria. The sensitivities of the microorganism species to the plant extract and isolated pure compounds were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <6mm were considered as not active against microorganisms. DMSO used as negative control during the whole test on bacteria. The results are calculated as averages of triplicate tests (Murai *et al*., 1995).

2.6. Antioxidant activity

The dichloromethane/methanol (1:1) extract was dissolved in four vials containing methanol to give 500, 250, 125 and 62 µg/mL. To each 1 mL of the above extracts, 4 mL of 0.04% DPPH was added and four vials with concentration of 100, 50, 25 and 12 µg/mL were prepared. The resulting solution was placed in an oven at 37ºC for 30 min and subjected to UV-Vis spectrophotometer to record absorbance at 517 nm. This was repeated for the acid-base extracts and isolated compounds. The percentage DPPH inhibition was calculated according to equation 1 (Maksyutina, 1971).

$$\%\ of\ radical\ scavenging\ activity = ABstandard - \frac{ABanalyte}{ABstandard} * 100 \quad (1)$$

2.7. Molecular docking study of isolated compounds

In this study, isolated compounds were subjected to molecular docking studies using the ADT version 1.5.2 and Auto Dock version 4.2 docking program to investigate the potential binding mode. The crystal structure of the enzyme (PHE196 and ALA51) with resolution 2.3 Å was chosen as the protein model. The structures of ligands were optimized using the HyperChem 7.0 software. Auto Dock version 4.2 was used to prepare the molecules and parameters before submitting it for docking analysis with Auto Dock. Polar hydrogen atoms were added while non-polar hydrogen atoms were merged and then, Gasteiger partial atomic charges were assigned to the ligands. All rotatable bonds of ligands, defined by default of the program, was allowed to rotate during the automated docking process and then prepared protein and ligand structures were saved in the PDBQT format suitable for calculating energy grid maps. A grid box size of 46×46×46 Å points with a grid spacing of 0.375 Å was considered. Lamarckian genetic algorithm (LGA) program with an adaptive whole method search in the Auto Dock was chosen to calculate the different ligand conformers. After 200 independent docking runs for each ligand, a cluster analysis was done. In according to the root mean square deviation (RMSD) tolerance of 2.0 Å conformations was clustered and ranked by energy of which the conformation with the best scored pose with the lowest binding energy was selected for these ligands (Mansourian et al., 2015; Morris et al., 1998).

3. Results and Discussions

Extraction of the roots of *B.antidysentrica* by acid-base extraction approach, dichloromethan/methanol (1:1), and methanol solvents afforded 5.1 g (1.46%), 12.77 g (3.65%), 9.4 g (2.68%) yields, respectively.

3.1. Phytochemical screening

Phytochemical screening test of dichloromethane/ methanol (1: 1) and methanol roots extracts revealed the presence of alkaloids, flavonoids, phytosterols, phenols, steroids, tannins, terpenoids, glycosides and saponins (Table 1). The findings of this test are in good agreement with the type of secondary metabolites reported earlier from the genus (Narihiko et al., 1986; Blech and Budzikiewicz, 1994; Ouyang et al., 1994) and these compounds might be responsible for the traditional medicinal use of the genus.

Table 1: Phytochemical screening tests of crude extracts

Phytochemical screening	Test	DCM:MeOH (1:1)	MeOH 100%
Flavonoids	Alkaline test	+	+
Saponins	Froth test	+	+
Phenols	Ferric chloride test	+	+
Tannins	Gelatin test	+	+
Terpenoids	Salkowski's test	+	+
Steroids		+	+
Phytosterols	Salkowski's test	+	+
Glycosides	Modified Borntrager'stest	+	+
Alkaloids		+	+

3.2. Characterization of compounds

Compound **1** was obtained as a brown solid (melting point: 155°C) with an R_f value of 0.44 (*n*-hexane/EtOAc (1:1) as eluent). A positive mayer's test was observed suggesting that the compound was an alkaloid. The IR (KBr disk) spectrum showed broad and sharp vibrations at 3441 and 1744 cm^{-1} attributed to hydroxyl (OH) and carbonyl moiety, respectively. The absorption bands at 1637, 1462 and 1230 cm^{-1} suggest the presence of aromatic system (C=C), C=N and C-O stretching vibrations, respectively. The presence of symmetric and asymmetric C-H stretching are clearly evident from vibrations at 2854 and 2927 cm^{-1}, respectively.

The ^{1}H NMR (400 MHz, $CDCl_3$, Table 2) spectrum revealed the presence of proton signals at δ_H 8.82 (*d*, H-13, *d, J* = 12 Hz), 7.98 (*d,* H-1, *J* = 4 Hz), 8.66 (*d*, H-4, *J* = 12 Hz), 7.00(*d*, H-4', *J* = 8), 8.09 (*d*, H-3', J = 8 Hz), 7.55 (H-6, *t*) and 7.72 (H-7, *t*). The presence of doublet of doublet peak was observed at δ_H 8.09 (H-3') suggest allylic and ortho coupled with δ_H 7.98 (H-1) and δ_H 7.00 (H-4'). The ^{13}C NMR and DEPT-135 spectra revealed a total of 20 well resolved carbon peaks. The presence of eleven aromatic carbon groups, one methylene (C-6'), one methyl and seven quaternary carbon atoms (of which four of them are aromatic carbon) are all evident. The existence of carboxylic acid carbonyl carbon at δc173.5 (C-9) and sp^2 oxygenated quaternary carbon at δc 159.5 (C-2') are clearly marked (Table 2). The peaks at δc 67.8 and 62.3 suggest oxygenated methylene and methyl signals forming CH_2-O-CH_3 moiety, also supported by DEPT-135 spectrum, where the former peak is pointing down whereas the later pointing up. Peaks at δc 139.2 and 145.5 suggest olefinic methine near to heteroatom, preferably next to nitrogen, supported by DEPT-135 spectrum. The above spectral data and comparison with literature revealed that the compound is indole alkaloid derivative of flazin methyl ether (**1**, Figure 2) previously isolated from juice of *Ribes nigrum* L. (Blech and Budzikiewicz, 1994). However, this is the first report of the compound from roots of *B. antidysentrica*.

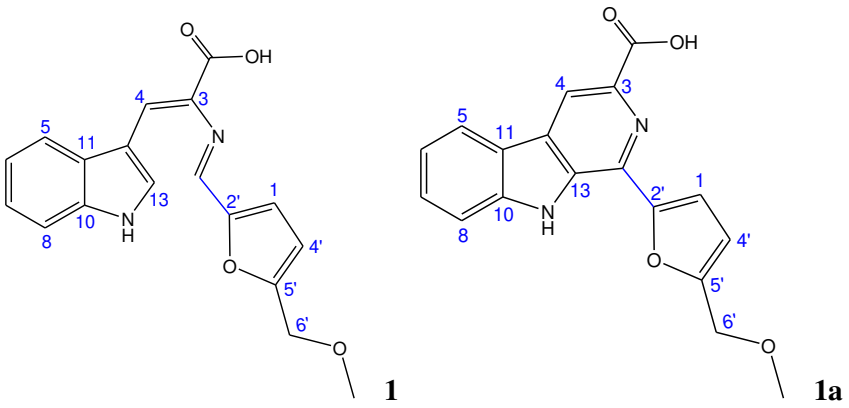


Figure 2: Structure of compound **1** and flazin methyl ether (**1a**)

Table 2: ^{1}H NMR, ^{13}C NMR and DEPT-135 spectral data of compound **1** in $CDCl_3$.

Position	Compound **1**			Flazin methyl ether (Blech and Budzikiewicz, 1994)	
	^{1}H (δ in ppm and J in Hz)	**^{13}C**	**DEPT-135**	**^{1}H** (δ in ppm and J in Hz)	**^{13}C**
1	7.98 (1H, *d*, *J*=4)	145.5	145.5	-	137.1
3	-	130.7	-	-	132.2
4	8.67 (1H, *d*, *J*= 4)	117.3	117.3	8.85 (1H, *s*)	115.9
5	7.98 (1H, *d, J*=8)	127.9	127.9	8.41 (1H, *d, J*=7.8)	122.0
6	7.55 (*t*)	128.1	128.1	7.34 (1H,*dd,J*=7.8/8.2)	120.5
7	7.72 (*t*)	129.1	129.1	7.65 (1H,*dd,J*=7.8/8.2)	128.9
8	7.00 (1H,*d*, *J*=8)	116.5	116.5	7.82 (1H, *d, J*=8.2)	112.9
10	-	139.6	-	-	141.5
11	-	124.3	-	-	120.9
12	-	127.10	-	-	129.9
13	8.82(1H, *d, J*=4)	139.2	139.2	-	132.0
2’		159.5	-	-	151.7
3’	8.09 (1H, *dd, J*=8, 4)	116.5	116.5	7.45 (1H, *d, J*=3.4)	111.1
4’	7.00 (1H, *d, J*=8)	116.5	116.5	6.77 (1H, *dd, J*=3.4)	111.9
5’	-	159.5	-		153
CO	-	173.5	-		166.5
CH_2	3.96 (2H, *s*)	68.7	68.7	4.64 (2H, *s*)	65.5
CH_3	3.31(3H,*s*)	62.3	62.3	3.34(3H, *s*)	57.2

Compound **2** was isolated as yellowish amorphous solid with mpt of 149°C and R_f value of 0.57 (EtOAc/*n*-hexane (4:1) as eluent). A positive mayer’s test was observed suggesting that the compound was an alkaloid. The IR (KBR) spectrum of the compound showed a broad vibration at 3451 cm^{-1}, sharp vibrations at 2924 cm^{-1} and 2854 cm^{-1}, 1637 cm^{-1}, and 1400 cm^{-1} attributed to amine group (N-H), sp^3 H-C, sp^2 C-H, imine bond C=N and C=C bonds, respectively. The ^{1}H NMR spectrum showed peaks at δ_H 8.07 (H-4, *d*, *J* = 7.8 Hz), 7.54 (H-10, *t*, *J* = 8 Hz), 7.72 (H-9, *t, J* = 8 Hz) and 8.82 (H-2, *d, J* = 8 Hz), 7.98 (H-1, *d, J* = 8 Hz), 7.00 (H-5, *d, J* = 8 Hz) and 8.11 (H-11, *d, J* = 8 Hz). The ^{13}C NMR and DEPT-135 spectra of showed the presence of fourteen carbon signals including aromatic peaks between δ_C 116.5-159.5, of which six of them are sp^2 quaternary at δ_C 159.5 (C-6), 139.5 (C-13), 136,09 (C-16), 132.06 (C-15) , 130.5 (C-14) and 124.36 (C-12). Upfeild chemical shift of carbonyl carbon at δ_C 159.5 (C-6) suggest that the carbonyl group is directly linked to nitrogen of indole moiety and it is also α,β-conjugated (δ_C 139.4 (C-4) and 129.0, C-5) suggesting that the compound have β-carboline alkaloid canthine-6-one skeleton.

The COSY spectrum supported correlations between H1↔H2, H4↔H5, H8↔H9, H9↔H10 and H10↔H11 (Table 3). The HSQC spectrum suggested direct connectivity between H1→C1, H2→C2, H4→C4, H5→C5, H8→C8, H9→C9, H10→C10, and H11→C11 (Table 3). The HMBC spectrum showed correlations between H1→C2,9,12; H2→C1,15,16; H4→C6,9; H5→C-6,16; H8→C12; H9→C11,13; H10→C8,12 and H11→C-3,15 (Table 3) in good agreement with canthine-6-one alkaloids. Thus, based on the above spectral data and comparison with literature (Ouyang et al., 1994), compound **2** was suggested to be identical with canthin-6-one (Figure 3) previously isolated from roots of *Brucea mollis* (Ouyang et al., 1994).

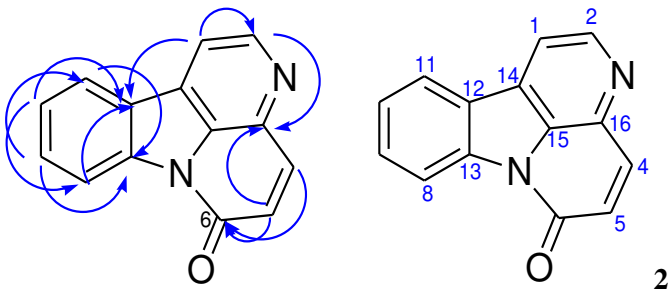
Compound **3** was isolated as yellowish amorphous solid with melting point of 180°C and R_f value of 0.41 (EtOAc/*n*-hexane (4:1) as eluent). The IR spectrum of compound showed the presence of amine group (N-H) at 3400 cm^{-1}, C-H band at 2924 cm^{-1}, C=O band at 1744 cm^{-1}, C=C and C=N at 1644 cm^{-1} and C-N at 1398 cm^{-1}. The ^{1}H NMR spectrum (Table 4) revealed the presence of methoxy protons as singlet at δ_H 4.08 (3H) and 4.23 (3H), and aromatic protons at δ_H 8.5 (*s*, H-1), 8.38 (*d*, J

= 8 Hz, H-8), 7.97(*d*, *J* = 8 Hz, H-4), 7.65 (*t*, *J* = 8 Hz, H-9), 7.01(*d*, *J* = 8 Hz, H-5) and 6.86 (*d*, *J* = 12 Hz, H-10). The ^{13}C NMR and DEPT-135 spectrum (Table 4) showed two methoxy carbon atom signals appearing at δ_C 57.7 and 56.2. The presences of eleven aromatic peaks were observed at δ_C 160.4, 155.9, 151.3, 140.1, 139.2, 132.1, 132.9, 131.6, 130.5, 113.1, 109.8 and 107.9.

In agreement with the spectral data of canthine-6-one (**2**), up-feild chemical shift of carbonyl carbon at δ_C 160.4 (C-6) suggests that the carbonyl group is directly linked to nitrogen of indole moiety and it is also α,β-conjugated (δ_C 139.15 (C-4) and 125.1 C-5) suggesting that the compound have *β*-carboline alkaloid canthine-6-one skeleton. Close inspection of the 2D NMR spectra (Table 4) showed the following correlations. COSY spectrum showed correlations between aromatic protons appearing at δ_H 7.97 (H-4) and δ_H 6.86 (H-5), δ_H 8.38 (H-8) and δ_H 7.65 (H-9), δ_H 7.65 (H-9) and δ_H 7.01 (H-10). HSQC spectrum showed ^{1}J correlations between aromatic protons and their respective carbons H2→C2, H4→C4, H5→C5, H8→C8, H9→C9, H10→C10, and methoxy groups (H17→C17 and H18→C18). HMBC spectrum (Table 4) showed correlations between H2→C1,11,14; H5→C6,9,16; H8→C10,12; H10→C11,8,12. The HMBC spectrum also indicated correlations of the methoxy protons at δH 4.23 (H-17) and 4.08 (H-18) with C-11 and C-1, respectively, confirming their location at C-1, 11 of the canthine-6-one skeleton. Thus, based on

Table 3: ^{1}H, ^{13}C, DEPT-135, COSY, HSQC and HMBC spectral data of canthin-6-one (**2**, Figure 3)

Position	Compound **2**						Canthin-6-one (Ouyang et al., 1994)	
	^{1}H (δ in ppm and J in Hz)	^{13}C	DEPT-135	COSY	HSQC	HMBC	^{1}H (δ in ppm and J in Hz)	^{13}C
1	7.98 (1H, *d, J*=8)	116.4	116.5	$H_1 \leftrightarrow H_2$	$H_1 \rightarrow C_1$	$H_1 \rightarrow C_{2,12,15}$	7.59(1H, *d, J*=5)	115.4
2	8.82 (1H, *d, J*=5)	145.7	145. 7		$H_2 \rightarrow C_2$	$H_2 \rightarrow C_{1, 14,16}$	8.58(1H, *d, J*=5)	144.8
4	8.07 (1H, *d, J*=8)	139.4	139.4	$H_4 \leftrightarrow H_5$	$H_4 \rightarrow C_4$	$H_4 \rightarrow C_{6,15,16}$	7.77(1H, *d, J*=9.7)	138.6
5	7.00 (1H, *d, J*=8)	129.0	129.0		$H_5 \rightarrow C_5$	$H_5 \rightarrow C_{6,16}$	6.75(1H, *d, J*=9.7)	127.9
6	-	159.5						158.2
8	8.69 (1H, *dd, J*=8,4)	117.3	117.3	$H_8 \leftrightarrow H_9$	$H_8 \rightarrow C_8$	$H_8 \rightarrow C_{12}$	8.28(1H, *d, J*=7.7)	116.3
9	7.72 (1H, *t*)	130.9	130.9	$H_9 \leftrightarrow H_{10}$	$H_9 \rightarrow C_9$	$H_9 \rightarrow C_{13}$	7.45(1H, *t*)	129.8
10	7.54 (1H, *t*)	125.7	125.7	$H_{10} \leftrightarrow H_{11}$	$H_{10} \rightarrow C_{10}$	$H_{10} \rightarrow C_{8,12}$	7.28(lH, *t*)	124.7
11	8.11 (1H, *dd, J*=8,4)	122.7	122.7		$H_{11} \rightarrow C_{11}$	$H_{11} \rightarrow C_{13,14}$	7.73(1H, *d, J*=7.7)	121.6
12	-	124.4						123.3
13	-	139.5						138.2
14	-	130.5						128.9
15	-	132.1						130.9
16	-	136.1						135.2

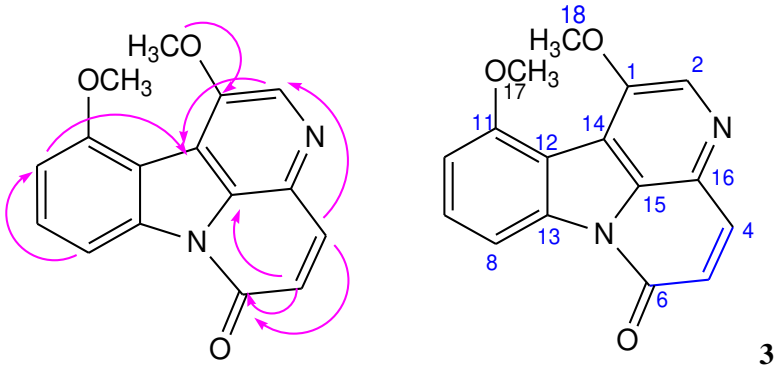


2

Figure 3: Structure of canthine-6-one (**2**)

Table 4: ^{1}H, ^{13}C, DEPT-135, COSY, HSQC and HMBC NMR spectral data of 1,11-dimethoxychan tin-6-one (**3**).

Position	Compound **3**			COSY	HSQC	HMBC	1,11-dimethoxychantin-6-one (Narihiko et al., 1986)	
	^{1}H (δ in ppm and J in Hz)	^{13}C	DEPT-135				^{1}H (δ in ppm and J in Hz)	^{13}C
1	-	155.9	-				-	155.6
2	8.5 (1H, *s*)	132.1	132.1		$H_2 \rightarrow C_2$	$H_2 \rightarrow C_{1,9,11,14}$	8.41 (1H, *s*)	131.3
4	7.97(1H,*d*,*J*=8)	139.15	-	$H_4 \leftrightarrow H_5$	$H_4 \rightarrow C_4$		7.91(1H,*d*,*J*=10)	139.0
5	6.86(1H,*d*,*J*=8)	131.6	125.1		$H_5 \rightarrow C_5$	$H_5 \rightarrow C_{6,16}$	6.79(1H,*d*,*J*=10)	131.8
6	-	160.4	-				-	160.2
8	8.38(1H,*dd*,*J*=8,4)	109.8	109.8	$H_8 \leftrightarrow H_9$	$H_8 \rightarrow C_8$	$H_8 \rightarrow C_{10,12}$	8.29(1H,*d*,*J*=8)	109.5
9	7.65(1H,*t*, *J*=8)	125.1	131.6	$H_9 \leftrightarrow H_{10}$	$H_9 \rightarrow C_9$	$H_9 \rightarrow C_{11,13}$	7.54(1H,*t*, *J*=8)	124.8
10	7.01(1H,*dd*, *J*=8,4)	107.9	107.9		$H_{10} \rightarrow C_{10}$	$H_{10} \rightarrow C_{12,8}$	6.86(1H,*d*,*J*=8)	107.5
11	-	151.3	-				-	151.0
12	-	113.1	-				-	116.6
13	-	132.9					-	132.6
14	-	113.1	-				-	112.8
15	-	130.5	-				-	130.2
16	-	140.1			$H_2 \rightarrow C_2$	$H_2 \rightarrow C_{1,14,16}$	-	139.8
OCH_3	4.23(3H,s)	57.7	57.7			$H_{17} \rightarrow C_{11}$	4.16(3H,s)	57.4
OCH_3	4.08(3H,s)	56.2	56.2			$H_{18} \rightarrow C_1$	4.0(3H,s)	56.0



3

Figure 4: Structure of 1,11-dimethoxycanthin-6-one (**3**)

the above spectral data and extensive comparison with literature, the compound was identified as 1,11-dimethoxycanthin-6-one (**3**, Figure 4), previously reported from stem of *B. antidysentrica* (Narihiko *et a*l., 1986).

3.3. Antibacterial activity

The antibacterial activity of the crude extracts and alkaloids (**1**-**3**) were examined at a concentration of 0.5 mg/mL against four pathogenic bacterial strains two Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) and two Gram-negative (*Escherichia coli* and *Salmonella thphimurium*). Antibacterial potential of crude extracts and alkaloids (**1**-**3**) were assessed in terms of zone of inhibition of bacterial growth (Table 5). The results revealed that the isolated compounds showed promising antibacterial activity against *S. aureus, B. subtilis, E. coli* and *S. thphimurium.* Canthine-6-one (**2**) showed medium activity (12.66±0.6 and 12.33±2.31) against *E. coli* and *S. thphimurium*, respectively, compared to ciprofloxacin (27.3±2.52 and 29±1.00). 1,11-dimethoxycanthin-6-one (**3**) showed promising antibacterial activity (12.5±0.87 and 12.3±1.65 mm) against *S. thphimurium* and *B. subtilis* compared to ciprofloxacin (29±1.00 and 33.3±3.22 mm). The DCM:MeOH (1:1) crude, acid-base crude extract and compound (**1**) showed moderate antibacterial activity against *S. aureus*, *E. coil, S. thphimurium* and *B. subtilis*, respectively.

3.4. Antioxidant activity

In the DPPH scavenging assay, crude extracts and alkaloids (**1**-**3**) were investigated through the free radical scavenging activity via their reaction with the stable DPPH radicals. The reduction of the DPPH was followed via the decrease in absorbance at 517 nm. The DPPH radical scavenging activities (in %) of extracts and isolated compounds were found to be 87.5 (canthine-6-one, **2**), 85.3 (compound **1**), 83.3 (DCM:Methanol (1:1) crude extract), 80.00 (acid-base crude extract), 78.4 (1,11-dimethoxycanthin-6-one, **3**) at 100 µg/ml (Table 6). It was observed that the DPPH scavenging activity increased with increasing concentration of the samples. For the various concentrations, canthine-6-one (**2**) exhibited the highest percent inhibition of the DPPH compared to ascorbic acid which showed maximum scavenging effect at very low concentration (Figure 5).

Table 5: Zone of bacteria growth inhibition diameter (mm)

Sample (0.5 mg/mL)	Inhibition diameter (mm) ±SD			
	E. coli	*S. thphimurium*	*B. subtilis*	*S. aureus*
Methanol/dichloromethane (1:1) extract	11.6±1.53	10.66± 1.15	11.33±1.15	9.33±0.58
Acid-base extract	11.33±2.08	11.0±1.73	12.0±3.74	11.33±0.58
Derivative of flazin methyl ether (**1**)	11.33±0.58	11.66±3.06	11.33±1.53	11.0±1.00
Canthin-6-one (**2**)	12.66±0.60	12.33±2.31	11.0±0.707	9.6±1.16
1,11-dimethoxycanthin-6-one (**3**)	11.3±0.58	12.5±0.87	12.3±1.65	10±1.00
Ciprofloxacin	27.3±2.52	29±1.00	33.6±3.22	23.5±0.58

SD- Standard deviation

Table 6: % of scavenging activity of the extracts and isolated compounds of *B. antidysentrica*

C (µg/mL)	Samples									
	DCM:MeOH(1:1) extract		Acid-base extract		**1**		**2**		**3**	
	A	% scavenging activity	A	% scavenging activity	A	% scavenging activity	A	% scavenging activity	A	% scavenging activity
100	0.2	83.3	0.26	80	0.15	85.3	0.1	87.5	0.28	78.4
50	0.3	75	0.27	79.2	0.23	77.4	0.2	83.3	0.31	76.1
25	0.35	70.8	0.3	76.9	0.3	70.5	0.3	75	0.34	73.8
12	0.4	66.6	0.4	69.2	0.4	60.7	0.38	68.3	0.45	65.4

A = Absorbance

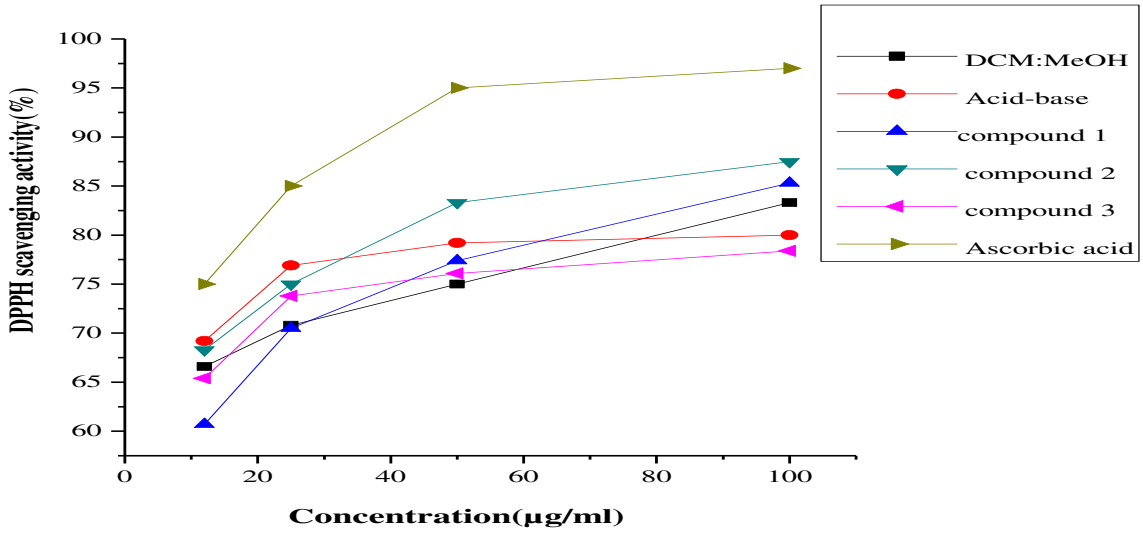


Figure 5: DPPH scavenging activity (%) of *B. antidysentrica* extracts, isolated compounds and positive reference (L-Ascorbic acid).

Table 7: Docking score of DNA gyrase protein and alkaloids (**1**-**3**).

Mode	Compound **1**			Canthin-6-one (**2**)			1,11-dimethoxycanthin-6-one (**3**)		
	Affinity (Kcal/mol)	dist from best mode		Affinity (Kcal/mol)	dist from best mode		Affinity (Kcal/mol)	dist from best mode	
		rmsd i.b.	rmsd u.b.		rmsd i.b.	rmsd u.b.		rmsd i.b.	rmsd u.b.
1	-5.8	0.000	0.000	-5.7	0.000	0.000	-5.7	0.000	0.000
2	-5.5	8.225	10.733	-5.7	22.422	25.040	-5.3	1.873	4.233
3	-5.5	1.802	5.669	-5.6	1.130	3.936	5.2	2.391	4.508
4	-5.4	8.135	11.181	-5.5	12.175	13.368	-5.0	15.021	16.977
5	-5.3	2.110	3.485	-5.3	22.536	25.215	-5.0	15.786	17.759
6	-5.3	7.920	11.682	-5.2	14.292	15.937	-4.9	16.498	18.132
7	-5.3	23.777	26.168	-5.2	14.977	16.780	-4.8	16.396	18.263
8	-5.1	7.742	10.427	-5.2	14.559	0.000	-4.8	13.336	15.474
9	-5.1	8.762	11.879	-5.1	14.289	25.040	-4.8	14.126	16.244

3.5. Molecular docking

In association with *in vitro* antibacterial activity, molecular docking studies were performed for three alkaloids in order to predict the orientation and binding affinity of ligand molecules at the active site of receptor with bacterial enzyme DNA gyrase. In docking study, all the compounds were found to have minimum binding energy ranging from -5.8 to -4.8 KJ/mol (Table 7). On comparative basis alkaloids (**1-3)** revealed minimum binding energy of -5.8, -5.7 and -5.7 kcal/mol, respectively (Table 7). These molecules were unwrapped by active site amino acid residues at the active site pocket region (as shown in Figure 6a-c). The protein (DNA-gyrase) comprises of fourteen active site residues, which are promiscuous to the ligands. Out of which only two (PHE198 and ALA 51) residue is directly interacting with the ligands. The other residues in close proximity to the inhibitor are hydrophobic in nature. "Compound (1) and 1,11-dimethoxycanthin-6-one (3) were found to show hydrogen bond interaction with active site amino acid residues PHE196 and ALA 51 at a distance of (1.5Ao), respectively.. The yellow dashed line indicates a possible hydrogen bond formed between the connections residues (Figure 6a-c) and red color also indicated the hydrogen bond interaction (Figure 7a-c). Canthin-6-one (**2**) had no hydrogen bond interaction with the protein. Compound (**1**) showed two hydrophobic interactions with TRY 218 and GLU 219, Canthin-6-one (**2**) showed six hydrophobic interactions with amino acid residues VAL201, LEU 52 LYS57, GLY200 ASN198 and ALA52 and 1,11-dimethoxycanthin-6-one (**3**) showed four hydrophobic interactions with ASN 198, LEU52, VAL201 and LYS57. All ligands displayed no pi to pi and pi to cation interactions with the protein. DNA gyrase is an essential bacterial enzyme that catalyzes the introduction of negative (-) supercoils into chromosomal and plasmid DNA. Gyrase was discovered soon after it was clear that in vitro recombination of bacteriophage λ required a negatively supercoiled DNA substrate. DNA gyrase cleave and relegate DNA to regulate DNA topology and are a major class of antibacterial drug targets (Reece and Maxwell, 1991). The docking of alkaloids (**1**-**3**) with DNA gyrase showed higher binding affinity as well as hydrogen bonding and good hydrophobic interaction with the receptor. Thus, from this study it can be concluded that the compounds **1**-**3** may act as potential inhibitors by hindering the function of DNA gyrase enzyme.

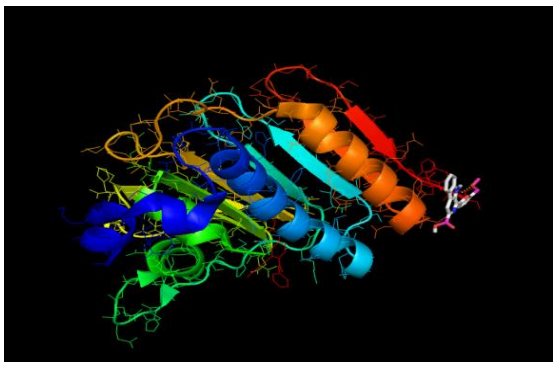


Figure 6a: Molecular docking of compound **1** with DNA gyrase receptor.

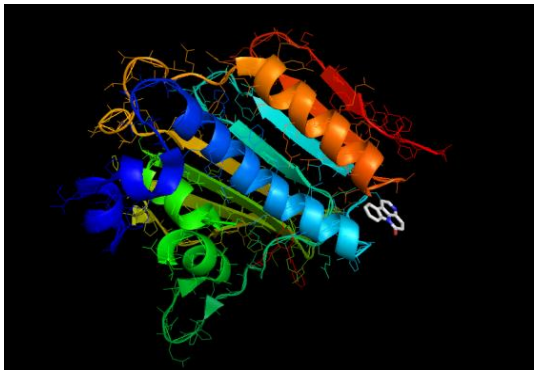


Figure 6b: Molecular docking of canthin-6-one (**2**) with DNA gyrase receptor.

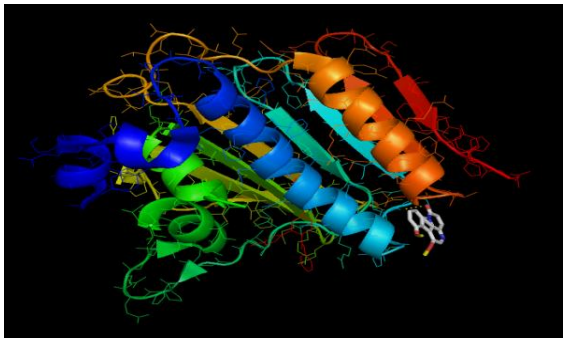


Figure 6c: Molecular docking of 1,11-dimethoxycanthin-6-one (**3**) with DNA gyrase receptor.

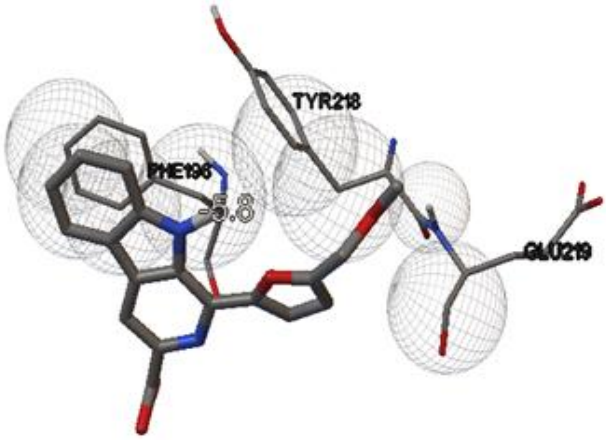


Figure 7a: Molecular docking of compound **1** with hydrophobic and hydrogen bond interactions.

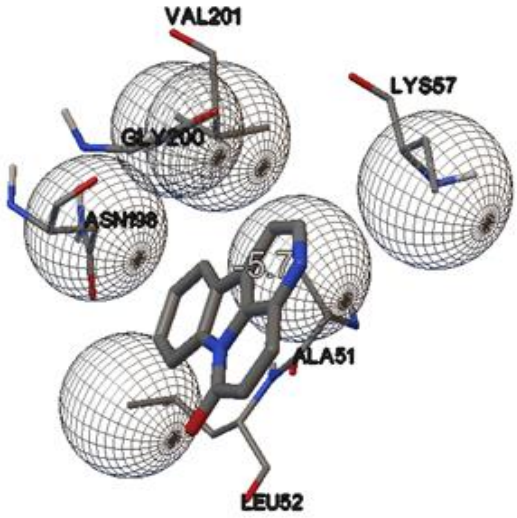


Figure 7b: Molecular docking of canthin-6-one (**2**) with hydrophobic and hydrogen bond interactions.

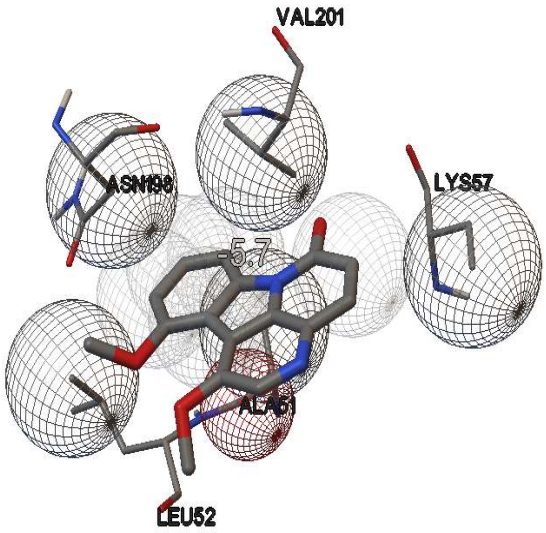


Figure 7c: Molecular docking of 1,11-dimethoxycanthin-6-one (**3**) with hydrophobic and hydrogen bond interactions.

4. Conclusion

For decades traditional medicines have been used and continue to be an alternative approach on treatment for various diseases. Due to the growing interest of consumers in substances of natural origin coupled with the increasing apprehension surrounding potentially harmful infectious diseases, our group did a comprehensive phytochemical analysis on the roots of *B. antidysentrica*. Silica gel column chromatographic separation of the crude extracts led to isolation of three indole alkaloids which are identified as compound **1** (derivative flazin methyl ether), canthin-6-one (**2**), and 1,11-dimethoxycanthin-6-one (**3**) all of which are identified for the first time from the species. In addition, compound **1** was isolated for the first time from the genus. The antibacterial test results revealed that the

isolated compounds showed promising antibacterial activity against *S. aureus*, *E. coli*, *S. thphimurium,* and *B. subtilis*. Compound **1** and canthin-6-one (**2**) exhibited medium (12.66±0.60 and 12.5±0.87 mm zone of inhibition) antibacterial activity against *E. coli* and *S. thphimurium* compared to that of ciprofloxacin (27.3±2.52 and 29±1.00 mm zone of inhibition). Canthin-6-one (**2**) had strong DPPH free radical scavenging activity (87.5% at 100 µg/mL) compared to ascorbic acid. Molecular docking analysis revealed good scoring pose (lowest energy) with value of -5.8 Kcal/mole, -5.7 Kcal/mol and -5.7 Kcal/mol for alkaloids **1**-**3**, respectively. The finding of these pharmacologically important secondary metabolites, the observed biological activity supported by molecular docking analysis and free radical activity support the traditional use of the plant to treat various infectious diseases.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Data availability statement

All spectral data can be freely accessed from supporting information.

Acknowledgment

The authors acknowledge Adama Science and Technology University (ASTU), Adama, Ethiopia for funding part of the work. Mr. Temesgen Asefa (staff of Department of Applied Biology, ASTU) is duly acknowledged for his assistance during antibacterial tests.

Reference

Abbink, J., (1995). Medicinal and ritual plants of the Ethiopian southwest: an account of recent Research, *Indigeneous Knowledge and Development Monitor,* 3: 6-8.

Adhikari, B.S., Babu, M.M., Saklani, P.I., Rewat, G.S., (2010). Medicinal plants diversity and their conservation status in wild life institute of indi (wii) campus, deheadun, *Ethnobotanical Leaflet*, 14:6-56.

Alhadi, E.A., Khalid, H.S., Alhassan, M.S., Kabbashi, A.S., Noor, O.M., (2015). Antimicrobial and phytochemical screening of *Cordia africana* in Sudan," *World Journal Of Pharmacological Researc*h, 4(3):257-269.

Alves, B.S., Miranda, M.H., Soares, A.L., Randau, P.K., (2014). Simaroubaceae family: botany, chemical composition and biological activities. *Rev. Bras. Farmacogn.* 24: 481-501.

Ayoola, G.A, Coker, H.A.B., Adesegun, S.A., Adepoju-Bello, A.A., Obaweya, K., (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in southwestern Nigeria, *Tropical Journal of Pharmacological Research*, 7:1019-1024.

Blech, S., Budzikiewicz, H., (1994). β-Carbonline alkaloids from *Ribes nigrum L*, *Journal of Nature Research,* 49:540-544.

Cragg, G.M., Newman, D.J., (2005). Plants as a source of anti-cancer agents," *Journal of Ethno pharmacology*, 100: 72-79.

Endashaw, Bekele. (2007). Study on Actual Situation of Medicinal plants in Ethiopia. Prepared for *JAICRF* (Japan Association for International Collaboration of Agriculture and Forestry), 70-74.

Evans, W.C., Trease, G.E., (1989). Phenols and phenolic glycosides," *in Textbook of Pharmacognosy*, 12: 343–383, Balliese, Tindall and Co Publishers, London, UK.

Fullas, A. (2001). Ethiopian traditional medicine: common medicinal plants in perspective." Sioux City, IA, USA, pp, 29.

Hedenberg, I., Edvans, S., (1989). Simaroubaceae. Flora of Ethiopia. Uppsala, 3: 437- 441.

Maksyutina, N.P., (1971). Hydroxycinnamic acids of *Plantago* major and *Pl. lanceolata*," *Chemistry of Natural Compounds,* 7:795.

Mansourian M., Fassihi A., Saghaie L., Madadkar-Sobhani A., Mahnam K., Abbasi, M., (2015). QSAR and docking analysis of A2B adenosine receptor antagonists based on non-xanthine scaffold, *Medicinal Chemistry Research*, 24:394-407.

Morris G.M., Goodsell D.S., Halliday R.S., Huey R., Hart W.E., Belew, Richard, K., (1998). Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function," *Journal of Computational Chemistry*, 19(14):1639-1662.

Murai M., Tamayam Y., Nishibe, S.,(1995). Phenylethanoids in the herb of *plantago lanceolata* and inhibitory effects on arachidonic acid-induced mouse ear edema," *Journal of Medicinal Plant research*, 61:479.

Narihiko, F., Masayoshi, O., Aratani, T., (1986). Antitumor Agents Cytotoxic Antileukemic Alkaloids from *Brucea antidysenterica, Journal of Natural Products,* 49:428-434.

Ouyang, Y., Koike, K., Ohimoto, T., (1994). Canthine-6-one alkaloids from roots of *Brucea mollis*, *Phytochemistry*, 36(6):1543-1546.

Pearce, D.W., Puroshothaman, S., (1992). Protecting Biological Diversity: the Economic Value of Pharmaceutical plants, CSERGE Global Environmental changes working paper," *Centre for Social and Economic Res*earch *on the Global Environment*, University College, pp. 92-127.

Reece, R.J., Maxwell, A., (1991). DNA Gyrase: Structure and Function". *Critical Reviews in Biochemistry and Molecular Biology,* 26(3-4): pp 335-337.

Roopashree T.S., Dang R., Rani R.H.S., Narendra, C., (2008). Antibacterial activity of antipsoriatic herbs: *Cassia tora, Momordica charantia and Calendula officinalis*," *International Journal of Applied Research in Natural Products*, 1(3:)20-28.

Saiprasana, B., Manohar, B., Roja, R., Prasanta, K., Rajeshree, P., (2012). Phytochemical investigation and study on antioxidant properties of *Ocimum canum* hydro-alcoholic leaf, *Journal of drug delivers and Therapeutics*, 2:122-123.

Schippman, U., Leaman, D.J., Cunningham, A.B., (2002). Impact of cultivation and gathering of medicinal plants on biodiversity: Global trends and issues. In: Biodiversity and Ecosystem Approach in Agriculture, Forestry and Fisheries," *Food and Agriculture organization,* Rome.

Sofowora, F.A., Debiyi, O.O., (1978). Phytochemical screening of Nigerian medical plants II," *Lloydia*, 41:234-246.

Ugochukwu, S., Uche, I., Ifeanyi, O., (2013). Preliminary phytochemical screening of different solvent extracts of stem, bark and roots of *Dennetia tripetala G*., Baker", Asian *Journal of plant Science Research*, 3:10-13.

Viswanad, V., Aleykutty, N. A., Jaykar, B., Zachariah, S. M., Thomas, L., (2011). Studies on antimicrobial and antioxidant activity of Methanolic extract of *Samadera indica. International Journal of Pharmaceutical Science Review and Research,* 11: 59-64.