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ARTICLE

Bioactive carbazole alkaloids from *Alysicarpus ovalifolius* (Schumach)

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Abstract Phytochemical and biological evaluation of the stem bark of *Alysicarpus ovalifolius* led to the isolation of three carbazole alkaloids identified as mohanimbine (**1**), koenimbine (**2**) and koenidine (**3**) along with quercetin 3-*O*-glucoside (**4**), kaempferol 7-*O*-glucoside (**5**), orientin (**6**), apigenin (**7**), quercetin (**8**), plumbagin (**9**) and stigmasterol (**10**). The structures of these compounds were elucidated using physical and spectroscopic methods as well as comparison with the literature data. Compound **3** showed strong activity against chloroquine-sensitive strain I (D6) and the multi-drug resistant Indochicha I (W2) of *Plasmodium falciparum* with IC_{50} values of 63.07 ± 0.01 and 54.19 ± 0.04 ng/mL, respectively. Compound **1** on the other hand exhibited moderate larvicidal against *Anopheles gambiae* larvae as well as antimicrobial activities against *Candida albicans* and gram-positive *Staphylococcus aureus,* respectively.

Keywords Alkaloids · *Alysicarpus ovalifolius* · Antiplasmodial · Larvicidal · Mosquitocidal · Fabaceae

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Introduction

The family Fabaceae consists of approximately 20,000 species spread in 650 genera and is rich in flavonoids, anthraquinones, alkaloids, terpenoids, lipids, and polysaccharides some of which have medicinal properties (Wojciechowski et al. 2004; Yenesew et al. 2004). The family is one of the most economically important plants in the provision of medicines, ornaments, dyes, timber, fodder, tannins, resins, essential oils, flavours, insecticides, piscicides, and even human food (Bentjee 1994; Mannetje 2002). The hitherto phytochemically uninvestigated *Alysicarpus ovalifolius* is used in folklore medicine in management of fever, wounds, ringworm, acute and chronic-troubled bleeding piles, and as a stimulant in birth control while leaf sap and root decoction are drunk to relieve cough (Lamers et al. 1996; Kokwaro 2009). In the current communication, phytochemical analysis of *n*-hexane, CH_2Cl_2 and MeOH extracts resulted in the isolation of three carbazole alkaloids (**1–3**) together with compounds (**4–10**). These compounds together with their activities are being reported from this plant for the first time.

Materials and methods

Experimentation, solvents and fine consumables

Melting points were determined using Gallenkamp melting point apparatus and are uncorrected. The NMR data were measured in $CDCl_3$ and $CDCl_3$-DMSO-d_6 on a JOEL NMR instrument operating 600 and 150 MHz, respectively. Some NMR analyses were done using Brucker AM 300 spectrometer operating at 300 and 75 MHz, respectively. TMS was used as internal standard. The mass spectral data were obtained using a Varian MAT 8200 A instrument. Column chromatography was performed using silica gel 60 (0.063–0.200 mm, Merck-Germany), while thin layer chromatography (TLC) was performed using silica gel 60 F_{254} (Merck) pre-coated plates. All solvents used were of analytical grade.

The stem bark of *Alysicarpus ovalifolius* was obtained from Shimba Hills (latitude: 4°19′39″S and longitude: 39°21′39″E) where it naturally grows as a wild plant. The plant materials were authenticated at the herbarium of the Museums of Kenya where voucher specimen (No: FAB/AO/2012) was preserved.

Extraction and isolation

The air dried and pulverized root bark (1 kg) of the plant was soaked sequentially in *n*-hexane (3 × 3 L), CH_2Cl_2 (3 × 3 L) and MeOH (3 × 3 L), each lasting 4 days at room temperature. The extracts were separately filtered and evaporated under reduced pressure to afford yellowish (7 g), brown (25 g) and reddish-brown (176 g) extracts of *n*-hexane, CH_2Cl_2 and MeOH, respectively. Five gram of n-hexane extract was mixed with silica gel in a minimum amount of dichloromethane and chromatographed over silica gel-packed column (2.0 × 60 cm, SiO_2 120 g) using *n*-hexane with increasing amount of CH_2Cl_2 up to 100 % of the latter. A total of 90 fractions, each 20 mL was collected and their homogeneity monitored by TLC (solvent systems: *n*-hexane EtOAc, 9:1 and 4:1). The eluants were grouped into three pools (1-III) depending on TLC profiles. Fractions 1–25 constituted pool I, which upon evaporation of solvent afforded a yellow oily compound that lost colour with time and was discarded. Pool II (fractions 30–55, 1.5 g) showed a single yellow spot of R_f 0.61 (solvent system: *n*-hexane–EtOAc, 4:1) which on further recrystallization gave **10** (90 mg). Pool III (fractions 57–85, 1.0 g) also crystallized out to give a colourless powder which on further recrystallization afforded **9** (75 mg). Dichloromethane extract (22 g) was adsorbed onto silica gel and then subjected to column chromatography (2.5 × 60 cm, SiO_2 240 g, pressure ≈1 bar) using *n*-hexane-CH_2Cl_2 gradient (increment 10 %) up to 100 % CH_2Cl_2 and elution concluded with ethyl acetate, collecting 20 mL each. The process afforded various sub-fractions (I–V) as determined by TLC profiles [solvent systems: *n*-hexane–CH_2Cl_2 (1:2, 1:1) and CH_2Cl_2–MeOH, 98:2 and 96:4)]. The sub-fraction I (fractions 1–15) showed no spot and solvent recovered. Sub-fraction II (fractions 20–60) produced a single spot R_f 0.43 (eluent: *n*-hexane–CH_2Cl_2, 1:1) that was Dragendorff's reagent positive which upon evaporation of solvent followed by crystallization in CH_2Cl_2–MeOH mixture afforded **1** (45 mg). Sub-fraction III (fractions 63–86, 3.7 g) afforded compounds of R_f values 0.43 and 0.38 and upon repeated chromatographic separation afforded a further **1** (15 mg) and **2** (20 mg), respectively. Sub-fraction IV (fraction 93–130, 3.4 g) showed one major spot R_f 0.60 (solvent system: CH_2Cl_2–MeOH, 97:3) and upon evaporation of solvent, followed by crystallization gave compound **2** in 165 mg. Fractions 134–180 constituted sub-fraction V (5 g) and was further purified by medium pressure chromatography (2.5 × 50 cm, SiO_2 150 g, pressure ≈1 bar) to give further compounds **2** and **3** in 50 and 70 mg, respectively.

Medium pressure chromatographic separation of MeOH extract (150 g) over 2 % oxalic acid solution-deactivated silica gel column using a mixture of CH_2Cl_2–methanol (5 % increment of MeOH) and MeOH neat gave a total of 80 fractions of 50 mL each. Fractions exhibiting similar TLC profiles were pooled together (Pools A–C). Pool A (fractions 7–20, 5 g) showed one major spot R_f 0.33 (eluant: CH_2Cl_2–MeOH, 97:3) along with minor ones and was further purified by crystallization to give **8** (180 mg). Fractions 25–45 (pool B, 7 g) was repeatedly fractionated over 2 % oxalic acid-deactivated silica gel (SiO_2 150 g; 2.5 × 50 cm; 2–3 % MeOH–CH_2Cl_2) affording sub-fractions which resulted into **8** (15 mg), **7** (35 mg) and **6** (70 mg). On the other hand, fraction C (5 g) yielded **5** (54 mg) and **4** (35 mg) under similar purification procedure.

*Compound **1**, colourless powder, mp 92–94 °C [lit. 88–90 °C (Abu Bakar et al. 2007)]*

^{1}H NMR ($CDCl_3$, 300 MHz), δ 7.69 (4-H, 1H, s), 7.96 (5-H, 1H, d, J = 7.5 Hz), 7.19 (6-H, 1H, d *J* = 7.5 Hz), 7.28 (7-H, 1H, d, *J* = 5.0 Hz), 7.36 (8-H, 1H, d, *J* = 7.2 Hz), 6.64 (3′-H, 1H, d, *J* = 9.6 Hz), 5.67 (4′-H, 1H, d, *J* = 9.6 Hz), 1.77 (5′-H, 2H, t, *J* = 8.1 Hz), 1.83 (6′-H, 2H, m), 5.13 (7′-H, 1H, t, *J* = 6.6 Hz), 1.62 (8′-H, s), 1.77 (9′-H, s), 1.49 (2′-CH_3, 3H, s), 2.38 (3-CH_3, 3H, s), 7.41 (1H, N–H, s).

13C NMR ($CDCl_3$, 75 MHz), δ 104.2 (C-1),149.9 (C-2), 131.7 (C-3), 117.5 (C-4), 119.5 (C-5), 119.3 (C-6), 121.2 (C-7), 110.4 (C-8), 134.9 (C-9), 139.5 (C-10), 118.4 (C-11), 116.7 (C-12), 78.2 (C-2′), 128.5 (C-3′), 124.2 (C-4′), 40.8 (C-5′), 22.7 (C-6′), 116.7 (C-7′), 131.7 (C-8′), 25.9 (C-9′), 25.7 (C-10′), 17.6 (2′-CH_3). IR ν_{max} (KBr) cm^{-1}: 3350,

2930, 2850, 1650, 1450, 1380, 1210, 760; 28^{1}H and ^{13}C NMR ($CDCl_3$) ppm: see Tables 1 and 2; ESI-MS (rel. int): *m/z* 332.3 $[M]^+$ (100), 331.3 (10), 276.2 (7), 250 (30), 248 (10), 210 (10).

*Compound **2**, colourless powder, mp. 196–197 °C [lit. 194–195 °C (Nayak et al. 2010)]*

^{1}H NMR ($CDCl_3$, 300 MHz), δ 7.61 (4-H, 1H, s), 7.41 (5-H, 1H, d, $J = 7.6$ Hz), 7.25 (6-H, 1H, d, $J = 7.2$ Hz), 7.42 (8-H, 1H, d, $J = 7.6$ Hz), 6.78 (3′-H, 1H, d, $J = 11$ Hz), 5.68 (4′-H, 1H, d, $J = 14.7$ Hz), 1.47 and 2.28 (2 × 2′-CH_3, 3H, s), 2.29 (3-CH_3, 3H, s), 3.87 (7-OCH_3, s), 7.61 (1H, N–H, s).

13C NMR ($CDCl_3$, 75 MHz), 103.7 (C-1), 150.9 (C-2), 125.3 (C-3), 122.1 (C-4), 103.7 (C-5), 114.2 (C-6), 155.2 (C-7), 112.3 (C-8), 136.6 (C-9), 132.9 (C-10), 118.5 (C-11), 116.0 (C-12), 77.1 (C-2′), 130.1 (C-3′), 118.1 (C-4′), 28.2, 16.5 (C-2′), 56.7 (6-OCH_3). IR ν_{max} (KBr) cm^{-1}: 3330, 2960, 2870, 1642, 1470, 1363, 1312, 1208, 690; 28^{1}H and ^{13}C NMR ($CDCl_3$) ppm: see Tables 1 and 2; ESI–MS (rel. int): *m/z* 294.3 $[M]^+$ (100), 296 (10), 293.3 (24).

*Compound **3**, pale yellow crystals, mp 225–226 °C [lit. 224–225 °C (Mohammad et al. 2013)]*

^{1}H NMR ($CDCl_3$, 300 MHz), δ 7.40 (4-H, 1H, s), 7.53 (5-H, 1H, s), 6.95 (8-H, 1H, s), 6.64 (3′-H, 1H, d, $J = 9.6$ Hz), 5.69 (4′-H, 1H, d, $J = 9.6$ Hz), 1.28 and 1.50 (2 × 2′-CH_3, 3H, s), 2.35 (3-CH_3, 3H, s), 3.99 (6-OCH_3, s), 3.97 (7-OCH_3, s), 7.30 (1H, N–H, s).

13C NMR ($CDCl_3$, 75 MHz), 103.4 (C-1), 146.7 (C-2), 133.9 (C-3), 115.7 (C-4), 101.1 (C-5), 142.5 (C-6), 146.9 (C-7), 93.7 (C-8), 133.9 (C-9), 117.1 (C-10), 117.1 (C-12), 74.2, (C-2′), 127.4 (C-3′), 118.7 (C-4′), 14.8 and 26.3(2 × 2′-CH_3), 55.3 (6-OCH_3), 54.8 (7-OCH_3). IR ν_{max} (KBr) cm^{-1}: 3150, 2940, 2880, 1640, 1450, 1344, 1110, 860; 28^{1}H and ^{13}C NMR ($CDCl_3$) ppm: see Tables 1 and 2; ESI–MS (rel. int): *m/z* 324.3 $[M]^+$ (100), 323.3 (50), 308 (5), 282 (5).

Table 1 In vitro antiplasmodial activity (IC_{50}) of crude extracts *A. ovalifolius* root bark and the pure isolates against D6 and W2 strains of *Plasmodium falciparum*

Test materials	IC_{50} values[a] (ng/mL)	
	W2 Clone	D6 Clone
n-Hexane extract	323.0 ± 0.01	546.0 ± 0.01
Dichloromethane extract	234.0 ± 0.01	482.0 ± 0.03
Methanol extract	265.0 ± 0.04	579.0 ± 0.02
Pure isolates		
Mohanimbine (**1**)	130.0 ± 0.01	279.0 ± 0.01
Koenimbine (**2**)	161.4 ± 0.02	176.7 ± 0.01
Koenidine (**3**)	63.0 ± 0.01	54.2 ± 0.04
Chloroquine (20 mg/mL)	17.5 ± 0.01	26.9 ± 0.01

[a] Values are mean ± SD of three replicates

Acid hydrolysis of compounds 4 and 5

A solution of **4** and **5** (each 10 mg) in a mixture of 8 % HCl (1 mL) and MeOH (20 mL) was separately refluxed for 2 h. The reaction mixtures were reduced in vacuo to dryness, dissolved in H_2O (2 mL) and neutralized with NaOH. The neutralized products were then subjected to TLC analysis (eluent: EtOAc–MeOH–H_2O–HOAc, 6:2:1:1). The chromatograms were sprayed with aniline hydrogen phthalate followed by heating at 100 °C for 2 min. The presence of glucose was confirmed after comparison with authentic samples.

Antiplasmodial assay

An in vitro antiplasmodial activity was carried out using the *P. falciparum* multi-drug resistant Indochicha I (W2) and chloroquine-sensitive Sierra Leone I (D6) strains

Table 2 Larvicidal and mosquitocidal activities of crude extracts and pure isolates as % mortality and LC_{50} values

Test materials	Larvicidal activity		Mosquitocidal activity	
	Mortality[a] (%)	LC_{50} (μg/mL)	Mortality[a] (%)	LC_{50} (μg/mL)
n-Hexane extract	25.9 ± 0.01	120.61	5.1 ± 0.01	500.56
Dichloromethane extract	87.7 ± 0.01	9.86	88.5 ± 0.01	17.83
Methanol	77.8 ± 0.01	85.64	5.1 ± 0.01	435.17
Pure isolates				
Mohanimbine (**1**)	82.3 ± 0.01	5.56	44.3 ± 0.01	213.90
Koenimbine (**2**)	53.6 ± 0.01	41.76	42.6 ± 0.01	260.46
Koenidine (**3**)	34.7 ± 0.01	87.54	30.7 ± 0.1	289.07

[a] Values are mean ± SD of three replicates recorded at a concentration of 250 μg/mL

according to procedures of Desjardins et al. (1979) and Chulay (1983) to determine the IC_{50}. The parasites were grown in a continuous culture supplemented with mixed gas (90 % nitrogen, 5 % oxygen), 10 % human serum and 6 % haematocrit of A+ red blood cell (Trager and Jensen 1976). When the cultures had reached a parasitemia of 3 % with at least a 70 % ring developmental stage present, parasites were transferred to a 96-well microtitre plate with wells pre-coated with the extract/isolates. The samples were diluted across the plate to provide a range of concentrations used to accurately determine IC_{50} values. The plates were then incubated in a mixed gas incubator for 24 h, then 3H-hypoxanthine was added and the parasite allowed to grow for 18 h more in triplicates. Cells were then processed with a plate harvester (TomTec) on a filter paper and washed to eliminate unincorporated radioisotope. Chloroquine was used as a standard drug.

Larvicidal and mosquitocidal tests

Batches of 20 third instar *Anopheles gambiae* larvae were transferred by means of droppers to a small disposable test cup each containing 100 mL of five concentrations of the isolates of 0, 10, 100, 250, 500 and 1000 µg/mL dissolved in acetone in triplicate vials according to (Globade et al. 2002). Acetone was used as a positive control, while distilled water acted as a negative control. Dead larvae were counted after 24 h. For insecticidal bioassays, sample solutions of 0, 10, 100, 250, 500 and 1000 µg/mL dissolved in acetone in triplicate vials were applied into several filter paper discs (5 cm diameter) then placed in perforated dishes (treated set). After drying the filter papers, ten unfed insects were introduced into each of the dishes through a hole and allowed to be in contact with the filter papers discs for 20 min, then transferred into cages with sugar water and observed for 24 h in triplicates (Gbolade et al. 2002). The mortality of the insects was monitored and toxicity levels of the test samples evaluated graphically to give LC50 values. Controls were distilled H_2O and solvent acetone.

Antifungal and antibacterial tests

The disc diffusion assay method was applied according to Singh, et al. 2002 using *Candida albicans* (HG 392), *Aspergillus fumigatus* (HG 420) and *Aspergillus niger* (ATCC 90028) as the representative fungi. *Staphylococcus aureus* (ATCC 25922), *Streptococcus faecalis* (ATCC 25925) and *Bacillus anthracis* (QST 713) were used as the representative gram-positive bacteria, while *Klebsiella pneumoniae* (ATCC 90028), *Salmonella typhimurium* (ATCC 25927), *Pseudomonas aeruginosa* (ATCC 25923) and *E. coli* (K 12) were representatives of gram-negative bacteria. Crude samples were tested in vitro at sample concentration of 1000 µg/mL dissolved in dimethylsulfoxide (DMSO) in triplicate vials, while pure isolates were tested at 100 µg/mL. Mueller–Hinton agar was aseptically aliquoted at volumes of 25 mL to Petri dishes and left to congeal. The agar was inoculated aseptically with test organisms using streaking method (Singh et al. 2002). Test discs (5 mm diameter) previously impregnated with 10 µL of test samples were placed approximately equidistant into the seeded agar using a sterile forceps. Disc containing 10 µL of the 20 µg/mL solution of the standard drug (Fluconazole and Amoxicillin) was used as the positive control. The agar plates were incubated at 37 °C for 72 h after which the inhibition zones were measured in millimeters (McChesney et al. 1991).

Results and discussion

Column chromatography separation of the CH_2Cl_2 extract (29 g) yielded three compounds (**1–3**), Fig. 1, which showed positive dragendorff's test for alkaloids. Compound **1** was isolated as colourless powder with a molecular formula $C_{23}H_{25}O$ as evidenced by a molecular ion peak at *m/z* 332.3 $[m]^+$. It showed a positive Dragendorff's test suggesting it is an alkaloid compound. The ^1H NMR spectrum of **1** (Fig. 2) showed four methyl singlet signals which appeared at δ 1.77 and 1.62 (3H each, vinyl methyls), 2.38 (3H, an aromatic methyl) and 1.49 (3H) besides a vinyl proton on a trisubstituted double bond at δ 5.13 (br t, $J = 6.6$ Hz). The latter peak correlated with the vinyl methyls in the HMBC spectrum suggesting the presence of a terminal $–CH_2CH{=}C(CH_3)_2$ group in the compound (Abu Bakar et al. 2007; Mohammad et al. 2013). In the ^1H NMR spectrum, a set of olefinic protons at C-3′ and C-4′ appeared as doublets at δ 6.64 ($J = 9.6$ Hz) and 5.67 ($J = 9.6$ Hz) which together with a ^{13}C NMR (Fig. 3) diagnostic peak at δ 78.2 signified the presence of carbon–carbon double bond in a pyran ring typical of carbazole alkaloids (Dheeref et al. 2014). Comparison of ^1H and ^{13}C NMR spectra of compound **1** with those of mohanimbine previously isolated from *Murraya koenigii* revealed close similarities (Chakraborty et al. 1978; Fiebig et al. 1985). Analysis of both ^1H and ^{13}C NMR data of compound **1** taking into consideration the fragmentation pattern in the ESI-MS confirmed the compound to be mohanimbine.

Compound **2**, a colourless powder also afforded a positive Drangedorff's test for alkaloids. The ESI-MS molecular ion peak at *m/z* 294.3 and the ^{13}C NMR data in combination with DEPT (90° and 135°) suggested a molecular formula $C_{19}H_{20}NO_2$ as deduced by comparative analysis of ^{13}C NMR (19 distinct signals); DEPT-^{13}C NMR

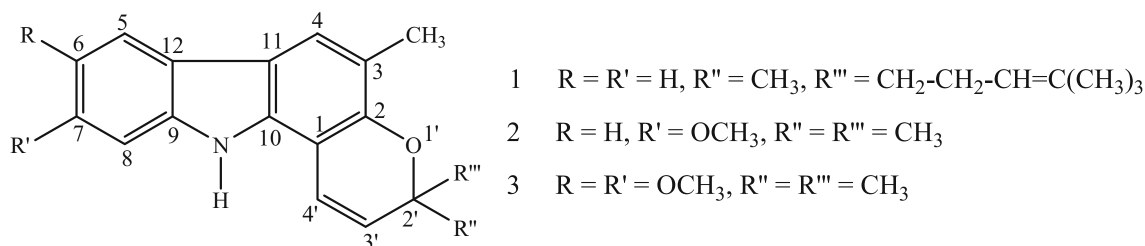

Fig. 1 Structures of compounds **1**, **2** and **3**

Fig. 2 ^{1}H NMR spectrum for compounds **1**, **2** and **3**

(90°: 6CH all SP^2); $\theta = 135^0$: 6CH and $4CH_3$ including a methoxy group at δ 56.7. The DEPT data comprising of 6CH and $4CH_3$ which totalled to $C_{10}H_9$ suggested the presence of either a hydroxyl or a –NH group in the compound. The ^{1}H NMR of compound **2** showed a set of olefinic signals at δ 6.78 (d, $J = 11.0$ Hz) and 5.68 (d, $J = 14.7$ Hz) which together with a broad singlet at δ 7.61 suggested that compound **2** is a carbazole alkaloid (Dheeref et al. 2014). Comparative analysis of both ^{1}H and ^{13}C NMR data of the compound taking into consideration of

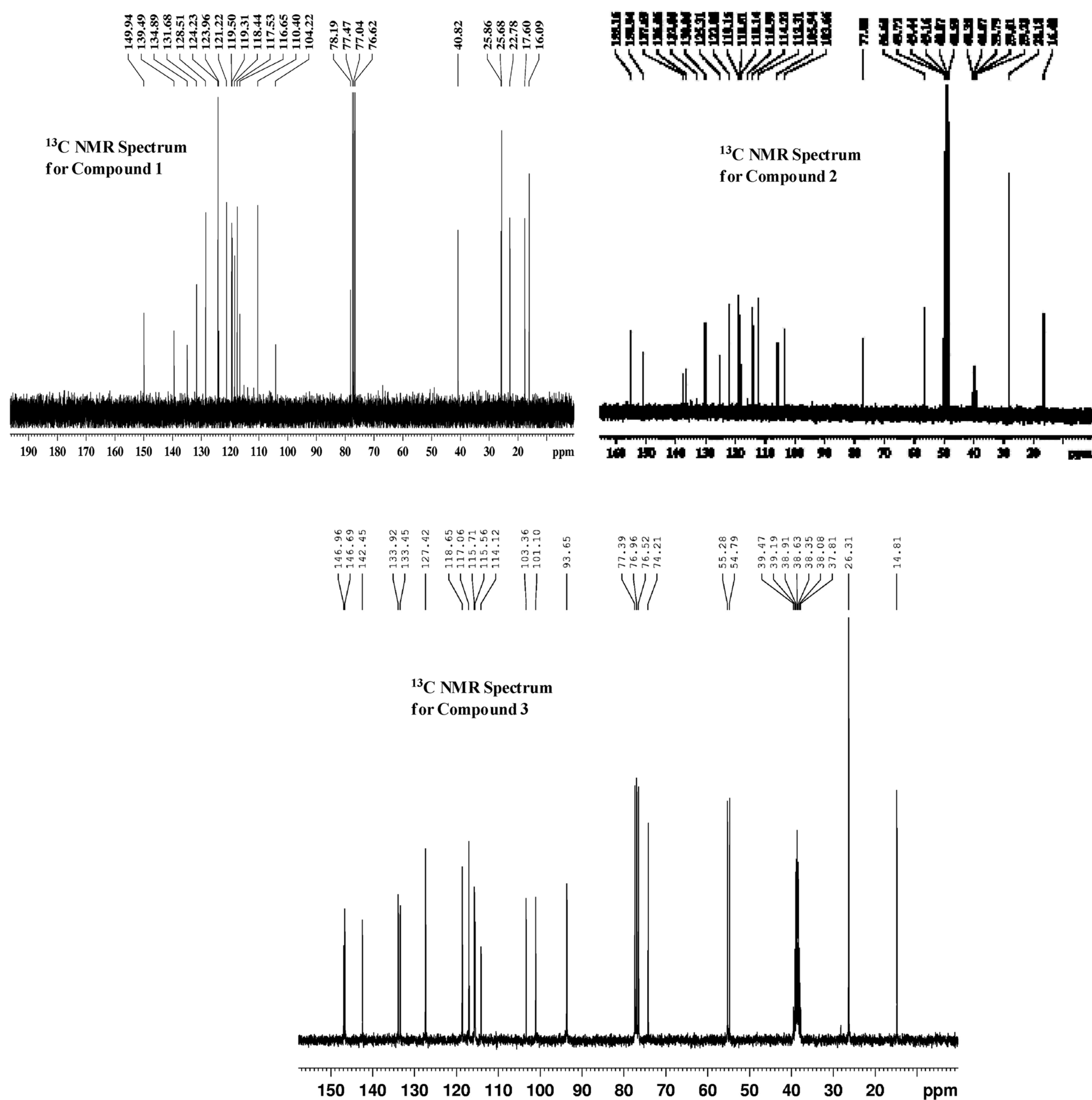

Fig. 3 ^{13}C NMR spectra for compounds **1**, **2** and **3**

the molecular ion *m/z* 294.3, and the fragmentation pattern in the ESI-MS revealed the compound to be koenimbine previously isolated from *M. koenigii* (Ito et al. 2006; Mohammed et al. 2013). On the basis of physical and spectroscopic data, compound **2** was confirmed to be koenimbine.

Compound **3** was obtained as pale yellow needles and the ESI-MS indicated a molecular ion peak at *m/z* 324.3 suggesting a molecular formula of $C_{20}H_{21}NO_3$ which is a 29 amu higher than that for compound **2**, thus suggesting that compound **3** is a derivative **2**. Comparison of the ^{1}H and ^{13}C NMR spectra of **3** with those of **2** (Figs. 2 and 3) showed close similarities with notable difference between the two compounds being the presence of additional methoxy group in **3** as evidenced by a ^{1}H NMR peak at δ 3.97 (s, 3H) with corresponding δ_C 54.8. Further confirmation of the structure of **3** was accomplished using HSQC and HMBC data which aided unequivocal assignment of all the signals associated with the compound. Thus, based on spectroscopic data coupled with reported literature data,

Table 3 In vitro antifungal and antibacterial activities of extracts and pure isolates of *A. ovalifolius*

Test microbe	Diameter of zones of inhibition* in mm							
	N-hex	DCM	MeOH	Cpd 1	Cpd 2	Cpd 3	Flu	Am
C. albicans	2.3 ± 0.01	**13.2 ± 0.1**	9.1 ± 0.1	14.5 ± 0.1	8.0 ± 0.2	10.4 ± 0.2	17.3 ± 0.2	–
A. fumigatus	5.2 ± 0.01	7.4 ± 0.1	7.2 ± 0.2	6.7 ± 0.1	6.5 ± 0.2	7.3 ± 0.3	19.5 ± 0.1	–
A. niger	ND	5.8 ± 0.2	6.5 ± 0.1	6 .5 ± 0.1	7.1 ± 0.3	8.5 ± 0.4	15.8 ± 0.3	–
S. aureus	7.2 ± 0.01	15.3 ± 0.1	12.3 ± 0.1	13.8 ± 0.1	6.4 ± 0.0	5.2 ± 0.1	–	19.5. ± 0.1
S. faecalis	4.5 ± 0.01	6.0 ± 0.1	6,2 ± 0.1	5.5 ± 0.3	6.3 ± 0.1	6.1 ± 0.1	–	19.3 ± 0.3
B. anthracis	ND	4.6 ± 0.1	5.6 ± 0.1	4.4 ± 0.3	2.1 ± 0.1	7.4 ± 0.2	–	16.7 ± 0.1
E. coli	ND	10.8 ± 0.1	8.1 ± 0.1	5.6 ± 0.3	6.8 ± 0.1	4.9 ± 0.1	–	18.5 ± 0.1
K. pneumonia	3.6 ± 0.01	10.3 ± 0.1	8.0 ± 0.2	7.2 ± 0.2	5.3 ± 0.1	6.2 ± 0.2	–	19.9 ± 0.2
S. typhimurium	ND	5.0 ± 0.1	13.3 ± 0.1	8.1 ± 0.2	5.0 ± 0.2	9.4 ± 0.1	–	19.6 ± 0.0
P. aeuruginosa	ND	4.1 ± 0.1	6.1 ± 0.2	5.3 ± 0.2	6.6 ± 0.2	8.8 ± 0.1		17.7 ± 0.0

Bold value represents the highest activity values observed

C. albicans (HG 392), *A. fumigatus* (HG 420), *A. niger* (ATCC 90028), *S. aureus*, (ATCC 25922), *S. faecalis* (ATCC 25925), *K. pneumonia* (ATCC 90028), *S. typhimurium* (ATCC 25927), *E. coli* (K 12), *P. aeuruginosa* (ATCC 25923), *B. anthracis* (QST 713)

Flu fluconazole, *Am* amoxicillin, *ND* not detected, – not done

* Values are mean ± SD of three determinations

compound **3** was established to be koenidine also previously isolated from *M. koenigii*.

The extracts and isolates (**1–3**) showed strong to moderate in vitro antiplasmodial activities against D6 and W2 strains as shown in Table 1. The lowest IC_{50} values of 234.0 ± 0.01 and 63.0 ± 0.01 ng/mL were observed for the dichloromethane extract and koenidine (**3**), respectively, for the W2 strain of *P. falciparum* compared with the standard drug (chloroquine) whose value was 17.5 ± 0.01 ng/mL. Compound **3** also exhibited strong activity against the D6 strain of *P. falciparum* with IC_{50} value of 54.2 ± 0.04 ng/mL compared with the standard drug (chloroquine) whose value was 26.9 ± 0.01 ng/mL. Thus, the strong activity of the dichloromethane extract could be attributed to the presence of the compound **3**. No antiplasmodial activity has been reported for the extracts/isolates.

The dichloromethane extract also exhibited the highest larvicidal activity at a concentration of 250 µg/mL with 87.7 ± 0.01 % mortality recorded by the first time interval of 24 h. The methanol extract was equally active with mortality of 77.8 ± 0.01 %. Compound **1** was the most active of the pure isolated with 82.3 ± 0.01 % mortality. The lowest LC_{50} values of 9.86 and 5.56 µg/mL were observed for dichloromethane extract and compound **1**, respectively. Results are summarized in Table 2. The dichloromethane extract had the highest mosquitocidal activity of 88.5 ± 0.01 % mortality at a concentration of 250 µg/mL after 100 min, while compound **1** showed 44.3 ± 0.01 % mortality with LC_{50} values of 17.83 and 213.90 mg/mL, respectively:

The crude extracts and pure isolates were subjected to in vitro antifungal and antibacterial activities against the yeast-like and filamentous fungi as well as some gram-positive and gram-negative bacteria using the disc diffusion method. Results are presented in Table 3. Again the dichloromethane extract showed strongest activity against *C. albicans* and *S. aureus* with zones of inhibition measuring 13.2 ± 0.1 and 15.3 ± 0.1 mm, respectively, compared to Fluconazole and Amoxicillin, which were used as the standard drugs with zones of inhibition of 17.3 ± 0.2 and 19.5 ± 0.1 mm. Methanol extract was most toxic to *S. typhimurium* and *S. aureus* with zones of inhibition of 13.3 ± 0.1 and 12.3 ± 0.1 mm, respectively. Mohanimbine (**1**) exhibited strongest inhibition against *C. albicans* and *S. aureus* (14.5 ± 0.1 and 13.8 ± 0.1 mm, respectively). These results concur with the previous investigations (Mohammad et al. 2013, Dheeraf et al. 2014) which reported the antimicrobial activities of compounds **1**, **2** and **3** isolated from *M. koenigii.* Antimicrobial activities of some *Alysicarpus* species were previously reported (Rameshkumar and Umarajan 2013; Kumar et al. 2014). The results from this study confirm the ethnomedicinal information of plant.

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