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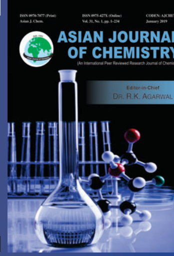

Isolation of Steroid Compounds from *Randia malabarica* (Triveng) and their Biological Activity

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Two steroidal compounds *i.e.*, 1-octacosanol (**1**) and β-sitosterol (**2**) were isolated from ethyl acetate fraction of *Randia malabarica* (Triveng). The antitumor activity of the compounds **1** and **2** were observed against A549 and HeLa using MTT assay. The isolated compounds exhibited a strong inhibitory effect on the two cancer cell lines with a significant effect of inhibition. Molecular docking studies have been performed for both the compounds, showed good inhibitory activity, thereby opening its applications to various clinical trials.

Keywords: 1-Octacosanol, *Randia malabarica*, Molecular docking, Antimicrobial activity, Antitumor activity.

INTRODUCTION

The drugs that discovered play a major role in the treatment of human diseases such as bacterial, fungal, oxidative activities, *etc*., The plant family Rubiaceae comprises 450 genera, 6500 species in tropical and subtropical distribution [1]. It is represented in Pakistan by 33 genera and 87 species [2]. A large number of plants from Rubiaceae family have shown the growth of pathogenic microorganisms like anti-inflammatory, anticonvulsant and antitumour activities [3-8].

Anti-inflammatory and antioxidant activities from the methanolic extract of *Randia hebecarpa* Benth. (Rubiaceae) leaves in two different methods by DPPH assay and the linoleic acid peroxidation method are reported [9]. In this article, 1-octacosanol (**1**) and β-sitosterol (**2**) are isolated from ethyl acetate fraction of *Randia malabarica* (Triveng). To our best of knowledge both the compounds from this species have been isolated first time and tested for antimicrobial and antitumour activity.

EXPERIMENTAL

Randia malabarica (Triveng) was collected from Madukkarai area, Coimbatore, India, during September to November 2014. The specimen was identified in Botanical Survey of India and the voucher number for the plant was BSI/SRC/5/23/2014-15/Tech/1244. The chemicals was used acetone, ethyl acetate, silicagel G_{60} (70-320 mesh), thin layer chromatography (TLC) and silica plate. Spectrum measurements were performed using a various of spectroscopies tools, infrared spectra were measured with Alpha Brucker-8400 S. ^{1}H and ^{13}C NMR spectra were measured using V + 400 MHz high resolution multi nuclear FT-NMR spectrometer which works at 400 MHZ (^{1}H NMR) and at 400 MHz (^{13}C NMR), 1-D and 2-D NMR with TMS as an internal standard and ES-MS spectrometry.

Extraction and isolation: A well grown plant were collected from *R. malabarica* and it is shade dried at room temperature for a week, then it was homogenized to a fine powder and extracted with acetone (95 %) (5 L × 2 h × 3) using soxhlet apparatus. After evaporation of acetone under reduced pressure, the aqueous residue (700 g) was diluted with water and then successively partitioned with petroleum ether (8 L × 3), EtOAc (8 L × 3). The petroleum ether extract (180 g) was subjected to column chromatography on silica gel (200-300 mesh, 3.6 kg) using a stepwise gradient elution of petroleum ether-EtOAc (50:1, 20:1, 10:1, 6:1, 4:1, 2:1, 1:2 and 0:1 v/v). Repeated chromatography of this fraction on silica gel column yielded pure compound **1** (0.98 g), (eluted with petroleum ether 100 %) and compound **2** (0.50 g) (eluted with petroleum ether:ethyl acetate 97:3). The compounds were recrystallized by hexane.

Test for steroid with Liebermann-Burchard reaction: Compounds **1** and **2** was dissolved in chloroform with few drops of conc. H_2SO_4 followed by addition of 2-3 drops of acetic

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anhydride, suddenly the colour changes from violet to green colour, which belongs to steroid [10].

Antibacterial assay: Plant crude extract and the isolated compounds **1** and **2** were tested for their *in vitro* antibacterial activity against five pathogenic bacteria used for the study were *Escherichia coli*, *Psedumonas aeruginosa*, *Salmonella paratypi*, *Staphylococcus aureus* and *Bacillus subtilis* using Muller-Hint agar medium by disc diffusion technique [11]. Dissolved in 1 mL of dimethyl sulfoxide in various concentrations and the commercially available sterile discs were soaked in the preparation for 0.5 h. Then it was placed in empty petri plates for air drying, then it was placed on the surface of the agar plates and gently pressed on the agar surface. The plates were incubated for 24-48 h at 40 ºC. The zone of inhibition was observed using microscope and measured its diameter.

Antifungal assay: The *in vitro* antifungal activity of plant crude extract and the isolated compounds **1** and **2** was studied against the five fungal cultures were *Candida albicans*, *Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus parasites* and *Monosus rubes* for antifungal, assays measuring inhibition of mycelia growth on agar media were used. Compounds was dissolved in 1 mL of sterile DMSO as a stock solution, then it was transferred to 4 mL Sabouraud dextrose agar (SDA) growth media in separate tubes and it was autoclaved for 15 min and cooled to 50 ºC, then each tube was loaded with various concentration of drug solution [12]. All these tubes were incubated at 28 ± 1 ºC for 10 days. A relative humidity was maintained at 40-50 % in the incubation room [13]. The zone of inhibition was measured.

Antitumour assay: *in vitro* Anticancer activity was investigated against human lung adeno carcinoma cell line (A549 cell line), cervical cancer cell line (HeLa cell line) for the compound 1-octacosanol by using MTT (4, 5-dimethylthiazol-2-yl)-2-5-diphenyl tetrazolium bromide assay method [14]. HeLa cells and A549 cells were treated with 20, 40, 60, 80 and 100 µg concentrations of the given two samples. Control cultures were treated with DMSO. After 36 h, 20 µL of MTT (5 mg/mL) solutions were added to each well and the cultures were further incubated for 4 h and then 200 µL of DMSO was added. The absorbance at 655 nm was measured using plate reader.

Molecular docking studies: Molecular docking study predicts the best orientation and the position of a ligand when it bounds to a protein receptor of known three-dimensional structures. The atom co-ordinate files of all multi drug resistance proteins were prepared through their respective PDB entry. Multi drug resistance proteins were docked with 1-octacosanol and β-sitosterol, using Autodockvina. Polar hydrogens were added to the protein structures using Discovery Studio Visualizer. Grid spacing angstrom was different for each protein. The search parameter genetic algorithm evaluates the fitness of each conformation [15]. Four multi drug resistance (MDR) proteins were involved in this study. Three dimensional X-ray structures of the four MDR proteins such as 5HVM from *Aspergillus fumigatus*, 5NPK from *Staphylococcus aureus*, 2ZOC from Human A549 cell line and 5I9B from Human Hela cell line were retrieved from Protein data bank (PDB). Using Uniprot database, the entry was identified for all the four proteins and their respective PDB ID was selected based on their position availability and resolution. The three-dimensional structure of the ligands β-sitosterol and 1-octacosanol were retrieved from pubchem database and was saved in .sdf format, later it was converted to .pdb format using Open Babel GUI.

1-Octacosanol (1): Yield: 2 g, white cystal, m.f. $C_{28}H_{58}O$; m.p 85-86 ºC. IR (KBr, ν_{max}, cm^{-1}): 3417, 2919, 2849, 1465, 723. ^{1}H NMR (400 MHz, δ_H, $CDCl_3$) δ ppm: 0.88 (3H, t, *J* = 6.8 Hz, H-28), 1.25 (50 H, br-s, H-3 to H-27), 1.54 (2H, m, H-2), 3.63 (2H, t, *J* = 6.8Hz, H-1). ^{13}C NMR (100 MHz, δC, $CDCl_3$) δ ppm: 63.11 (C-1), 14.1 (C-28), 22.1 (C-27), 25.7 (C-3), 29.6 (C-4 to C-25), 31.9 (C-26), 32.8 (C-2). *m/z* (relative intensity %): 393 $[M-H_2O]^+$ (12), 111 (23), 97 (51), 83 (51), 83 (71), 69 (72), 57 (100). HR ESI-MS *m/z*: 411 $[M+1]^+$.

β-Sitosterol (2): Yield: 0.5 g, white shiny cystal, m.f. $C_{29}H_{50}O$; m.p 136 ºC. IR (KBr, ν_{max}, cm^{-1}): 3430, 2924, 2852, 1639, 1462, 1377, 1052, 961. ^{1}H NMR (400 MHz, δ_H, $CDCl_3$) δ ppm: 0.79 (3H, S, H-18), 0.80 (3H, d, *J* =7.0 Hz, H-27), 0.81 (3H, d, *J* = 6.5 Hz, H-26), 0.83 (3H, t, *J* = 7.0 Hz, H-29), 0.86 (3H ,d, *J* = 6.5 Hz, H-21), 0.99 (3H, s, H-19), 3.54 (1H, m, H-3), 5.33 (1H, m, H-6). ^{13}C NMR (100 MHz, δ_C, $CDCl_3$) δ ppm: 12.4 (CH_3, C-29), 17.0 (CH_3, C-18), 17.4 (CH_3, C-21), 19.2 (CH_3, C-27), 21.7 (CH_2, C-11), 23.3 (CH_2, C-28), 24.7 (CH_2, C-15), 25.7 (CH_2, C-23), 28.7 (CH_2, C-16), 30.8 (CH, C-25), 30.4 (CH_2, C-2), 31.4 (CH_2, C-7), 31.7 (CH, C-8), 31.8 (CH_2, C-22), 33.0 (CH, C-20), 36.9 (C, C-10), 38.9 (CH_2, C-1), 42.3 (C, C-13), 42.9 (CH_2, C-4), 45.1 (CH, C-24), 49.9 (CH, C-9), 54.4 (CH, C-17), 71.4 (CH, C-3), 121.7 (CH, C-6), 140.8 (C, C-5). *m/z* (relative intensity, %) : 414 (2),129 (13), 111 (16), 97 (36), 83 (45), 71 (54), 57 (100). HR ESI-MS: *m/z* 414 $[M]^+$.

RESULTS AND DISCUSSION

Compound **1** obtained as a white crystalline powder has the molecular formula $C_{28}H_{58}O$ as determined by HR-ESI-MS spectral data, which showed the molecular ion peak at *m/z* 411. The resulting mass spectrum demonstrated fragmentation pattern typical of a long-chain alcohol, exhibiting a peak at 392 $[M-H_2O]$ and masses differing by 14 mass units. The IR spectra of compound **1** suggested the presence of (OH) peak around 3417 cm^{-1}. Interpretation of ^{1}H NMR indicates the presence of 25 methylene groups at δ_H 1.25, leading to the final formulae CH_3-$(CH_2)_{27}$-OH. The ^{13}C NMR spectrum confirms the presence of only one methyl group at δ_C 14.10 and all other signals were found to be methylene groups . The ^{13}C peak at δ_C 63.11 is assigned for OH at C-1. From the above spectral characterization, compound **1** was confirmed as consisting of 1 methyl and 27 methylene carbon and hence possessing the structure CH_3-$(CH_2)_{27}$-OH [16].

Compound **2** was obtained as white shiny crystals. HR ESI-MS spectrum of the compound indicated the MI peak at 414 thus confirming to the molecular formula $C_{29}H_{50}O$.The IR spectrum displayed characteristic absorptions attributable to hydroxyl stretching peak at 3430 cm^{-1}, carbon-carbon stretching at 2924 and 2852 cm^{-1}, C=C absorption peak at 1639 cm^{-1}, methylene peak at 1462 cm^{-1} and cycloalkane peak at 1052 cm^{-1}. The ^{1}H NMR spectrum of compound **2** (400 MHz, $CDCl_3$) has revealed a proton multiplet at δ_H 3.54, the position and multiplicity of which was indicative of H-3 of steroid nucleus. The typical H-6 of the steroidal skeleton was evident as a

TABLE-1
ANTIBACTERIAL ACTIVITY OF PLANT CRUDE EXTRACT AND 1-OCTACOSANOL AND β-SITOSTEROL

S. No.	Organisms	Zone of inhibition (mm)			
		Std. ciprofloxacin (10 μg/disc)	Samples (100 μg/disc)		
			Plant crude extract	1-Octacosanol	β-Sitosterol
1	*Escherichia coli*	12 ± 0.48	11 ± 0.45	12 ± 0.48	13 ± 0.25
2	*Pseudomonas aeruginosa*	10 ± 0.40	09 ± 0.37	10 ± 0.40	15 ± 0.15
3	*Salmonella paratyphi*	12 ± 0.45	12 ± 0.46	12 ± 0.45	14 ± 0.65
4	*Staphylococcus aureus*	20 ± 0.42	28 ± 0.30	20 ± 0.42	21 ± 0.55
5	*Bacillus subtilis*	15 ± 0.51	12 ± 0.47	15 ± 0.51	10 ± 0.42

TABLE-2
ANTIFUNGAL ACTIVITY OF PLANT CRUDE EXTRACT AND 1-OCTACOSANOL AND β-SITOSTEROL

S. No.	Organisms	Zone of inhibition (mm)			
		Std. clotrimazole (10 μg/disc)	Samples (100 μg/disc)		
			Plant crude extract	1-Octacosanol	β-Sitosterol
1	*Candida albicans*	29 ± 0.54	07 ± 0.27	08 ± 0.37	11 ± 0.79
2	*Aspergillus niger*	35 ± 0.57	09 ± 0.45	10 ± 0.47	14 ± 0.29
3	*Aspergillus fumigatus*	31 ± 0.51	12 ± 0.55	06 ± 0.24	16 ± 0.45
4	*Aspergillus parasitus*	30 ± 0.49	16 ± 0.47	12 ± 0.56	11 ± 0.62
5	*Monoscus ruber*	30 ± 0.50	10 ± 0.37	09 ± 0.37	07 ± 0.42

multiplet at δ_H 5.33 that integrated for one proton. The ^{1}H NMR spectrum showed two doublets centered at δ_H 0.81 (J = 6.5 Hz) and δ_H 0.80 (J = 7.0 Hz) which could be attributed to two methyl groups at C-26 and C -27, respectively. The doublet at δ_H 0.99 (J = 6.5 Hz) was demonstrative of a methyl group at C-19. On the other hand, the triplet of three proton intensity at δ_H 0.83 could be assigned to the primary methyl group at C- 29. The ^{13}C NMR shows recognizable signals at δ_C140.8 and δ_C 121.7 which were assigned to C-5 and C-6 double bonds, respectively. The spectra showed 29 carbon signals, including 6 methyl, 9 methylenes, 11 methane and 3 quaternary carbons. The above spectral features are in close agreement with those observed for β-sitosterol according to the literature [17].

Biological assay: In the present study, the plant crude extract, compound **1** (1-octacosanol) and compound **2** (β-sitosterol) was studied against 5 pathogenic bacterial strains of both gram positive and gram negative bacteria. The results (Table-1) reveals that both crude extract and the compounds displayed potential antibacterial activity against all tested organisms. The crude extract, compounds **1** and **2** showed highest antibacterial activity against *S. aureus* with inhibition zone of 28 ± 0.30, 20 ± 0.42 and 21 ± 0.55 mm respectively. The antifungal activity was determined against 5 fungal strains. The plant crude shows good activity against *A. parasites*, moderate activity against *C. albicans* and the lowest activity against *A. fumigates*. The isolated compounds **1** and **2** showed good activity against *A. fumigates* and *A. parasites* (Table-2).

Compounds **1** and **2** was screened for its anticancer activity against HeLa and A549 cell lines using MTT assay with bufalin as a positive control. Compound **1** exhibited strong inhibitory effects against HeLa cells with IC_{50} value of 58.47 μg/mL and IC_{50} value of 67.94 μg/mL against A549 cells. Similarly, the compound **2** exhibited strong inhibitory effects against HeLa cells with IC_{50} value of 52.46 μg/mL and IC_{50} value of 74.52 μg/mL against A549 cells. Cytotoxic changes observed was cell aggregation, cell rounding and cell death. The overall results indicate the promising baseline information for the potential uses of the isolated compounds **1** and **2** from *R. malabarica* aerial part as an anticancer agent.

Docking studies: Multi-drug resistance proteins from various organisms were involved in this study 5HVM from *Aspergillus fumigatus*, 5NPK from *Staphylococcus aureus*, 2ZOC from Human A549 cell line and 5I9B from Human Hela cell line were docked separately with β-sitosterol and 1-octacosanol [18]. Molecular docking was performed for protein-ligand complexes with the predicted active site residues, the dimension of grid box differs for each protein depends on its active site residues. The number of torsions was set. The lowest binding energy of the ligand conformation was considered as the most stable conformation. Binding energies of each protein with the ligands are listed in Table-3. Among all the four MDR proteins, the docking of 5npk of *Staphylococcus aureus* with the ligand β-sitosterol gives the best binding energy and considered as the best protein for further studies.

TABLE-3
DOCKING SCORE AND NUMBER OF HYDROGEN BONDS FORMED BETWEEN THE PROTEINS AND COMPOUND 1-OCTACOSANOL AND β-SITOSTEROL

Proteins	1-Octacosanol		β-Sitosterol	
	Docking score (KCal/mol)	H-bond	Docking score (KCal/mol)	H-bond
5hvm	-6.4	3	-9.4	4
5npk	-5.8	1	-3.7	2
2zoc	-5.9	3	-9.5	3
5i9b	-5.4	1	-7.7	2

Conclusion

It is suggested that some compounds isolated from aerial parts of *R. malabarica* could be responsible for its antimicrobial activity. It is proved that ethyl acetate fraction of *Randia malabarica* had potential cytotoxicity against lung cancer and cervical epithelial carcinoma cells. In this study, the molecular docking was applied to explore the binding mechanism and to

correlate its docking score with the activity of the compounds β-sitosterol and 1-octacosanol. The results of present work show the better inhibitory activity against the antimicrobial and anticancer activities.

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CONFLICT OF INTEREST

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

REFERENCES

1. D.I. Mabberley, Mabberley's Plant Book, Cambridge University Press: Cambridge (1987).
2. S. Nazimuddin and M. Qaiser, eds.: S.I. Ali and E. Nasir, Rubiaceae, In: Flora of Pakistan, vol. 190, pp. 1-145 (1989).
3. M. Del Carmen Recio, R.M. Giner, S. Manez and J.L. Ríos, *Planta Med.*, **60**, 232 (1994); https://doi.org/10.1055/s-2006-959465.
4. J. Sun, H. Lou, S. Dai, H. Xu, F. Zhao and K. Liu, *Phytochemistry*, **69**, 1405 (2008); https://doi.org/10.1016/j.phytochem.2008.01.008.
5. J.Y. Tao, S.J. Dai, F. Zhao, J.F. Liu, W.S. Fang and K. Liu, *J. Asian Nat. Prod. Res.*, **14**, 97 (2012); https://doi.org/10.1080/10286020.2011.628839.
6. N. Li, L. Cao, Y. Cheng, Z.Q. Meng, Z.H. Tang, W.J. Liu, Z.-Z. Wang, G. Ding and W. Xiao, *Pharm. Biol.*, **52**, 1445 (2014); https://doi.org/10.3109/13880209.2014.895910.
7. H.Y. Wang, K. Liu, R.X. Wang, S.H. Qin, F.L. Wang and J.Y. Sun, *Nat. Prod. Res.*, **29**, 644 (2015a); https://doi.org/10.1080/14786419.2014.980255.
8. H.Y. Wang, R.X. Wang, Y.X. Zhao, K. Liu, F.L. Wang and J.Y. Sun, *Chem. Biodivers.*, **12**, 1256 (2015b); https://doi.org/10.1002/cbdv.201400289.
9. A.S. Nazari, S.A. Dias, W.F. da Costa, C.A. Bersani-Amado, G.J. Vidotti, M.C. de Souza and M.H. Sarragiotto, *Pharm. Biol.*, **44**, 7 (2006); https://doi.org/10.1080/13880200500496504.
10. J. Harborne, Phytochemical Methods; A Guide to Modern Techniques of Plant Analysis, Chapman & Hall, London, edn. 3, pp. 129-138 (1998).
11. S. Umadevi, G.P. Mohanta, V. Chelladurai, P.K. Manna and R. Manavalan, *J. Nat. Rem.*, **3**, 185 (2003).
12. J.N. Eloff, *Planta Medica*, **64**, 711 (1998).
13. S. Khan, G.M. Khan, S. Mehsud, A. Rahman and F. Khan, *Gomal J. Medical Sci.,* **2**, 40 (2004).
14. J.P. Yuan, G.H. Wang, H. Ling, Q. Su, Y.H. Yang, Y. Song and C. Huang, *World J. Gastroenterol.*, **10**, 2731 (2004); https://doi.org/10.3748/wjg.v10.i18.2731.
15. L. Korrodi-Gregório, V. Soto-Cerrato, R. Vitorino, M. Fardilha and R. Pérez-Tomás, *PLoS One*, **11**, E0165973 (2016); https://doi.org/10.1371/journal.pone.0165973.
16. S. Firdous, K. Khan, S. Zikr-Ur-Rehman, Z. Ali, S. Soomro, V.U. Ahmad, M. Rasheed, M.A. Mesaik and S. Faizi, *Rec. Nat. Prod.*, **8**, 51 (2014).
17. J. Escudero, C. Lopez, R.M. Rabanal and S. Valverde, *J. Nat. Prod.*, **48**, 128 (1985); https://doi.org/10.1021/np50037a025.
18. B. Muthukala, K. Sivakumari and K. Ashok, *Int. J. Curr. Pharm. Res.*, **7**, 13 (2015).