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Synthesis, characterization and antimicrobial activity of some novel hydrazone derivatives of anacardic acid

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ABSTRACT

Hydrazone derivatives are found to possess anti-inflammatory, anti-tubercular, anti-convulsant anti-microbial, anti-mycobacterial, analgesic and anti-platelet activities. The present paper describes the synthesis, characterization and antimicrobial evaluation of some novel hydrazone derivatives ***6a-j*** *of anacardic acid. The structural assignment of the newly synthesized hydrazone derivatives were confirmed by spectroscopic techniques like,* ^{1} *H NMR, MS and IR data.*

Keywords: Anacardic acid, Antibacterial, Antifungal, CSNL, Hydrazones, Synthesis

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INTRODUCTION

Anacardic acid (pentadecyl salicylic acid) is a phenolic constituent present in CNSL; (*Anacardium occidentale L.*) which is a by-product of cashew nut industry and these are salicylic acid derivatives with a non-isoprenoid alk(en)yl side chain [1]. Anacardic acid derivatives exhibited various biological activities like, specific activator of kinase, activity of Aurora Kinase A [2], affect of the structure of the enzyme [3], suppresses expression of nuclear factor-kB regulated gene products leading to potentiation of apoptosis [4] as a modulators of histone acetyltransferases [5] and as a inhibitor of the HAT activity of recombinant *Plasmodium falciparum* GCN5 [6]. In addition to the above activities, anacardic acid derivatives exhibit biological activities like soybean lipoxygenase-1 inhibitory activity [7,8] and antimicrobial activity [9,10]. Recently, N S Reddy *et al.* reported the synthesis and antibacterial activity of 6-substituted anacardic acid derivatives [11, 12], urea and thiourea derivatives at C-8 alkyl chain of anacardic acid [13] and sulfonamide derivatives of **a**nacardic acid [14, 15].

Hydrazone, schiff bases of acyl, aroyl and heteroacroyl compounds are obtained depending on the experimental conditions; which have application as biologically active compounds [16] and as analytical reagents [17]. Hydrazone derivatives are molecules containing highly reactive azomethine group (CO-NH-N=CH), these are found to possess anti-tumoral [18], anti-convulsant [19], anti-microbial [20], analgesic [21], anti-platelet [22], anti-mycobacterial [23], anti-inflammatory [24] and anti-tubercular [25] activities.

Infectious diseases such as bacterial and fungal infections have been reported to increase dramatically worldwide in recent times and one of the major causes is suppressed immunity [26]. Increasing incidence of microbial resistance to the majority of antibiotics is a major concern and threat to the public health and warrants development of novel

and effective antibiotics to combat drug resistance [27]. Due to the growth of population and changes in climatic conditions several new diseases are likely to affect the human beings. So, there is a continuous need for the synthesis of new biologically active organic compounds by using a fast and efficient approach which may act as potential antimicrobial agents.

Inspired by the various pharmacological, biological importance associated with hydrazone and anacardic acid derivatives, the present paper describes the synthesis, characterization and antimicrobial evaluation of some novel hydrazone derivatives linked with anacardic acid.

MATERIALS AND METHODS

The uncorrected melting points of compounds were taken in an open capillary in a paraffin bath. All reagents used were commercial and laboratory grade, melting points were determined in open capillaries and are uncorrected. IR spectra were recorded on potassium bromide disks on a Perkin-Elmer 383 spectrophotometer. ^{1}H NMR spectra were obtained on Varian 400 MHz instrument and Varian 200 MHz, with TMS as internal Standard and chemical shifts are expressed in δ ppm solvent used in $CDCl_3$ & DMSO-d_6 and mass spectrum on a Hewelett Packard mass spectrometer operating at 70 ev, purity of the compounds were checked by TLC, which is performed with E. Merck pre coated silica gel plates (60 F-254) with iodine as a developing agent. Acme, India silica gel, 60-120 mesh for column chromatography is used. All compounds were purified by column chromatography using ethylacetate in hexane.

Experimental methods

Isolation of anacardic acid ene mixture (1)

Commercially available, CNSL (50 g) was dissolved in MeOH: water mixture (8:2, 250 m L). To the above solution was added activated charcoal (10 g) and heated at 50 ^{o}C for 15 min, filtered over celite bed to remove insoluble materials and colour impurities. The clear filtrate was taken into a 1L round bottom flask fitted with reflux condenser and mechanical stirrer. To this solution $Ca(OH)_2$ (25 g) was added portion wise over a period of 45 min at 50-55 ^{o}C and the reaction mixture was heated to 60 ^{0}C for 5 h. The precipitated calcium anacardate was filtered and washed thoroughly with MeOH (300 mL) and isopropylacetate (200 mL) to afford off-white solid which was poured into ice cold water, and adjusted to pH ~ 2 using 6N HCl and stirred for 30 min. The aqueous solution was extracted with isopropylacetate (4 x 50 mL), the combined organic layer was washed with water (2 x 75 mL), brine solution (2 x 100 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield an ene mixture of anacardic acid **1** (32.5 g, reddish brown liquid).

Synthesis of 2-Hydroxy-6-pentadecylbenzoic acid (2)

An ethanol solution (500 mL) of anacardic acid ene mixture **1** (10 g, 29.21 mmol) was hydrogenated in a 1L Parr-apparatus at 50 psi of H_2 pressure in presence of 10 % Pd/C (1 g, 10 %) for 2 h. The reaction mixture was filtered through celite bed and the filtrate concentrated under reduced pressure to obtain **2,** which was recrystallized in petroleum ether to obtain the pure 2-hydroxy-6-pentadecylbenzoic acid **2** (7 g, 68.8 %) as a white solid. M.P: 85-86 °C; FT-IR (KBr pellet): ν_{max} 3002, 2918, 2851, 1655, 1450, 1246, 1214 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.89 (t, 3H, J = 6.8 Hz), 1.27 (brs, 24H), 1.57-1.63 (m, 2H), 2.98 (t, 2H, J = 8.0 Hz), 6.78 (d, 1H, J = 7.6 Hz), 6.88 (d, 1H, J = 8.4 Hz), 7.37 (t, 1H, J = 8.0 Hz), 11.02 (brs, 1H, D_2O exchangeable COOH); ESIMS: m/z 349 $(M+H)^+$.

Synthesis of methyl 2-hydroxy-6-pentadecylbenzoate (3)

To a stirred suspension of 2-hydroxy-6-pentadecylbenzoic acid **2** in methanol, taken in a sealed tube was added Amberlyst -15 (20% w/w) and heated to reflux for 24 h. After completion of the reaction (monitored by T.L.C), the reaction contents were filtered and the filtrate was evaporated under reduced pressure to obtain methyl 2-hydroxy-6-pentadecylbenzoate **3** as a pale yellow liquid. The crude compound was taken to next step without further purification. Yield: 1.45 g, 70%; IR (KBr): 3419, 3066, 2978, 2951, 2915, 2851, 2643, 1942, 1665, 1606, 1578, 1472, 1448, 1336, 1315, 1296, 1250, 1236, 1214, 1203, 1163, 1121, 1106, 1067, 1019, 987, 971, 945, 880, 841, 817, 748, 710, 646, 591, 542; cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.79 (s, 1H, -OH), 7.14 (t, J = 8.0 Hz, 1H, aromatic -H), 6.69 (dd, J = 8.0, 12.4 Hz, 2H, aromatic -H), 3.76 (s, 3H), 2.43 (t, J = 7.6 Hz, 2H, -CH_2-side chain), 1.45 (brs, 2H, -CH_2-side chain), 1.23 (brs, 24 H, -$(CH2)_6$-side chain), 0.85 (t, J = 6.8 Hz, 3H, -CH_3-side chain); ESIMS: m/z, 361.0 $(M-H)^+$.

Synthesis of 2-hydroxy-6-pentadecylbenzohydrazide (4)
To a stirred solution of 2-hydroxy-6-pentadecylbenzoate **3** (1.45 g, 4.160 mol) in methanol, taken in a sealed tube, was added hydrazine-hydrate (99% w/v, 41.60 mmol) and heated to reflux for 48 h. After completion of the reaction (judged by T.L.C), the reaction mixture was cooled to 10 ^{o}C and filtered to obtain compound **4**. The crude compound was repeatedly washed with water followed by n-hexane to obtain 2-hydroxy-6-pentadecylbenzohydrazide **4**. White solid, M.P.: 78 ^{o}C; Yield: 725 mg, 50%; IR (KBr): υ_{max} 3339, 3322, 3312, 3018, 2960, 2917, 2849, 2558, 1667, 1633, 1596, 1585, 1512, 1464, 1376, 1350, 1333, 1291, 1250, 1236, 1173, 1161, 1111, 1064, 987, 948, 936, 903, 889, 810, 798, 786, 765, 749, 739, 646, 585, 541 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ: 9.38 (s, 1H, -OH), 9.13 (s, 1H, -NH), 7.04 (t, J = 7.6 Hz, 1H, aromatic -H), 6.62 (t, J = 8.8 Hz, 2H, aromatic -H), 4.30 (brs, 2H, -NH2), 2.40 (t, J = 7.6 Hz, 2H, -CH$_2$-side chain), 1.47 (brs, 2H, -CH$_2$-side chain), 1.22 (brs, 24 H, -(CH2)$_6$-side chain), 0.84 (t, J = 6.8 Hz, 3H, -CH$_3$-side chain); ESIMS: *m/z* 363.0 (M+H)$^{+}$.

General Procedure for the Synthesis of (E)-N'-substituted benzylidene-2-hydroxy-6-pentadecyl benzohydrazide derivatives (6a-6j)
The (*E*)-N'-substituted benzylidene-2-hydroxy-6-pentadecylbenzohydrazide derivatives **6a-j** were synthesized by refluxing 2-hydroxy-6-pentadecylbenzohydrazide **4** (0.275 mmol) with aryl aldehydes **5a-j** (0.275 mmol) in ethanol (1.0 mL) for 30 minutes. The progress of reaction was monitored by TLC. After completion of reaction, the precipitated solids were filtered and dried under vacuum to obtain corresponding hydrazone derivatives **6a-j** in quantitative yields.

(*E*)-N'-Benzylidene-2-hydroxy-6-pentadecylbenzohydrazide (6a):
White solid; M.P.: 86-88 ^{o}C; IR (KBr): υ_{max} 3309, 3154, 2920, 2850, 1662, 1619, 1606, 1587, 1524, 1486, 1466, 1447, 1357, 1336, 1317, 1288, 1269, 1226, 1136, 1111, 1063, 1023, 990, 953, 919, 877, 843, 798, 761, 721,696, 552, 445, 417 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d$_6$*): δ 11.62 (s, 1H), 9.67 (* 9.38, s, 1H), 8.20 (* 7.98, s, 1H), 7.67-7.31 (* m, overlapped protons, 5H), 7.12 (d, J = 7.2 Hz, 1H), 6.74-6.65 (m, 2H), 2.54 (brs, 2H), 1.52 (brs, 2H), 1.22-1.07 (m, 24 H), 0.85 (* 0.84 , brs, 3H) (* Rotamers); ESI-MS: m/z, 451.0 (M+1).

(*E*)-N'-(4-Hydroxybenzylidene)-2-hydroxy-6-pentadecylbenzohydrazide (6b)
White solid; M.P.: 116-118 ^{o}C; ^{1}H NMR (400 MHz, DMSO-*d$_6$*): δ 11.40 (s, 1H), 9.89 (* 9.75, s, 1H), 9.62 (* 9.33, s, 1H), 8.09 (* 7.91, s, 1H), 7.67 (* 7.49, d, J = 8.6 Hz, 2H), 6.84 (* 6.63, d, J = 8.6 Hz, 2H), 7.17-7.10 (m, 1H), 6.72-6.67 (m, 3H), 2.42 (t, J = 6.8 Hz, 2H), 1.48 (brs, 2H), 1.22-1.09 (m, 24 H), 0.85 (brs, 3H) (* Rotamers); ESI-MS: m/z, 466.2 (M+1).

(*E*)-N'-(3,4-Dimethoxybenzylidene)-2-hydroxy-6-pentadecylbenzohydrazide (6c)
White solid; M.P.: 101-102 ^{o}C; Yield: 85%; IR (KBr): υ_{max} 3569, 3398, 3248, 3079, 3002, 2953, 2921, 2851, 2730, 2033, 1663, 1600, 824, 1536, 1514, 1464, 1440, 1422, 1375, 1366, 1332, 1268, 1246, 1206, 1168, 1138, 1073, 1060, 1036, 1023, 975, 962, 949, 900, 850, 803, 766, 753, 722, 622, 594, 558 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d$_6$*): δ 11.53 (* 11.50, s, 1H), 9.64 (* 9.35, s, 1H), 8.12 (* 7.87, s, 1H), 7.31 (* 7.02, s, 1H), 7.13 (brs, 2H), 6.89 (brs, 1H), 6.73-6.68 (m, 2H), 3.82 (* 3.80, s, 6H), 3.71 (* 3.57, s, 3H), 1.52 (s, 2H), 1.22-1.06 (m, 24 H), 0.84 (brs, 3H) (* Rotamers); ESI-MS: m/z, 510.2 (M+1).

(*E*)-N'-(3,4,5-Trimethoxybenzylidene)-2-hydroxy-6-pentadecylbenzohydrazide (6d):
White solid; M.P.: 77-78 ^{o}C; Yield: 92%; IR (KBr): υ_{max} 3607, 3584, 3572, 3565, 3372, 3237, 3137, 3053, 3002, 2954, 2920, 2850, 2728, 1665, 1632, 1613, 1575, 1537, 1505, 1463, 1417, 1376, 1362, 1326, 1309, 1281,1268, 1240, 1165, 1076, 1061, 1002, 996, 944, 897, 842, 828, 798, 756, 721, 650, 639, 591 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d$_6$*): δ 11.65 (* 11.61, s, 1H), 9.66 (* 9.37, s, 1H), 8.13 (* 7.85, s, 1H), 7.13 (* 7.08, t, J = 7.6 Hz, s, 1H), 6.98 (* 6.64, s, 2H), 6.72 (* 6.66, t, J = 8.0 Hz, 2H), 3.83 (* 3.70, brs, 2H), 1.53 (* 1.07, brs, 2H), 1.22-1.17 (m, 24 H), 0.84 (t, J = 6.4 Hz, 3H); ESI-MS: m/z, 541.0 (M+1).

(*E*)-N'-(4-Acetamidobenzylidene)-2-hydroxy-6-pentadecylbenzohydrazide (6e)
White solid; M.P.: 118-119 ^{o}C; Yield:92%; IR (KBr): υ_{max} 3313, 3283, 3263, 3210, 3120, 3064, 2921, 2851, 1680, 1628, 1612, 1591, 1555, 1537, 1510, 718, 1466, 1410, 1370, 1317, 1287, 1267, 1234, 1175, 1164, 1143, 1114, 1073, 1061, 1016, 961, 940, 918, 885, 856, 843, 825, 798, 756, 721, 670, 590, 533 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d$_6$*): δ 11.52 (brs, 1H), 10.12 (* 10.03, brs, 1H), 9.65 (* 9.36, brs, 1H), 8.13 (* 7.91, brs, 1H), 7.65 (* 7.52, d, J = 8.4 Hz, s, 2H), 7.59 (* 7.24, d, J = 8.0 Hz, s, 2H), 7.13 (brs, 1H), 6.70 (t, J = 8.4 Hz, 2H), 2.56 (brs, 2H), 2.06 (* 2.01, s, 3H), 1.52 (brs, 2H), 1.25-1.06 (m, 24 H), 0.85 (brs, 3H) (* Rotamers); ESI-MS: m/z, 508.0 (M+1).

(*E*)-N'-(4-fluorobenzylidene)-2-hydroxy-6-pentadecylbenzohydrazide (6f)
Pale yellow solid; M.P.: 92-94 ^{o}C; Yield: 80%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.62 (brs, 1H), 9.60 (* 9.38, brs, 1H), 8.20 (* 7.96, s, 1H), 7.73 (brs, 2H), 7.36 (* 7.12, brd, J = 6.8 Hz, 1H), 7.26 (brs, 2H), 6.70 (dd, J = 8.0, 10.8 Hz, 2H), 2.56 (brs, 2H), 1.50 (brs, 2H), 1.20-1.06 (m, 24 H), 0.83 (brs, 3H) (* Rotamers); ESI-MS: m/z, 468.2 (M+1).

(*E*)-N'-(4-(trifluoromethoxy)benzylidene)-2-hydroxy-6-pentadecylbenzohydrazide (6g)
Pale yellow solid; M.P.: 121-122 ^{o}C; Yield: 78%; IR (KBr): υ_{max} 3562, 3182, 2925, 2854, 1646, 1618, 1586, 1511, 1505, 1465, 1379, 1343, 1262, 1219, 1204, 1162, 1139, 1101, 1017, 962, 921, 879, 847, 805, 757, 712, 513 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.72 (brs, 1H), 9.69 (* 9.40, brs, 1H), 8.23 (* 8.01, brs, 1H), 7.81 (* 7.44, brs, 2H), 7.44 (* 7.33, brs, 2H), 7.12 (brs, 1H), 6.68 (brs, 2H), 2.40 (brs, 2H), 1.51 (brs, 2H), 1.21-1.04 (m, 24 H), 0.85 (brs, 3H) (* Rotamers); ESI-MS: m/z, 533.1 (M-1).

(*E*)-N'-(4-Cyanobenzylidene)-2-hydroxy-6-pentadecylbenzohydrazide (6h)
Brown solid; M.P.: 130-132 ^{o}C; Yield: 80%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.89 (brs, 1H), 9.73 (* 9.40,s, 1H), 8.26 (* 8.03, s, 1H), 7.89 (* 7.77, d, J = 8.0 Hz, 2H), 7.86 (* 7.48, d, J = 8.0 Hz, 2H), 7.17-7.10 (m, 1H), 6.75-6.68 (m, 2H), 2.55 (brs, 2H), 1.50 (brs, 2H), 1.23-1.02 (m, 24 H), 0.85 (t, J = 6.4 Hz, 3H) (* Rotamers); ESI-MS: m/z, 476.2 (M+1).

(*E*)-N'-(4-(methylsulfonyl)benzylidene)-2-hydroxy-6-pentadecylbenzohydrazide (6i)
White solid; M.P.: 111-112 ^{o}C; Yield: 80%; IR (KBr): υ_{max} 3348, 3252, 3050, 3027, 3005, 2918, 2849, 1687, 1667, 1607, 1583, 1547, 1520, 1487, 1464, 1354, 1305, 1283, 1250, 1148, 1128, 1086, 965, 921, 917, 849, 827, 796, 775, 754, 735, 621, 578, 549, 533, cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6): δ 11.88 (brs, 1H), 9.73 (* 9.44, s, 1H), 8.28 (* 8.16, brs, 1H), 8.00 (* 7.86, d, J = 8.0 Hz, 2H), 7.95 (* 7.57, d, J = 8.0 Hz, 2H), 7.15 (brt, J = 8.0 Hz,1H), 6.75-6.70 (m, 2H), 3.28 (* 3.16, s, 3H), 2.55 (brs, 2H), 1.52 (brs, 2H), 1.22-1.07 (m, 24 H), 0.84-0.83 (m, 3H) (* Rotamers); ESI-MS: m/z, 529.0 (M+1).

(*E*)-N'-(4-Nitrobenzylidene)-2-hydroxy-6-pentadecylbenzohydrazide (6j)
Yellow solid; M.P.: 70-72 ^{o}C; Yield: 80%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.96 (s, 1H), 9.74 (* 9.46, s, 1H), 8.88 (* 8.40, brs, 1H), 8.30 (* 7.95, d, J = 8.8 Hz, 2H), 8.15 (* 7.56, d, J = 8.8 Hz, 2H), 7.13-7.15 (m, 1H), 6.75-6.72 (m, 2H), 2.41 (t, J = 6.8 Hz, 2H), 1.48 (brs, 2H), 1.23-1.01 (m, 24 H), 0.85 (t, aj = 6.8 Hz, 3H) (* Rotamers); ESI-MS: m/z, 596.1 (M+1).

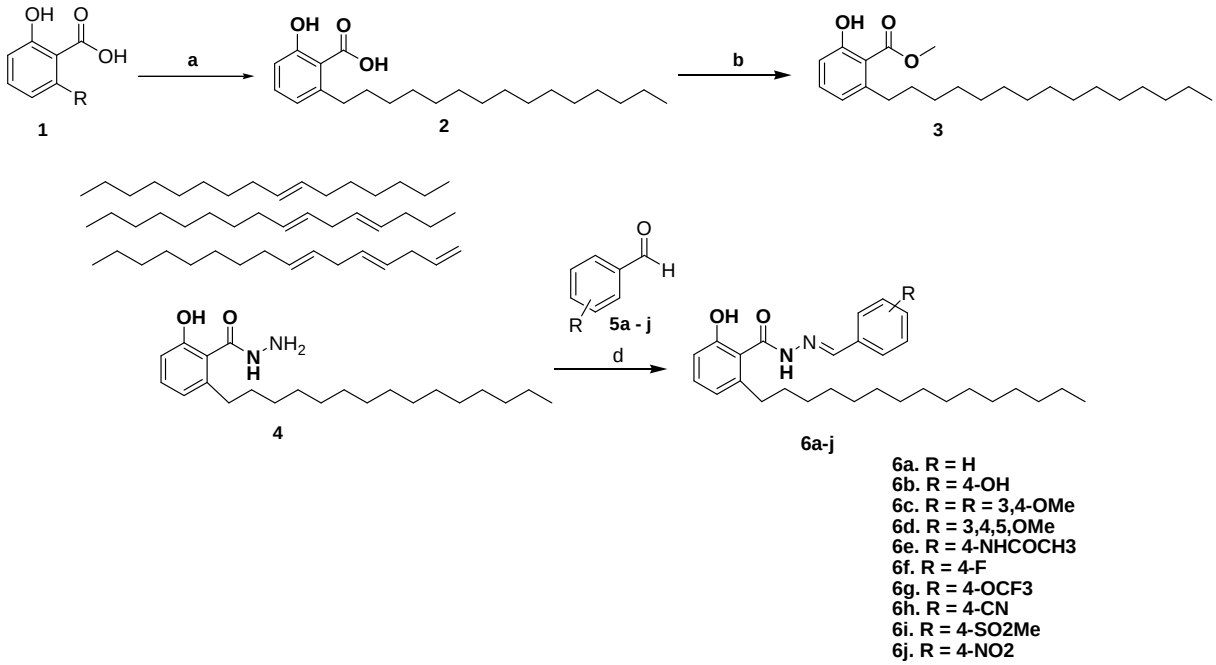
ANTIBACTERIAL AND ANTIFUNGAL BIOASSAY
The antimicrobial activities of the synthesized compounds (**6a- j**) were carried out by agar well diffusion method [28]. The synthesized compounds were tested for antibacterial activity against Gram negative bacteria *viz.*, *Pseudomonas aeruginosa* (MTCC 424) and *Escherichia coli* (MTCC 443) *and* Gram positive bacteria *viz.*, *Staphylococcus aureus* (MTCC 96) and *Streptococcus pyogenes* (MTCC 442) *and against fungal strain, Aspergillus.niger (MTCC 282)* and *Candida.albicans* (*MTCC 227*) at concentrations of 25, 50, 100, 250 µg/mL.

Soyabean casein digest agar Media (40g, Hi-Media) mixed with 1000 mL Milli Q water and then sterilized in autoclave at 15lb pressure for 20 minutes. The sterile Soyabean casein digest agar media solution is allowed to cool at 45°C. To this 1 ml of specified bacterial or Fungi test organism is added. These preparations are then poured into Petri dishes of 90 mm diameter and allowed to solidify medium. The inoculation was done under aseptic conditions and when the medium was in molten state. The solidified plates were bored with 8mm diameter cork borer. The plates with wells were used for the antimicrobial studies.

Test solutions was prepared by dissolving the synthesized compounds (**6a-j**) in DMSO at various concentrations such as 25, 50, 100, 250 µg/mL. 100 µl each concentration of these compounds was added in the bored place. Ampicillin and Greseofulvin was used as a standard reference (in case of bacterial and fungal activity) and DMSO was used as a control solvent (added by using a micropipette) which did not possess any inhibition zone. The plates were incubated at 30-35ºC for 24 hours for bacterial activity and at at 20-25°C for 24 hrs for fungal activity. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated.

RESULTS AND DISCUSSION

To the best of our knowledge, the synthesis of 2-hydroxy-6-pentadecylbenzohydrazide **4** and the subsequent hydrazone derivatives **6a-j** is not reported in the literature so far. The synthesis of novel (*E*)-N'-substituted-benzylidene-2-hydroxy-6-pentadecylbenzohydrazide derivatives **6a-j** was synthesized from 2-hydroxy-6-pentadecylbenzohydrazide **4** and the synthetic sequence is illustrated in **scheme-1**. 2-hydroxy-6-pentadecylbenzohydrazide **4,** which in turn was prepared from methyl 2-hydroxy-6-pentadecylbenzoate **3** in presence of hydrazine-hydrate (aqueous 99% w/v) in methanol for 48 h in sealed tube. The methyl ester derivative **3** was prepared from anacardic acid **2** in presence of amberlyst-15 (20% w/w) in methanol at 50 ^{o}C in sealed tube for 24 h. The 2-hydroxy-6-pentadecylbenzohydrazide **4** obtained was recrystallized from ethanol to afford good yields of product with high purity. Next, (*E*)-N'-substituted-benzylidene-2-hydroxy-6-pentadecylbenzohydrazide derivatives **6a-j** were prepared through the condensation reaction of 2-hydroxy-6-pentadecylbenzohydrazide **4** with different aromatic aldehydes **5a-j** in ethanol at reflux for 30 min. Anacardic acid **2** was obtained by hydrogenation of anacardic ene mixture **1** as per the recently reported literature procedure [11-15]. The synthesis and exploration of biological activity of some more new hydrazone derivatives from the condensation of 2-hydroxy-6-pentadecylbenzohydrazide **4** with various aryl and hetroaryl aldehydes is an ongoing research in our group and will be published elsewhere.



Scheme-1: Synthesis of some novel hydrazone derivatives of anacardic acid (6a-j)

Experimental conditions: *Reagents and Conditions:* a) 10% Pd-C, EtOH, H_2, 50 psi, 2 h; b) Amberlyst-15 (20% w/w), methanol, reflux, sealed tube, 24 h; c) aqueous; Hydrazine-hydrate (99% w/v), methanol, reflux, sealed tube, 48 h; c) aryl aldehydes **5a-j**, ethanol, reflux, 30 minutes

The structural confirmation of (*E*)-N'-substituted benzylidene-2-hydroxy-6-pentadecylbenzohydrazide derivatives **6a-j** was accomplished by spectroscopic techniques, including ^{1}H NMR, IR and mass spectroscopic methods. Two sets of signals were observed for all groups in the ^{1}H NMR spectra of each compound indicating the possibility of equilibrium and interconversion between rotamers (and/or configurational isomers) in solution, determination of E- or Z- geometry of C=N bond by ^{1}H NMR was based on the earlier report, that *N*-acylhydrazones derived from aromatic aldehydes in solution remained in the *E* form, because of the hindered rotation on the imine bond [29], we considered *E*- geometry in our case [30]. Moreover, all compounds were found to exist as a mixture of two rotameric forms in solution [30, 31] e.g. antiperiplanar (*ap*) and synperiplanar (*sp*) as indicated by their ^{1}H NMR

spectra. As an example, the ^{1}H NMR spectra of (*E*)-N'-(3,4,5-trimethoxybenzylidene)-2-hydroxy-6-pentadecylbenzohydrazide (**6d**) is discussed as follows: The proton signals resonating at 11.65 (* 11.61, singlet, 1H) ppm, 9.66 (* 9.37, singlet, 1H) ppm and 8.13 (* 7.85, singlet, 1H) ppm corresponds to –O**H**, -CO-N**H**- and –N=C**H**- groups in the scaffold respectively. The two triplet signals in the aromatic region at 7.13 (*7.08, 1H) ppm and 6.72 (* 6.66, 2H) ppm corresponds to the anacardic phenyl ring while the signal at 6.98 (* 6.64, singlet, 2H) corresponds to the 3,4,5-trimethoxy phenyl ring. The signals at 3.83 (* 3.70, br.s, 2H) ppm, 1.53 (*1.07, brs, 2H), 1-22-1.17 (multiplet, 24H) and 0.84 (triplet, 3H) corresponds to the side chain of the anacardic acid. The mass spectra of compounds showed (M+1) peaks, is in agreement with their molecular formula. The IR spectral data of the compounds **6a-j** were found to be in the expected range and gave the evidence for the expected functional groups.

Table-1 Results of Antibacterial Bioassay of Compounds 6a-j (concentration in DMSO)

Compound No.	Concentration of the compound µg/ml	Zone inhibition					
		Gram negative bacteria		Gram positive bacteria		fungi	
		Pseudomonas aeruginosa	*Escherichia coli*	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Candida albican*	*Aspergillus niger*
6a	**25**	-	-	-	-	-	-
	50	15	13	12	13	-	-
	100	16	15	14	15	9	12
	250	18	18	16	17	11	14
6b	**25**	-	-	-	-	-	-
	50	13	13	11	12	--	-
	100	15	16	13	14	16	17
	250	17	18	16	17	18	21
6c	**25**	-	-	-	-	-	-
	50	17	18	17	19	-	-
	100	21	21	19	20	10	12
	250	24	23	21	22	11	12
6d	**25**	-	-	-	-	-	-
	50	18	18	16	17	-	-
	100	19	18	17	17	16	19
	250	22	22	20	21	18	23
6e	**25**	-	-	-	-	-	-
	50	-	-	-	-	-	-
	100	9	10	8	8	11	13
	250	11	12	12	11	10	13
6f	**25**	-	-	-	-	-	-
	50	12	12	11	10	-	-
	100	14	15	13	13	12	14
	250	17	17	16	16	12	14
6g	**25**	-	-	-	-	-	-
	50	15	16	15	16	-	-
	100	17	18	17	18	10	12
	250	18	18	16	16	10	13
6h	**25**	-	-	-	-	-	-
	50	-	-	-	-	-	-
	100	14	13	11	10	11	14
	250	16	15	13	12	11	12
6i	**25**	-	-	-	-	-	-
	50	19	19	18	19	-	-
	100	21	19	17	17	17	22
	250	23	22	20	20	18	20
6j	**25**	-	-	-	-	-	-
	50	14	14	13	12	-	-
	100	16	15	15	13	10	13
	250	18	17	16	15	10	15
Ampicillin	**250**	20	20	18	19	--	--
Greseofulvin	**250**					24	28

"—" *no activity*

Antibacterial and antifungal evaluation

The antibacterial and antifungal evaluation data for compounds **6a-j** is presented in **Table-1**. The antibacterial activity of the samples is assessed using the different concentration of the sample i.e., moderate, good and excellent

activity. The zone of inhibition was measured in mm, on three different concentrations 50, 100, 250 µg/mL. Maximum inhibitory activity was observed for 250 µg/mL and compounds showed their effect in a dose dependant manner. The zone of inhibition increased as the concentration of the sample increased. From **table-1**, it is observed that compound **6c** with 3.4-dimethoxy substitution, compound **6i** with 4-methyl sulphonyl substitution and **6d** with 3,4,5-OMe, exhibited excellent antibacterial activity with reference to the standard drug ampicillin (tested against the gram positive and gram negative bacteria). Compounds **6a** (R = H), **6b** (R = 4-OH), **6j** (R = 4-NO2), **6f** (4-F), **6g** (R = 4-OCF_3) showed good antibacterial activity while the compounds **6e** and **6h** with substitutions 4-$NHCOCH_3$ and 4-CN showed moderate antibacterial activity and compound **6j** with R = 4-CN showed nil antibacterial activity.

In case of antifungal activity, most of the compounds **6a-j** showed moderate to weak fungal activity at concentration 100 and 250 µg/mL against the standard drug Greseofulvin.

CONCLUSION

The newly prepared (*E*)-N'-substituted-benzylidene-2-hydroxy-6-pentadecylbenzohydrazide derivatives **6a-j** were synthesized from 2-hydroxy-6-pentadecylbenzohydrazide **4** as the key intermediate. The structural confirmation of these derivatives **6a-j** was accomplished by spectroscopic techniques, including ^{1}H NMR, IR and mass spectroscopic methods. The antibacterial and antifungal activities of the synthesized compounds (**6a- j**) were carried out by well diffusion method. The zone of inhibition increased as the concentration of the sample increased. It is observed compound **6c** with 3.4-dimethoxy substitution, compound **6i** with 4-methyl sulphonyl substitution and **6d** with 3,4,5-OMe, exhibited excellent antibacterial activity with reference to the standard drug ampicillin (tested against the gram positive and gram negative bacteria). Most of the compounds **6a-j** showed moderate to weak fungal activity against the standard drug Greseofulvin

REFERENCES

[1] J.H.P. Tyman, *Chem. Soc. Rev.*, **1979**, 8, 499.
[2] A. Hari Kishore, B.M. Vedamurthy, K. Mantelingu, S. Agrawal, B.A. Ashok Reddy, S. Roy, K.S. Rangappa, T.K. Kundu, *J. Med. Chem.*, **2008**, 51,792.
[3] K. Mantelingu, A.H. Kishore, K. Balasubramanyam , G.V. Kumar, M. Altaf, S.N. Swamy, R. Selvi, C. Das, C. Narayana, K.S. Rangappa, T.K. Kundu, *J.Phys. Chem. B.*, **2008**, 111, 4527.
[4] B. Sung, M.K. Pandey, K.S. Ahn , T. Yi, M.M. Chaturvedi, M. Liu, B. Aggarwal, *Blood.*, **2008**, 111**,** 4880.
[5] G. Sbardella, S. Castellano, C. Vicidomini, D. Rotili, A. Nebbioso, M. Miceli, L. Altucci, A. Mai, *Bioorg. Med. Chem. Lett.*, **2008**, 18, 2788.
[6] L. Cui , J. Miao, T. Furuya, Q. Fan , X. Li , P.K. Rathod, X.Z. Su, L. Cui, *Eukaryotic Cell.*, **2008**, 7, 1200.
[7] S.V. Shoba, C.S. Ramadoss, B. Ravindranath, *J. Nat. Prod.*, **1994**, 57**,** 1755
[8] T.J. Ha, I. Kubo, *J. Agric. Food Chem.*, **2005**, 53**,** 4350.
[9] I. Kubo, H. Muroi, M. Himejima, Y. Yamigiwa, *J. Agric. Food Chem.*, **1993**, 41**,** 1016.
[10] J.L. Gillerman, N.J. Walsh, N.K. Werner, H. Schlenk, *Can. J. Microbiol*., **1969**, 15, 1219.
[11] N. Subhakara Reddy, V. Ravi Kumar, V. Himabindu, B. Ram, A. Srinivas Rao., *Letters in Drug Design & Discovery.*, **2011**, 8, 626.
[12] N. Subhakara Reddy, V. Ravi Kumar, V. Himabindu, B. Ram, A. Srinivas Rao., *Letters in Drug Design & Discovery.*, **2011**, 8, 972.
[13] N. Subhakara Reddy, A. Srinivas Rao, M. Adharvana Chari, V. Ravi kumar, V. Jyothi, V. Himabindu, *International Journal of Organic Chemistry.*, **2011**, 1, 167.
[14] N. Subhakara Reddy, A. Srinivas Rao, M. Adharvana Chari, V. Ravi kumar, V. Jyothi, V. Himabindu, *Letters in Organic Chemistry.*, **2012**, 9, 287-293.
[15] N. Subhakara Reddy, A. Srinivas Rao, M. Adharvana Chari, V. Ravi kumar, V. Jyothi, V. Himabindu, *Journal of chemical sciences*., **2012**, 124, 723.
[16] J.A. Sclafani, M.T. Maranto, T.M. Sisk, S.A. Van Arman , *J Org Chem.*, **1996**, 61 3221.
[17] J.P. Corhnelissen, J.H. Van Diemen, L.R. Groeneveld, J.G. Haasnoot, A.L. Spek, J ReediskInorgChem., **1992**, 31, 198..
[18] M. T. Cocco, C. Congiu, V. Lilliu and V. Onnis, *Bioorganic & Medicinal Chemistry.*, **2006**, 14, 366.
[19] J. R. Dimmock, S. C. Vasishtha, J. P. Stables, *Eur.J.Med.Chem.*, **2008**, 35, 241.
[20] A. Cukurovali, B. Yilmaz, S. Gur , C. Kazaz, *Eur.J. Med.Chem.*, **2006**, 41, 201.

[21] P. C. Lima, L. M. Lima, K. C. Silva, P. H. Leda, A. L. P. Miranda, C. A. M. Fraga and E. J. Barreiro, European Journal of Medicinal Chemistry, Vol. 35, No. 2, **2000**, pp. 187-203.
[22] G. A. Silva, L. M. M. Costa, F. C. B. Brito, A. L. P Miranda, E. J. Barreiro, C. A. M. Fraga, *Bioorg. Med.Chem.*, **2004**, 12, 3149.
[23] M. G. Mamolo, V. Falagiani, D. Zampieri, U. Vio, E. Banfi and G. Scialino, *IL Farmaco.*, **2004**, 58, 631.
[24] G. U. Salgin, K. N. Gokham, O. Gostal, Y. Koysal, E Kilici, S. Isik, G. Aktay, M. Ozalp, *Bioorg.Med.Chem.*, **2007**, 15, 5738.
[25] A. Imramovsky, S. Polanc, J. Vinsova, M. Kocevar, J. Jampitek, Z. Reckova, J. A. Kaustova, *Bioorg.Med.Chem.*, **2007**, 15, 2551.
[26] K.D. Olczak, M. Daszkiewicz, K. Slawinska, D.B. Baginska, D. Gozdowski, P. Daszkiewicz, B. Fronc, K. Semczuk, J. Oral Pathol.Med., **2012**, 41, 568.
[27] M. Danishuddin, L. Kaushal, B.M. Hassan, A.U. Khan, AMDD: antimicrobial drug database, Genomics Proteomics Bioinformatics., **2012**, 10, 360.
[28] R. D. Biljana, S. R. Niko, S. D. Vidoslav, R. D. Vukicević, R. M. Palić, *Molecules*., **2010**, 15, 2246.
[29] G. Palla, G. Predieri, P. Domiano, *Tetrahedron.*, **1986,** 42, 3649.
[30] Sarbani Pal *et al*. *J. Chem. Pharm. Res.*, **2010**, 2, 393.
[31] R.B. Palmer, N.H. Andersen, *Bioorg. Med. Chem. Lett*., **1996,** 6, 2173.