



Synthesis and antimicrobial potential of novel polyphenolic dendrimers

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Abstract

In the present work, eleven dendrimeric compounds (11-21) were synthesized by Michael addition reaction to avoid the structural defects at higher generations by using large excess of Michael donor (EDA). The synthesized compounds were characterized by IR, NMR and Mass Spectroscopy. Progress of the chemical reactions and the purity level of the compounds was evaluated using TLC plates. The biological studies of synthesized dendrimers – (11-21) were carried out for anti-bacterial and anti-fungal activities. The anti-bacterial activity was carried out by using Gram +ve strains viz. *B. subtilis* and Gram -ve strains viz. *E. coli*, *P. aeruginosa*, *S. aureus*. The anti-fungal activity was carried out by using anti-fungal strains viz. *C. albicans*, *A. niger*, *T. mentagrophytes* and *M. audouinii*. The anti-microbial activity was evaluated by Modified Kirby-Bauer method by using Ciprofloxacin as standard drug for anti-bacterial activity and Griseofulvin for anti-fungal activity at 10 mg mL⁻¹ conc. respectively.

Key words: Michael addition, TLC plates, anti-bacterial, anti-fungal and Griseofulvin.

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Introduction

Supramolecular chemistry can be defined as designing and development of novel functional systems through joining of multiple chemical components by non-covalent interactions. This chemistry has various synonyms like non-molecular chemistry, chemistry beyond the molecules and chemistry of molecular assemblies having intermolecular bonds (1). The first publication in the field of supramolecular chemistry was published in 2012. This chemistry was established as a discipline by Nobel Prize awarded scientists (Charles J. Pedersen, Donald J. Cram and Jean-Marie Lehn in 1987) which is recently being explored in a lot of areas such as logic gates, molecular vehicles, molecular motors and molecular wires. Again, in 2016, Nobel Prize was awarded to Bernard L. Feringa, J. Fraser Stoddart and Jean-Pierre Sauvage to the field of Supramolecular chemistry for design and development of molecular machines (2). Since,

from last few years, the area of Supramolecular chemistry has aroused as a multidisciplinary area for researchers involved in different fields such as analytical chemistry, chemical science, material science, biological and physical science to provide facilities for the developments of these different fields. By considering the vast scope of Supramolecular chemistry in mind, it is almost impossible to cover all the fields and challenges which has been emerged from this rich field (3).

Dendrimers are a unique class of polymers that are distinguished from all other synthetic macromolecules by their globular shape resulting from their perfectly branched architecture and their monodisperse nature (4). Dendritic macromolecules play an increasingly important role in materials and surface sciences, where applications utilizing polymer thin films can benefit from their distinctive chemical and physical properties (5). Recent investigations have highlighted the use of dendrimers as



functional surfaces and as interfacial materials for applications in membranes, adhesion, microelectronics and in chemical and biological sensing (6). Dendrimers are radially symmetric molecules having nano size, monodisperse and homogenous structures consisting of tree like branches or arms (7). Fritz Vogtle in 1978, first time discovered these hyper branched molecules followed by Donald Tomalia and co-workers in the early 1980s. Other scientists named these synthesized macromolecules as arborols which means in Latin tree. Dendrimers also known as cascade molecules but this name is not as much established as Dendrimers (8). Dendrimers are the molecules having symmetric branching units constructed around a linear core of polymer and also known as monodisperse macromolecules. The regularity of structure and monodispersity of Dendrimers has arisen from layer by layer and controlled arrangement through synthesis (9). The generation number and the number of surface groups of Dendrimers increase generally by layer addition.

Materials and Methods

Experimental

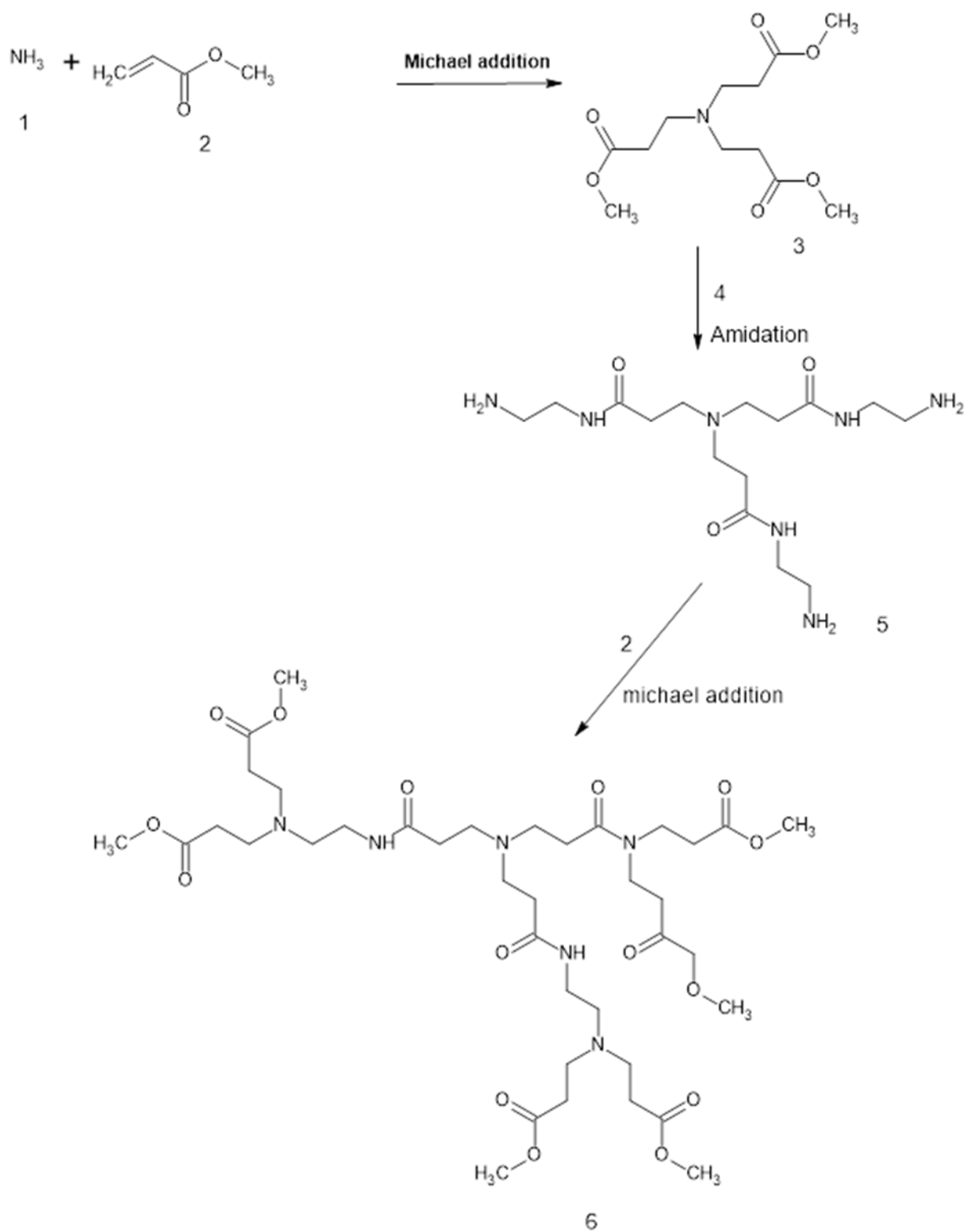
The chemicals used for experimental work were commercially procured from diverse chemical

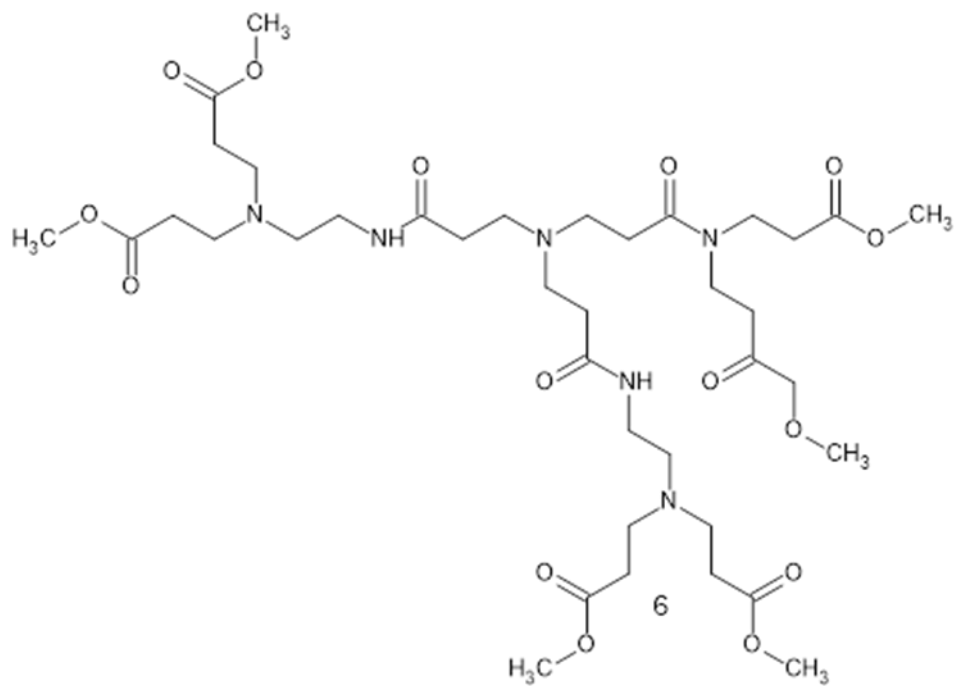
Synthetic scheme:

organizations - E. Merck India Ltd., C.D.H., S.D. Fine Chem. Ltd. and have been of L.R. Grade and purified by using widespread manner earlier than their use. MR-VIS Visual melting point apparatus (LAB India) was used to record the melting points of synthesized compounds and were uncorrected. The IR spectra had been recorded on Bruker Tensor-27, USA, spectrophotometer using KBr. ¹H-NMR spectra were recorded by using Bruker NMR spectrophotometer at 400 MHz by dissolving the compounds in DMSO in reference to Tetramethylsilane as internal standard and shift (δ) values are reported in parts per million (ppm). Water UPLC-TQD Mass spectrophotometer operated at 70.21 eV was used to record the mass data of compounds. The results are observed by molecular ion peaks. The spectral analysis was carried out at Punjab University, Chandigarh (10).

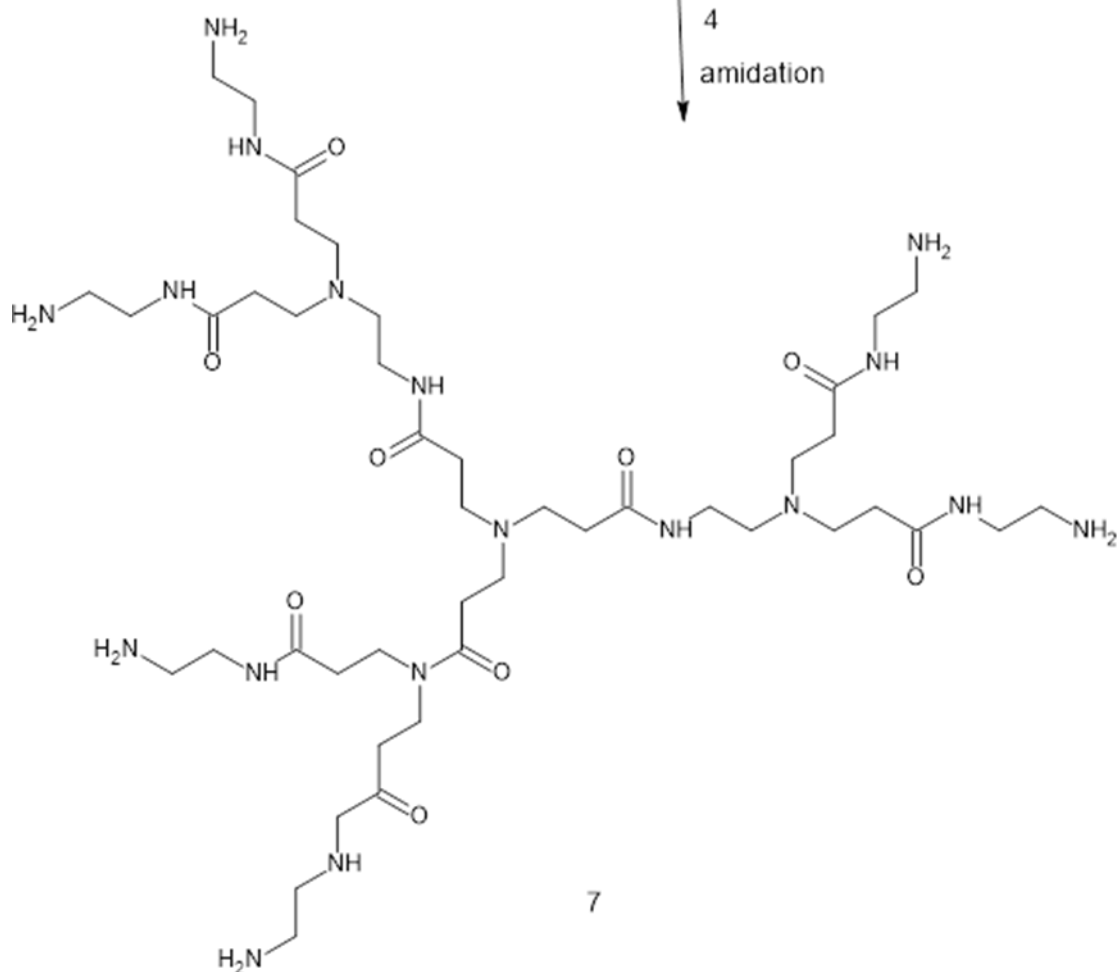
Progress of the chemical reactions and the purity level of the compounds was evaluated using TLC plates by solvent systems: CH₂Cl₂:CH₃OH - (9:1). The visualization of spots on TLC plates was carried out in iodine chamber and UV cabinet at long wavelength under UV lamp (11).

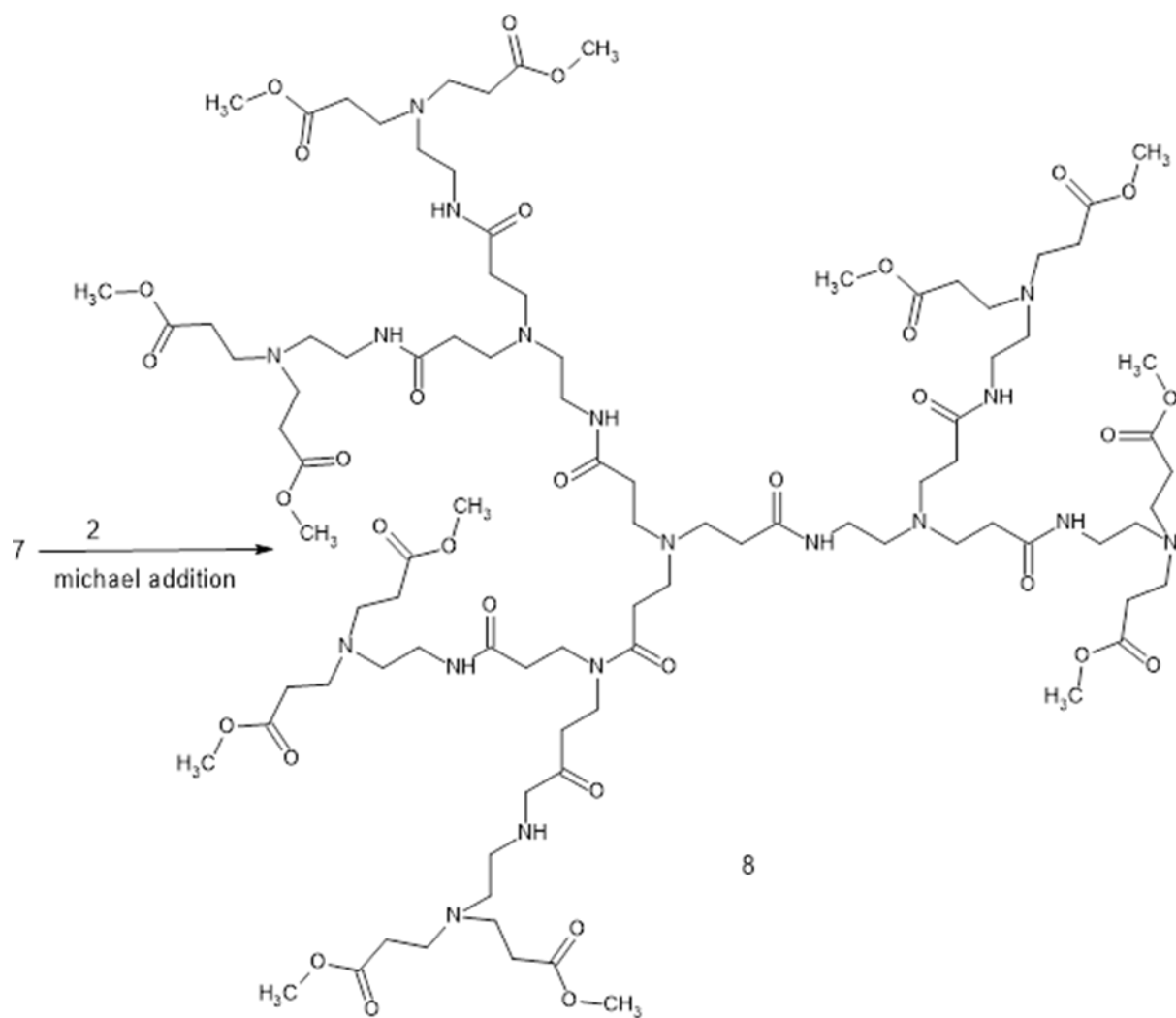






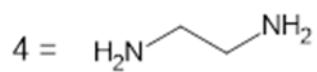
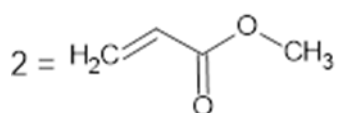
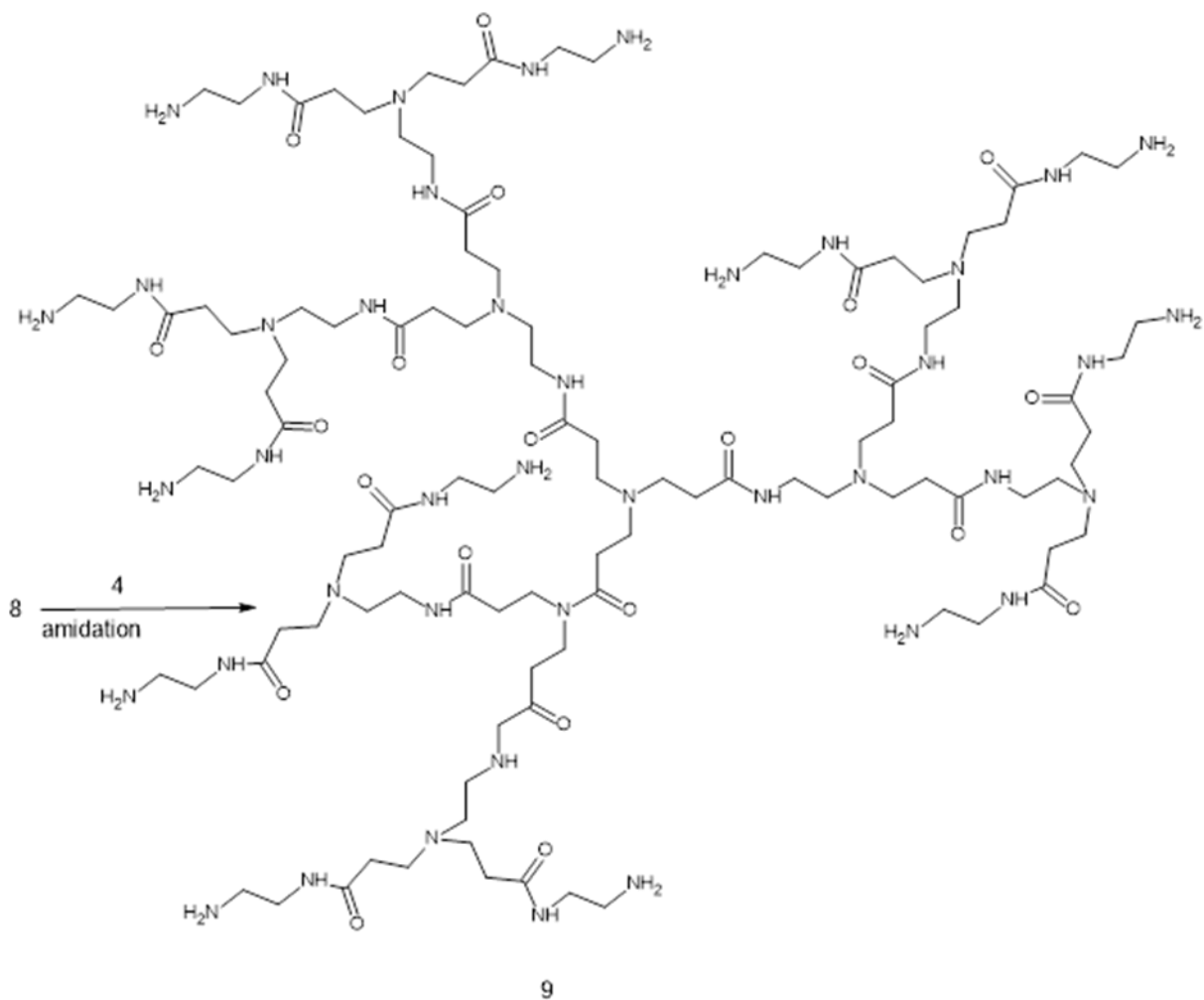
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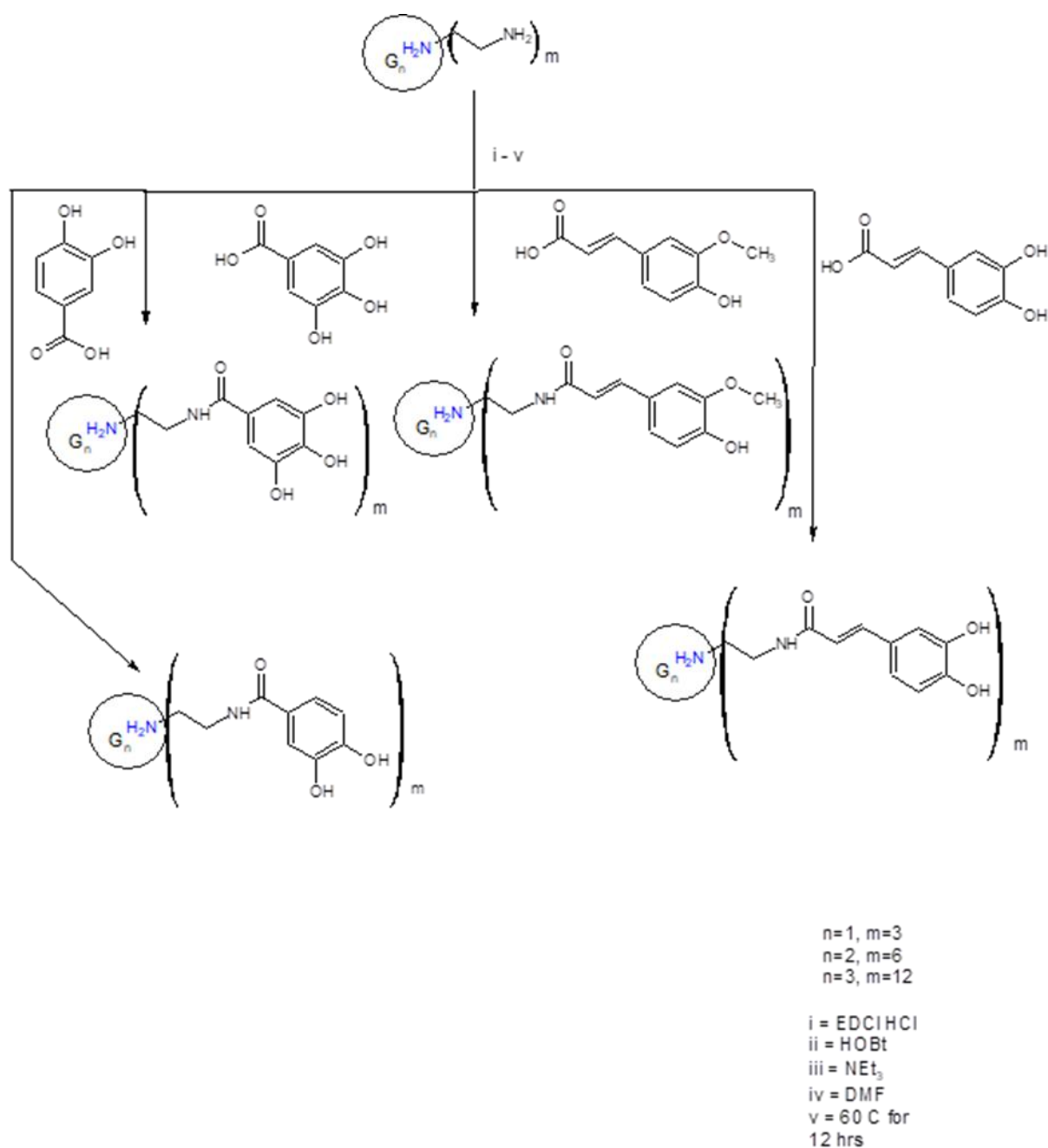


Fig. 1

Procedures used to synthesize the dendrimers:

I. The titled compounds were synthesized according to following steps:

1. Ammonia reacts with methyl prop-2-enoate according to Michael addition to form compound (3).
2. Amidation of Compound (3) yielded compound (5).
3. Compound (5) reacted with methyl prop-2-enoate to form compound (6).

4. Amidation of Compound (6) yielded compound (7).
5. Compound (7) reacted with methyl prop-2-enoate to form compound (8).
6. Amidation of Compound (8) yielded compound (9).
7. Then, Galic acid reacted with compounds (5,7 & 9) in the presence of EDCL.Hcl, HoBt, NEt₃ in DMF at 60°C for 12 hrs to give final compounds (10-13).



8. Propanedioic acid reacted with compounds (5,7 & 9) in the presence of EDCL.Hcl, HoBt, NEt3 in DMF at 60°C for 12 hrs to give final compounds (13-16).

9. 3,4-dihydrocinnamic acid reacted with compounds (5,7 & 9) in the presence of EDCL.Hcl, HoBt, NEt3 in DMF at 60°C for 12 hrs to give final compounds (17-21).

II. Synthetic procedures:

The dendrimeric compounds were synthesized by using divergent approach. All the titled compounds were synthesized by using divergent synthesis approach by using EDA (ethylenediamine) as initiation molecule followed by Michael addition reaction in which four arms are added on nitrogen of EDA. After this, in second step, EDA was again reacted on these formed four arms through amidation reaction shown in fig. 1 above under scheme of synthesis (12).

The final synthesized dendrimers were characterized by following spectral methods:

Compound-5:

IR (KBr): ν 3102.36 (C–H stretch CH₃), 2932.68 (C–H asym stretch CH₂), 2831.74 (C–H sym stretch CH₂), 1689.23 (C=O stretch), 1430.28 (C–C stretch skeletal bands), 1022.09 cm⁻¹(C–N stretch).

¹H NMR (DMSO): δ 1.7231 (s, 9H, -COOCH₃), 2.8533-2.9303 (m, 12H, CH₂-CH₂N-).

Yield: 60.8 %; M.P.: 82-84° C. The purity of compound was checked by TLC using CH₂Cl₂:CH₃OH – (9:1) as solvent system and iodine vapours as visualising agent and only one spot was obtained at R_f value 0.51.

Compound-7:

IR (KBr): ν 3304.40 (N–H stretch amide), 2973.46 (C–H stretch CH₃), 2945.73 (C–H asym stretch CH₂), 2855.29 (C–H sym stretch CH₂), 1686.44 (C=O stretch 2° amide), 1536.02 (NH bend 2° amide), 1468.53 (C–C stretch skeletal bands), 1081.87 cm⁻¹(C–N stretch amide).

¹H NMR (DMSO): δ 2.3030 (s, 18H, -COOCH₃), 2.5272-2.6549 (m, 6H, CH₂-CH₂CONH-), 6.2767 (s, 2H, NH, CONH-).

Yield: 57.7 %; M.P.: 87-89° C. The purity of compound was checked by TLC using

CH₂Cl₂:CH₃OH – (9:1) as solvent system and iodine vapours as visualising agent and only one spot was obtained at R_f value 0.46.

Compound-9:

IR (KBr): ν 3289.96 (N–H stretch amide), 3070.12 (aromatic C–H stretch), 2977.63 (C–H stretch CH₃), 2886.63 (C–H stretch CH₂), 1688.16 (C=O stretch 2° amide), 1572.66 (NH bend 2° amide), 1469.49 (C–C stretch skeletal bands), 1051.01 cm⁻¹(C–N stretch amide).

¹H NMR (DMSO): δ 1.7752 (s, 108H, -COOCH₃), 2.1567-2.1767 (m, 24H, CH₂-CH₂CONH-), 2.2765-2.2945 (m, 24H, CH₂-CH₂CONH-), 3.0675-3.1165 (m, 12H, CH₂, CH₂NH-), 3.2260-3.2503 (m, 12H, CH₂, CH₂NH-), 7.2139-7.2245 (m, 6H, NH, CONH-), 7.7752 (s, 3H, NH, CONH-).

Yield: 52.3 %; M.P.: 94-96° C. The purity of compound was checked by TLC using CH₂Cl₂:CH₃OH – (9:1) as solvent system and iodine vapours as visualising agent and only one spot was obtained at R_f value 0.49.

Compound-10:

IR (KBr): ν 3467.38 (O–H stretch), 3350.71 (N–H stretch), 3078.80 (aromatic C–H stretch), 2972.86 and 2864.49 (C–H stretch CH₃), 1681.69 (C=O stretch), 1494.56 (aromatic C=C stretch), 1584.24 (NH bend 2° amide), 1428.99 (C–C stretch skeletal bands), 1099.23 cm⁻¹(C–N stretch).

¹H NMR (DMSO): δ 4.6533-4.9303 (m, 12H, CH₂-CH₂CONH-), 4.5363-4.5533 (m, 12H, CH₂-CH₂NHCOAr), 6.3231-6.4539 (m, 6H, aromaticH), 9.5330 (s, 6H, NH, CONH-), 10.4767 (s, 6H, aromatic OH).

FABMS m/z: 769[(M+1)⁺6], 768[(M+H)⁺, 100]

Yield: 46.4 %; M.P.: 99-102° C. The purity of compound was checked by TLC using CH₂Cl₂:CH₃OH – (9:1) as solvent system and iodine vapours as visualising agent and only one spot was obtained at R_f value 0.33.

Compound-11:

IR (KBr): ν 3395.95 (O–H stretch), 3035.90 (aromatic C–H stretch), 2974.04, 2939.91 (C–H asym stretch CH₂), 2856.99 (C–H sym stretch CH₂), 1722.65 (C=O stretch), 1469.49 (aromatic C=C stretch), 1322.93 (O–H bend), 1011.48 cm⁻¹(C–N stretch).



¹H NMR (DMSO): δ 2.8567-2.8945 (m, 6H, CH₂-CH₂CONH-), 4.1931(m, 6H, CH₂,CH₂NH-), 4.5359-4.5536 (m, 6H,CH₂,CONHCH₂-), 4.6255 (m, 6H, CH₂-CH₂NHCOAr), 4.6549-4.9308 (m, 6H, aromatic H), 9.6030 (s,6H, NH, CONH),10.3767 (s, 9H, aromatic OH).

FABMS m/z: 817[(M+1)⁺ 10], 816[(M+H)⁺, 100]

Yield: 69.7 %; M.P.: 134-136° C. The purity of compound was checked by TLC using CHCl₃:CH₃OH (9.5:0.5) as solvent system and iodine vapours as visualising agent and only one spot was obtained at R_f value 0.37.

Compound-12:

IR (KBr): ν 3352.64 (N-H stretch amide), 3098.83 (aromatic C-H stretch), 2940.67 (C-H stretch CH₃),1733.44, 1688.73 (C=O stretch 2° amide), 1630.60 (C=N stretch), 1590.02 (NH bend 2° amide), 1465.63 (aromatic C=C stretch), 1438.50 (C-C stretch skeletal bands), 1178.29, 1050.05 cm⁻¹ (C-N stretch 2° amide).

¹H NMR (DMSO): δ 2.1567-2.1767 (m, 3H, CH, -CH=CH-), 2.2765-2.2945 (m, 3H, CH, -CH=CH-) 3.0675 (s, 9H,CH₃,OCH₃), 3.2260-2.8945 (m, 6H,CH₂-CH₂CONH-), 4.5363 (m, 6H, CH₂,CH₂NH-), 4.9163-4.9203 (m, CH₂,6H, CONHCH₂-), 5.4303 (m, 6H, CH₂-CH₂NHCOAr), 6.9139-6.9245(m, 9H, aromatic H), 7.5231 (s, 3H, aromatic OH), 8.1767(s, 6H, NH, CONH-).

FABMS m/z: 877[(M+1)⁺ 9], 876[(M+H)⁺, 100]

Yield: 52.4 %; M.P.: 138-140° C. The purity of compound was checked by TLC using CH₂Cl₂:CH₃OH - (9:1) as solvent system and iodine vapours as visualising agent and only one spot was obtained at R_f value 0.49.

Compound-13:

IR (KBr): ν 3420.14 (O-H stretch), 3308.22 (N-H stretch amide), 3088.71 (aromatic C-H stretch), 2913.35 (C-H asym stretch CH₂), 2850.95 (C-H sym stretch CH₂),1685.84 (C=O stretch 2° amide), 1587.13 (NH bend 2° amide), 1483.96 (aromatic C=C stretch), 1438.64 (C-C stretch skeletal bands), 1079.94 cm⁻¹ (C-N stretch 2° amide).

¹H NMR (DMSO): δ 2.1145-2.1460 (m, 12H, CH₂-CH₂CONH-, CH₂NH-), 2.2460 (m, 12H, CH₂,CONHCH₂-, -CH₂NHCOAr), 2.5165-2.5365 (m, 6H, -CH=CH-), 4.9163-4.9203 (m, 9H, aromatic H), 5.3303 (s, 3H, NH, CONH-), 8.1767 (s, 3H, NHCOAr), 8.7767 (s, 6H, aromatic OH).

FABMS m/z: 835[(M+1)⁺ 5], 834[(M+H)⁺, 100]

Yield: 63.5 %; M.P.: 133-135° C. The purity of compound was checked by TLC using CH₂Cl₂:CH₃OH - (9:1) as solvent system and iodine vapours as visualising agent and only one spot was obtained at R_f value 0.44.

Compound-14:

IR (KBr): ν 3465.46 (O-H stretch), 3349.19 (N-H stretch amide), 3051.37 (aromatic C-H stretch),2955.64 (C-H asym stretch CH₂), 2878.68 (C-H sym stretch CH₂),1681.84 (C=O stretch 2° amide), 1582.31(NH bend 2° amide), 1494.29 (aromatic C=C stretch), 1441.53 (C-C stretch skeletal bands), 1070.30 cm⁻¹(C-N stretch amide).

¹H NMR (DMSO): δ 2.5167 (m, 36H, CH₂-CH₂CONH-, CH₂NH-), 2.5265-2.5365 (m, 36H, CH₂,CONHCH₂-, -CH₂NHCOAr), 4.5481-4.5533 (m, 18H, aromaticH), 5.3390(s, 9H, NH, CONH-), 8.1890 (s, 12H, aromaticOH), 8.8765(s, 6H, NHCOAr).

FABMS m/z: 1833[(M+1)⁺ 11], 1832[(M+H)⁺, 100]

Yield: 72.3%; M.P.: 148-150° C. The purity of compound was checked by TLC using CH₂Cl₂:CH₃OH - (9:1) as solvent system and iodine vapours as visualising agent and only one spot was obtained at R_f value 0.21.

Compound-15:

IR(KBr): ν 3345.33 (N-H stretch amide), 3095.90 (aromatic C-H stretch),1687.77 (C=O stretch 2° amide), 1552.42(NH bend 2° amide), 1466.85 (aromatic C=C stretch), 1432.07 (C-C stretch skeletal bands), 1078.98 cm⁻¹(C-N stretch amide).

¹H NMR (DMSO): δ 3.0675-3.2381 (m, 36H, CH₂-CH₂CONH-, CH₂NH-), 3.2503 (m, 36H, CH₂,CONHCH₂-, -CH₂NHCOAr), 4.9163-4.9303 (m, 12H, aromatic H), 6.8539 (s, 9H, NH, CONH-), 7.5231(s, 6H, NHCOAr), 8.8765 (s, 18H, aromatic OH).

FABMS m/z: 1929[(M+1)⁺ 4], 1928[(M+H)⁺, 100]

Yield: 60.7 %; M.P.: 153-155° C. The purity of compound was checked by TLC using CH₂Cl₂:CH₃OH - (9:1) as solvent system and iodine vapours as visualising agent and only one spot was obtained at R_f value 0.51.

Compound-16:



IR (KBr): ν 3431.71 (O–H stretch), 3304.40 (N–H stretch amide), 3042.41 (aromatic C–H stretch), 2973.46 (C–H stretch CH₃), 2945.73 (C–H asym stretch CH₂), 2855.29 (C–H sym stretch CH₂), 1720, 1686.44 (C=O stretch 2° amide), 1585.20, 1536.02 (NH bend 2° amide), 1484.98 (aromatic C=C stretch), 1468.53 (C–C stretch skeletal bands), 1081.87 cm⁻¹ (C–N stretch amide).

¹H NMR (DMSO): δ 1.7581 (s, 18H, CH₃, OCH₃), 2.1145-2.1439 (m, 12H, -CH=CH-) 2.5167-2.5265 (m, 36H, CH₂-CH₂CONH-), 2.5365 (m, 36H, CH₂,CH₂NH-), 4.9181-4.9245 (m, 18H, aromatic H), 5.3390 (s, 9H, NH, CONHCH₂-), 8.1890 (s, 6H, NH, -CH₂NHCOAr), 8.8765 (s, 6H, aromatic OH).

FABMS m/z: 2073[(M+1)⁺7], 2072[(M+H)⁺, 100]

Yield: 74.5 %; **M.P.:** 163-165° C. The purity of compound was checked by TLC using CH₂Cl₂:CH₃OH - (9:1) as solvent system and iodine vapours as visualising agent and only one spot was obtained at R_f value 0.38.

Compound-17:

IR (KBr): ν 3472.20 (O–H stretch), 3252.67 (N–H stretch amide), 3072.05 (aromatic C–H stretch), 1689.34 (C=O stretch 2° amide), 1573.63, 1512.37 (NH bend 2° amide), 1475.28 (aromatic C=C stretch), 1430.32 (C–C stretch skeletal bands), 1095.15 cm⁻¹ (C–N stretch amide).

¹H NMR (DMSO): δ 1.6339-1.8539 (m, 36H, CH₂-CH₂CONH-), 2.8539-2.8945 (m, 36H, CH₂,CH₂NH-), 3.9131-3.9201 (m, 12H, -CH=CH-), 5.3530 (s, 9H, NH, CONHCH₂-), 7.5586-7.6263 (m, 18H, aromatic H), 8.3039 (s, 6H, NH, -CH₂NHCOAr), 8.8739 (s, 12H, aromatic OH).

FABMS m/z: 1989[(M+1)⁺2], 1988[(M+H)⁺, 100]

Yield: 79.8 %; **M.P.:** 174-176° C. The purity of compound was checked by TLC using CH₂Cl₂:CH₃OH - (9:1) as solvent system and iodine vapours as visualising agent and only one spot was obtained at R_f value 0.42.

Compound-18:

IR (KBr): ν 3302.44 (N–H stretch amide), 3037.09 (aromatic C–H stretch), 1694.48 (C=O stretch 2° amide), 1570.74 (NH bend 2° amide), 1472.38 (aromatic C=C stretch), 1432.96 (C–C stretch skeletal bands), 1074.80 cm⁻¹ (C–N stretch amide).

¹H NMR (DMSO): δ 3.0675-3.2260 (m, 48H, CH₂-CH₂CONH-), 3.2381-3.2503 (m, 120 H, CH₂,CH₂N-

), 4.9163-4.9203 (m, 36H, aromatic H), 4.9303 (s, 21H, NH, CONHCH₂-), 7.5231 (s, 12H, NH, -CH₂NHCOAr), 8.8767 (s, 24H, aromatic OH).

FABMS m/z: 4019[(M+1)⁺1], 4018[(M+H)⁺, 100]

Yield: 64.2%; **M.P.:** 183-185° C. The purity of compound was checked by TLC using CH₂Cl₂:CH₃OH - (9:1) as solvent system and iodine vapours as visualising agent and only one spot was obtained at R_f value 0.63.

Compound-19:

IR (KBr): ν 3336.28 (N–H stretch amide), 2950.05 (C–H asym stretch CH₂), 2849.52 (C–H sym stretch CH₂), 1682.86 (C=O stretch 2° amide), 1575.26 (NH bend 2° amide), 1526.68 (aromatic C=C stretch), 1448.14 (C–C stretch skeletal bands), 1080.77 cm⁻¹ (C–N stretch amide).

¹H NMR (DMSO): δ 1.7316-1.7545 (m, 48 H, CH₂-CH₂CONH-), 2.5175-2.5365 (m, 120 H, CH₂,CH₂N-), 4.5403-4.5590 (m, 24 H, aromatic H), 5.3333 (s, 21H, NH, CONHCH₂-), 8.1767 (s, 12H, NH, -CH₂NHCOAr), 8.7767 (s, 36 H, aromatic OH).

FABMS m/z: 4211[(M+1)⁺3], 4210[(M+H)⁺, 100]

Yield: 63.6 %; **M.P.:** 188-190° C. The purity of compound was checked by TLC using CH₂Cl₂:CH₃OH - (9:1) as solvent system and iodine vapours as visualising agent and only one spot was obtained at R_f value 0.73.

Compound-20:

IR (KBr): ν 3298.98 (N–H stretch amide), 3054.81 (aromatic C–H stretch), 2974.78 (C–H stretch CH₃), 2904.34 (C–H asym stretch CH₂), 2875.67 (C–H sym stretch CH₂), 1691.70 (C=O stretch 2° amide), 1595.46 (NH bend 2° amide), 1499.18 (aromatic C=C stretch), 1431.94 (C–C stretch skeletal bands), 1046.18 cm⁻¹ (C–N stretch amide).

¹H NMR (DMSO): δ 1.8539 (s, 48 H, CH₃, OCH₃), 2.8539-2.8945 (m, 120 H, CH₂, CH₂N-), 3.2067 (m, 48 H, CH₂-CH₂CONH-), 3.6503 (m, 24 H, -CH=CH-), 5.3530 (s, 21H, NH, CONHCH₂-), 7.5586-7.8703 (m, 36 H, aromatic H), 8.3039 (s, 12 H, NH, -CH₂NHCOAr), 8.8739 (s, 12H, aromatic OH).

FABMS m/z: 4500[(M+1)⁺2], 4499[(M+H)⁺, 100]

Yield: 76.3%; **M.P.:** 194-196° C. The purity of compound was checked by TLC using CH₂Cl₂:CH₃OH - (9:1) as solvent system and iodine vapours as visualising agent and only one spot was obtained at R_f value 0.67.



Compound-21:

IR (KBr): ν 3471.82 (O–H stretch), 3380.53 (N–H stretch amide), 3044.77 (aromatic C–H stretch), 2928.36 (C–H asym stretch CH₂), 2851.61 (C–H sym stretch CH₂), 1680.58 (C=O stretch 2° amide), 1457.03 (aromatic C=C stretch), 1095.80 cm⁻¹(C–N stretch).

¹H NMR (DMSO): δ 2.1567-2.2765 (m, 48 H, CH₂, -CH₂CONH-), 3.0675-3.2381 (m, 120 H, CH₂, CH₂N-), 3.5839-3.5945 (m, 24 H, -CH=CH-), 7.0261 (s, 21 H, NH, CONHCH₂-), 7.2245-7.6231(m, 36H, aromatic H), 8.8767 (s, 12 H, NH, -CH₂NHCOAr), 9.8303 (s, 24 H, aromatic OH).

FABMS m/z: 4332[(M+1)⁺ 1], 4331[(M+H)⁺, 100]

Yield: 79.4%; **M.P.:** 197-199° C. The purity of compound was checked by TLC using CH₂Cl₂:CH₃OH - (9:1) as solvent system and iodine vapours as visualising agent and only one spot was obtained at R_f value 0.49.

BIOLOGICAL EVALUATION

The anti-bacterial activity was carried out by using Gram +ve bacteria- *B. subtilis* (ATCC 23857) and Gram -ve bacteria- *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and *S. aureus* (ATCC 25923). The anti-fungal activity was carried out by using i.e. *C. albicans* (ATCC 10231), *A. niger* (ATCC 1015), *T. mentagrophytes* (ATCC 90112) and *M. audouinii* (ATCC 1029). To carry out the anti-microbial activity of newly formed dendrimers following nutrient media were used to culture the bacterial and fungal strains (13).

Procedure of Anti-microbial Activity: (Modified Kirby-Bauer method): It is a disc

diffusion method (14) which was used for the determination of anti-microbial efficacy of the synthesized dendrimers:

1. The test as well as standard drugs solutions were prepared in concentration of 10 mg/ml.
2. The previously grown bacterial and fungal strains were inoculated in petri plates and four wells, two for test compounds, one for standard drug and one for control. The standard drugs, ciprofloxacin and griseofulvin for anti-bacterial and anti-fungal activity, respectively and DMF as control was used.
3. Then filter disks previously sterilized in autoclave at 121° C temperature were impregnated with test solution, standard drug solution and with DMF (control), were placed in corresponding wells of petri plates.
4. Then petri plates were incubated for a period of 24 h for bacterial cultures at temperature 37° C and 48 h at a temperature of 25° C for cultures of fungi (15 & 16).
5. The, zones of inhibition (in mm) were measured and the average was calculated for three sets of zones of inhibition. The zones of inhibition of test were compared with those of standard drugs i.e, ciprofloxacin for anti-bacterial and griseofulvin for anti-fungal activity. The data of average zones of inhibition is listed in

Table -1:

Table - 1: Results of Anti-microbial activity of Synthesized Dendrimers:

Compounds	Average zone of inhibition (mm)							
	Bacterial strains				Fungal strains			
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>	<i>Microsporum audouinii</i>	<i>Aspergillus niger</i>	<i>Trichophyton mentagrophytes</i>
C11	17.5±0.85	21.7±0.42	20.4±1.16	17.2±0.90	18.3±1.05	17.5±1.00	21.9±0.59	18.0±0.53
C12	10.8±0.61	15.4±0.61	18.4±0.50	15.9±0.73	23.4±0.58	18.7±0.56	19.2±0.27	18.4±0.89



C13	18.5±0.67	20.2±0.61	18.5±1.00	20.6±0.70	22.1±0.75	18.8±0.36	20.1±0.95	19.9±0.48
C14	12.9±0.81	21.7±0.38	21.2±0.53	16.7±0.40	19.4±0.61	19.0±0.37	17.8±0.45	21.8±0.26
C15	19.7±0.53	20.6±0.86	22.3±0.85	17.3±0.32	22.2±1.07	13.9±0.55	21.2±1.01	14.9±0.67
C16	20.7±0.51	19.7±0.47	17.3±0.55	19.8±0.70	22.5±0.81	20.8±0.46	17.7±0.68	19.3±0.81
C17	13.5±0.85	13.3±0.53	15.6±0.70	15.8±0.62	22.4±0.99	19.5±0.55	16.2±0.23	20.9±0.81
C18	20.7±0.53	21.6±0.77	19.6±0.91	13.7±0.65	19.4±0.70	16.8±0.50	21.0±0.34	20.5±0.45
C19	19.4±0.42	11.8±0.54	17.9±0.45	16.3±0.68	20.4±1.00	18.2±0.68	18.8±0.46	20.2±0.85
C20	19.4±0.47	11.8±0.58	17.9±0.35	16.3±0.93	20.4±0.53	18.2±0.39	18.8±0.54	20.2±0.50
C21	15.1±0.12	18.5±0.85	21.9±0.41	15.8±1.00	21.3±1.10	18.8±0.43	14.4±0.67	22.9±0.39

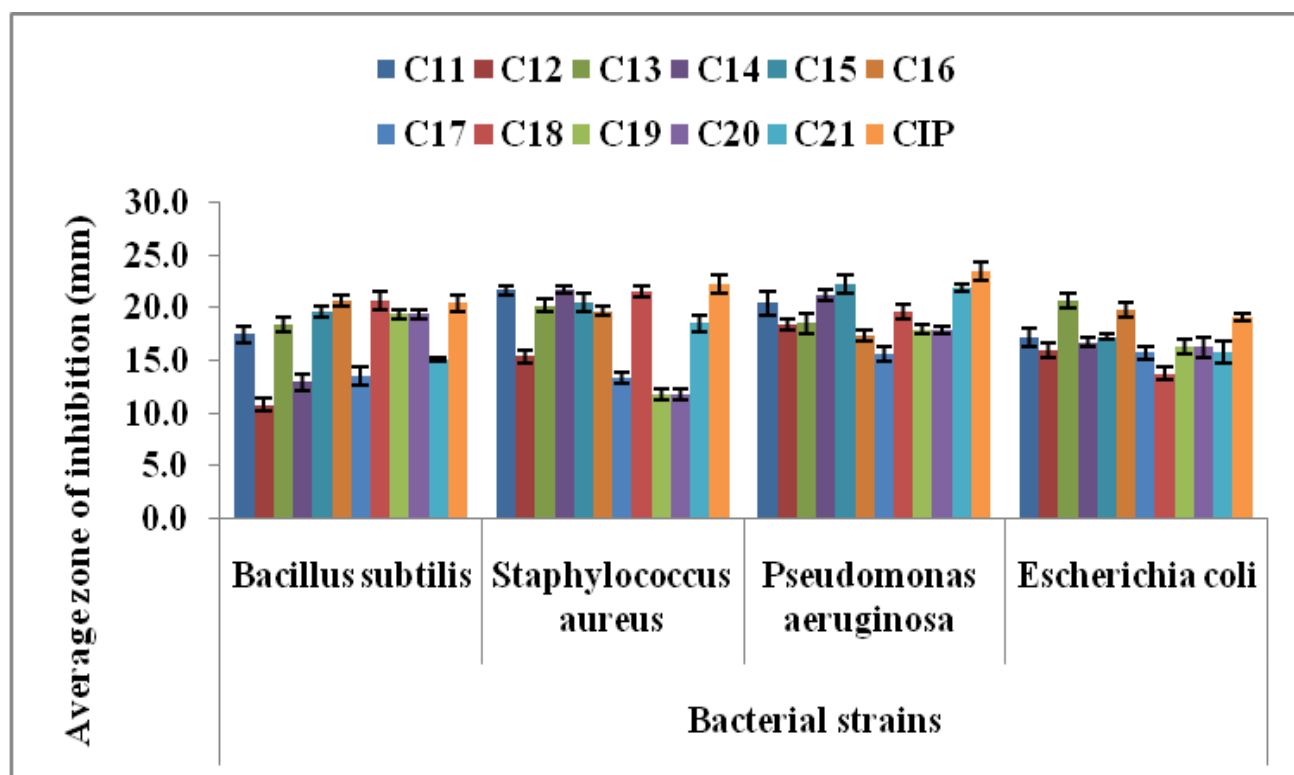


Fig. 2 Anti-bacterial studies of synthesized Dendrimers

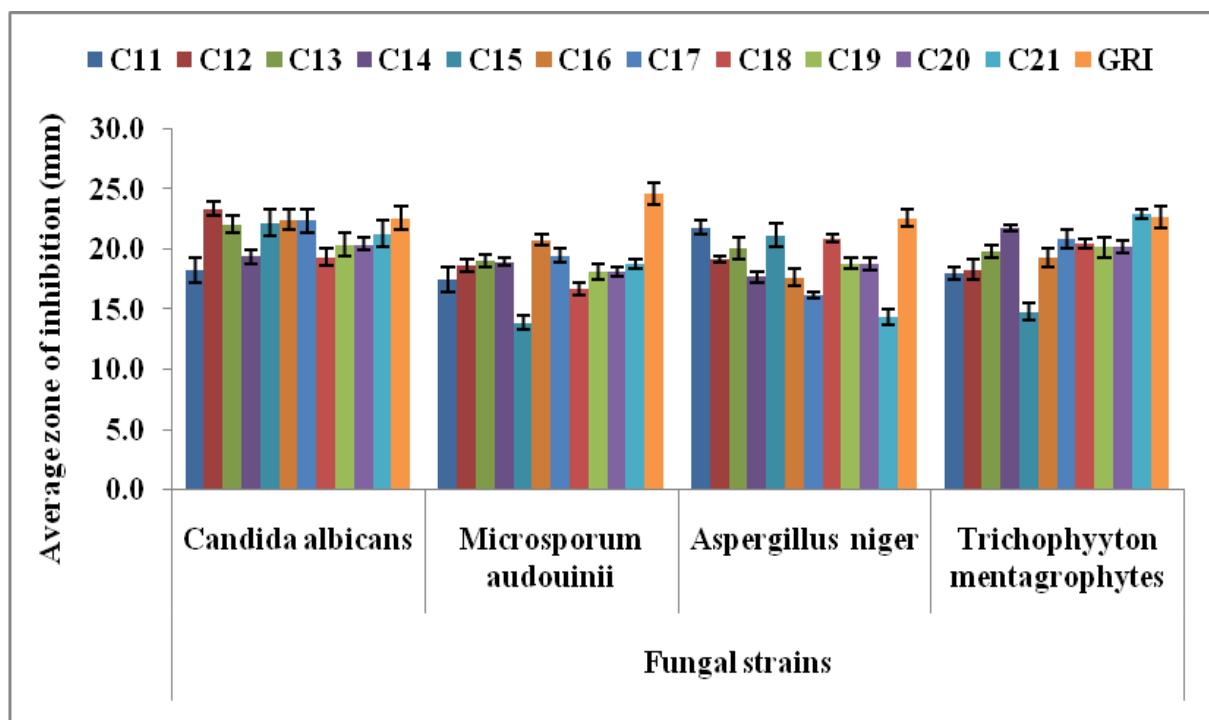


Fig. 3 Anti-fungal studies of synthesized Dendrimers

RESULT AND DISCUSSION

Chemical analysis- Dendrimers were characterized on the basis of spectral data presented as follows:

Compound (11):

The synthesis of Compound (11) was accomplished with 69.7% yield and characterized by the prominent peaks at 3395.95 (O–H stretch), 3035.90 (aromatic C–H stretch), 2974.04, 2939.91 (C–H asym stretch CH₂), 2856.99 (C–H sym stretch CH₂); 2.8567-2.8945 (m, 6H, CH₂-CH₂CONH-), 4.1931(m, 6H, CH₂CH₂NH-), 4.5359-4.5536 (m, 6H, CH₂CONHCH₂-), 10.3767 (s, 9H, aromatic OH) and by presence of pseudo molecular ion peak at *m/z* 816.

Compound (12):

The synthesis of compound (12) was accomplished with 52.4% yield and characterized by the prominent peaks at 3352.64 (N–H stretch amide), 3098.83 (aromatic C–H stretch), 2940.67 (C–H stretch CH₃), 1733.44, 1688.73 (C=O stretch 2° amide), 1630.60 (C=N stretch); 2.1567-2.1767 (m, 3H, CH, -CH=CH-), 2.2765-2.2945 (m, 3H, CH, -CH=CH-) 3.0675 (s, 9H, CH₃OCH₃), 3.2260-2.8945 (m, 6H, CH₂-

CH₂CONH-), 8.1767(s, 6H, NH, CONH-) and by existence of ionic pseudo molecular peak at 876 (*m/z*).

Compound (13):

The synthesis of compound (13) was accomplished with 63.5% yield and characterized by the prominent peaks at 3420.14 (O–H stretch), 3308.22 (N–H stretch amide), 3088.71 (aromatic C–H stretch), 2913.35 (C–H asym stretch CH₂), 2850.95 (C–H sym stretch CH₂); 2.1145-2.1460 (m, 12H, CH₂-CH₂CONH-, CH₂NH-), 2.2460 (m, 12 H, CH₂CONHCH₂-, -CH₂NHCOAr), 2.5165-2.5365 (m, 6H, -CH=CH-), 8.7767 (s, 6 H, aromatic OH) and by existence of ionic pseudo molecular peak at 834 (*m/z*).

Compound (14):

The synthesis of compound (14) was accomplished with 72.3% yield and characterized by the prominent peaks at 3465.46 (O–H stretch), 3349.19 (N–H stretch amide), 3051.37 (aromatic C–H stretch), 2955.64 (C–H asym stretch CH₂), 2878.68 (C–H sym stretch CH₂); 2.5167 (m, 36H, CH₂-CH₂CONH-, CH₂NH-), 2.5265-2.5365 (m, 36H, CH₂CONHCH₂-, -CH₂NHCOAr), 4.5481-4.5533 (m, 18H, aromatic H) and by existence of ionic pseudo molecular peak at 1832 (*m/z*).



Compound (15):

The synthesis of compound **(15)** was accomplished with 60.7% yield and characterized by the prominent peaks at 3345.33 (N–H stretch amide), 3095.90 (aromatic C–H stretch), 1687.77 (C=O stretch 2° amide), 1552.42 (NH bend 2° amide); 3.0675-3.2381 (m, 36H, CH₂-CH₂CONH-, CH₂NH-), 3.2503 (m, 36H, CH₂CONHCH₂-, -CH₂NHCOAr), 8.8765 (s, 18 H, aromatic OH) and by existence of ionic pseudo molecular peak at 1928 (*m/z*).

Compound (16):

Cordyheptapeptide A (88):

The synthesis of compound **(16)** was accomplished with 74.5 % yield and characterized by the prominent peaks at 3431.71 (O–H stretch), 3304.40 (N–H stretch amide), 3042.41 (aromatic C–H stretch), 2973.46 (C–H stretch CH₃), 2945.73 (C–H asym stretch CH₂); 1.7581 (s, 18 H, CH₃OCH₃), 2.1145-2.1439 (m, 12H, -CH=CH-) 2.5167-2.5265 (m, 36H, CH₂-CH₂CONH-), 2.5365 (m, 36 H, CH₂CH₂NH-) and by presence of pseudo molecular ion peak at *m/z* 2072.

Compound (17):

The synthesis of compound **(17)** was accomplished with 79.8% yield and characterized by the prominent peaks at 3472.20 (O–H stretch), 3252.67 (N–H stretch amide), 3072.05 (aromatic C–H stretch), 1689.34 (C=O stretch 2° amide); 1.6339-1.8539 (m, 36H, CH₂-CH₂CONH-), 2.8539-2.8945 (m, 36H, CH₂CH₂NH-), 3.9131-3.9201 (m, 12H, -CH=CH-) and by existence of ionic pseudo molecular peak at 1988 (*m/z*).

Compound (18):

The synthesis of compound **(18)** was accomplished with 64.2% yield and characterized by the prominent peaks at 3302.44 (N–H stretch amide), 3037.09 (aromatic C–H stretch), 1694.48 (C=O stretch 2° amide), 1570.74 (NH bend 2° amide); 3.0675-3.2260 (m, 48H, CH₂-CH₂CONH-), 3.2381-3.2503 (m, 120H, CH₂CH₂N-), 4.9163-4.9203 (m, 36 H, aromatic H), 4.9303 (s, 21H, NH, CONHCH₂-) and by existence of ionic pseudo molecular peak at 4018 (*m/z*).

Compound (19):

The synthesis of compound **(19)** was accomplished with 63.6% yield and characterized by the prominent peaks at 3336.28 (N–H stretch amide), 2950.05 (C–H asym stretch CH₂), 2849.52 (C–H sym stretch CH₂), 1682.86 (C=O stretch 2° amide), 1575.26 (NH bend 2° amide); 1.7316-1.7545 (m, 48 H, CH₂-CH₂CONH-), 2.5175-2.5365 (m, 120 H, CH₂CH₂N-), 4.5403-4.5590 (m, 24 H, aromatic H) and by existence of ionic pseudo molecular peak at 4210 (*m/z*).

Compound (20):

The synthesis of compound **(20)** was accomplished with 76.3% yield and characterized by the prominent peaks at 3298.98 (N–H stretch amide), 3054.81 (aromatic C–H stretch), 29747.85 (C–H stretch CH₃), 2904.34 (C–H asym stretch CH₂), 2875.67 (C–H sym stretch CH₂); 1.8539 (s, 48H, CH₃OCH₃), 2.8539-2.8945 (m, 120H, CH₂CH₂N-), 3.2067 (m, 48H, CH₂-CH₂CONH-), 3.6503 (m, 24H, -CH=CH-) and by existence of ionic pseudo molecular peak at 4499 (*m/z*).

Compound (21):

The synthesis of compound **(21)** was accomplished with 79.4% yield and characterized by the prominent peaks at 3471.82 (O–H stretch), 3380.53 (N–H stretch amide), 3044.77 (aromatic C–H stretch), 2928.36 (C–H asym stretch CH₂); 2.1567-2.2765 (m, 48H, CH₂-CH₂CONH-), 3.0675-3.2381 (m, 120H, CH₂CH₂N-), 3.5839-3.5945 (m, 24H, -CH=CH-) and by existence of ionic pseudo molecular peak at 4331 (*m/z*).

Biological evaluation:

The observation of anti-microbial activity suggested that compound **(15)** displayed very good anti-bacterial efficacy against *P. aeruginosa* but moderate anti-fungal activity against *C. albicans* and *A. niger* at 10 mg level in comparison to Ciprofloxacin and Griseofulvin respectively with inhibition values 22.3, 22.2 and 21.2.

The observation of anti-microbial activity showed that compound **(12)** displayed potent anti-fungal activity against pathogenic fungi *C. albicans* with inhibition values 23.4 at 10 mg level in comparison to Griseofulvin, as standard drug and compound **(18)** exhibited moderate anti-bacterial activity against *S. aureus* with inhibition value 21.6. The compounds **(21)** and



(14) exhibited moderate anti-bacterial activity against gram -ve bacterial strains *P. aeruginosa* with inhibition values 21.9 and 21.2, respectively while compound **(11)** exhibited moderate anti-bacterial activity against *S.aureus* and anti-fungal activity against *A. niger* with inhibition values 21.7 and 21.9. Lastly, compound **(16)** exhibited modest anti-fungal as well as anti-bacterial activity against *C. albicans* and *B. subtilis* with inhibition values 22.5 and 20.7, respectively.

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