Research Article

Chemical Constituents from *Cycas beddomei* and Their Bioactivity Studies

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ABSTRACT

Potentials antimicrobial from crude acetone and chloroform extracts of *Cycas beddomei* were evaluated. Bio-essay guided acetone and chloroform extracts were led to the identification of isolation and identification of eight compounds. Among the isolated compounds 2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol (**1a**), kaempferol (**2**), taxfolin-3-*O*-α-L-rhamnopyranosidfe (**4**) and quercetin-O-L-rhamnoside (**6**) showed antimicrobial activity.

Keywords: *Cycas beddomei*, flavonoids and antimicrobial activity.

INTRODUCTION

Medicinal plants are the "backbone" of traditional and ayurveda medicine, which means more than 2 billion people in the less developed countries utilize medicinal plants on a regular basis[1]. Nearly 2000 ethnic groups in the world, and almost each group has its own habitual medical knowledge and experiences[2,3]. India has wide-ranging climates and geographical regions that have caused a wide distribution of individual medicinal plant species such that each tribe has its own plants and customs[4]. The genus *Cycas* of Cycadaceae family is rich in flavonoids[5]. *Cycas beddomei* dyer is a tall shrub endemic to Tirumala hills, Andhra Pradesh, India[6] and some of the *Cycas* species are extensively used in traditional Indian medicine as stimulants, narcotics, aphrodisiac and expectorants in the treatment of malignant ulcers[7]. Previous investigation of the leaves of this species has led to the isolation of biflavanone, tetrahydrohinokiflavone along with amentoflavone[8]. Epidemiological and animal studies have correlated flavonoid consumption with reduced rates of heart disease and some cancers[9-16]. The biological activities of some of the isolated bi-flavonoids are well investigated. For instance, amentoflavone and its derivatives possess inhibitory effects on lipid peroxidation[17] and hinokiflavone inhibits the expression of the EPSTEIN Barr Virus (EBV) genes[18]. They are also known to possess antituberculosis, antifungal, antiviral, anti HIV and antimalarial activities[19-24]. The specific role of flavonoids in human health and disease, however, is far from well understood. This is partly because quantitative information regarding their metabolism and distribution after consumption from common foods is extremely scarce.

In the present effort we have carried out the isolation of biologically active compounds from *Cycas beddomei.* The compounds were characterized on the basis of various spectral techniques including NMR and Mass spectral data and tested for bioactivity studies.

EXPERIMENTAL

General Procedures

Melting points were recorded on a Fisher scientific melting point apparatus and were uncorrected. Mass spectra were recorded on an Agilent LC/MSD trap SL 1100 series with a 70 ev (ESI probe) and IR spectra were recorded on a Thermo Nicolet Nexus 670 FTIR spectrometer. ^{1}H-NMR, ^{13}C-NMR experiments were recorded on a Bruker 300 MHz spectrometer using TMS as an internal standard. All the solvents used were of analytical grade. Flash column chromatography was performed on silica gel (60-120 & 230-400mesh, Acm's make, Mumbai, India). Thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (E. Merck, Darmstadt, Germany). Visualization was performed by spraying the TLC plates with 5% H_2SO_4 solution followed by heating.

Plant Material

The whole plant of *Cycas beddomei* was collected from Tirumala forest, India and identification was made by Dr. K. Madavachetty, Department of Botany, Sri Venkateswara University, Tirupati.

Extraction and isolation

The air dried and powdered stem (5 kg) was extracted with acetone and Methanol for two days sequentially. As both these extracts are similar to each other and hence they were mixed and concentrated under vacuum to obtain a syrupy residue for about 150 g and it was subjected to column chromatography over silica gel (60-120 mesh) using n-hexane: acetone (100:0 to 0: 100) for 10 fractions and chloroform: acetone (100:0 to 0: 100) for 5 fractions i.e. F1-F10 & F11-F15). Fractions 1-3 on further flash column separation using n-hexane: acetone (95:05) yielded three closely related compounds designated as compound **1a** (5 mg), compound **1b** (4 mg) and **1c** (6 mg). Fraction 4 and 5 on further column chromatography using n-hexane: acetone 92:08, yielded compounds **2** (5 mg) and **3** 10 mg. Fraction 6 and 7 on repeated column chromatography (solvent system n-hexane: acetone 85:15) eluted compound **4** (8 mg).

Fraction 11 on further chromatographic analysis (using Chloroform: acetone 95:05) yielded compound **5** (4 mg), fraction 12 using chloroform: acetone 85:15, yielded compound **6** (7 mg). The compounds are identified by chemical and spectral methods as 2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol (**1a**), 2-(4-hydroxyphenyl)chroman-3,5,7-triol (**1b**) 2-(4-hydroxy-3-methoxyphenyl)chroman-3,5,7-triol (**1c**), kaempferol (**2**), kaempferol 3-O-α-L-rhamnopyranoside (**3**), taxifolin-3-α-L-rhamnopyranoside (**4**), kaempferol-3-O-β-D-glucopyranoside (**5**), and quercetin-3-O-L-rhamnoside (**6**).

2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol (1a)

^{1}H NMR (300 MHz, DMSO-d_6): δ 4.46 (1H, d, 7.5 Hz , H-2), 3.87 (1H, m, H-3), 2.75 (1H, m, H-4) & 2.40 (1H, m, H-4), 5.83 (1H, d, *J* = 2.2 Hz , H-6), 5.75 (1H, d, *J* = 2.07 Hz, H-8), 6.74 (1H, d, *J* = 1.5 Hz, H-2^1), 6.67 (1H, dd, *J* = 7.9 Hz, H-5^1), 6.62 (1H, dd, *J* = 7.9 & 1.7 Hz, H-6^1); ^{13}C NMR (75 MHz, DMSO-d_6): 82.91 (C-2) , 68.86 (C-3), 28.56 (C-4), 156.96 (C-5), 96.34 (C-6),157.88 (C-7), 96.55 (C-8),157.61(C-9), 100.87 (C-10), 132.27 (C-1^1), 115.3 (C-2^1), 146.28 (C-3^1,4^1), 116.11 (C-5^1), 120.07 (C-6^1); Mass (EIMS, 70 ev): 291 [M+H]$^+$.

2-(4-hydroxyphenyl)chroman-3,5,7-triol (1b)

^{1}H NMR (300 MHz, DMSO-d_6): 4.56 (1H, d, *J* = 7.5 Hz, H-2), 3.99 (1H, m, H-3), 2.9 (1H, m, H-4), 2.5 (1H, m, H-4), 5.90 (1H, s, H-6), 5.86 (1H, bs, H-8), 7.21 (1H, d, *J* = 8.3 Hz, H-2^1, 6^1), 6.77 (1H, d, *J* = 8.3, H-3^1, 5^1); ^{13}C NMR (75 MHz, DMSO-d_6): 80.88 (C-2), 67.09 (C-3), 27.94 (C-4), 155.41 (C-5), 95.54 (C-6), 156.25 (C-7), 95.54 (C-8), 155.54 (C-9), 99.24 (C-10), 129.37 (C-1^1), 127.89 (C-2^1, 6^1), 114.51 (C-3^1,5^1), 154.94 (C-4^1); Mass (EIMS, 70 ev): 275 [M+H]$^+$.

2-(4-hydroxy-3-methoxyphenyl)chroman-3,5,7-triol (1c)

^{1}H NMR (300 MHz, DMSO-d_6): 5.05 (1H, d, *J* = 7.4 Hz, H-2), 4.49 (1H, m, H-3), 2.83 (1H, m, H-4) & 2.58 (1H, m, H-4), 5.82 (1H, d, *J* = 1.5 Hz, H-6), 5.86 (1H, d, *J* = 1.5 Hz, H-8), 6.97 (1H, bs, H-2^1), 6.77 (1H, d, *J* = 7.2 Hz, H-5^1), 6.75 (1H, d, *J* = 7.2 Hz , H-6^1), 3.83 (3H, s, OMe); **^{13}C** NMR (75 MHz, DMSO-d_6): 85.7 (C-2), 67.7 (C-3), 28.1 (C-4), 157.2 (C-5), 95.3 (C-6), 157.8 (C-7), 94.8(C-8), 157.3(C-9), 99.4 (C-10), 131.1 (C-1^1), 110.1 (C-2^1), 147.6 (C-3^1), 146.0 (C-4^1), 115.7 (C-5^1), 120.7 (C-6^1), 56.1 (OMe); Mass (EIMS, 70 ev): 305 [M+H]$^+$.

Kaempferol (2)

^{1}H NMR (300 MHz, DMSO-d_6): δ 12.15 (2H, s, OH-5, OH-3), 9.70 (1H, brs, OH-7), 9.10 (1H, brs, OH-4'), 8.14 (2H, d, *J* = 9.0 Hz, H-2', 6'), 7.0 (2H, d, *J* = 9.0 Hz, H-3', 5'), 6.52 (1H, d, *J* = 2.1 Hz, H-8), 6.26 (1H, d, *J* = 2.1 Hz, H-6); Mass (ESIMS, 70 ev): 286 [M]$^+$, 285 [M-1]$^+$.

Kaempferol 3-O-α-L-rhamnopyranoside (**3**)

^{1}H NMR (300 MHz, DMSO-d_6): 12.68 (1H, s, OH-5), 9.40 (2H, brs, OH-7), 7.84 (2H, d, *J* = 8.9 Hz, H-2', 6'), 7.32 (2H, d, *J* = 8.9 Hz, H-3', 5'), 6.45 (1H, d, *J* = 2.1 Hz, H-8), 6.25 (1H, d, *J* = 2.1 Hz, H-6), 5.53 (1H, d, *J* = 1.0 Hz, H-1"), 3.10-4.23 (4H, m, H-2", 3", 4", 5"), 0.89 (3H, d, *J* = 6.0 Hz, rhamnosyl CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$+DMSO–d_6): 177.7 (C-4), 163.9 (C-7), 161.4 (C-5), 159.7 (C-4'), 157.0 (C-8a), 156.4 (C-2), 134.2 (C-3), 130.1 (C-2', 6'), 120.6 (C-1'), 115.2 (C-3', 5'), 104.3 (C-4a), 101.3 (C-1"), 98.6 (C-6), 93.4 (C-8), 71.5 (C-4"), 70.7 (C-3"), 70.1 (C-2"), 69.9 (C-5"), 17.0 (CH3-6"); Mass: EIMS 70 ev 432 [M$^+$], 286 [M-rhamphonyl]$^+$.

Taxfolin-3-*O*-α-L-rhamnopyranoside (**4**)

^{1}H NMR (300 MHz, DMSO-d_6): δ 11.80 (1H, s, OH-5), 9.07 (3H, br s, OH-7, 3', 4'), 6.87 (1H, s, H-5'), 6.72 (2H, s, H-2', 6'), 5.88 (1H, d, *J* = 2.0 Hz, H-8), 5.86 (1H, d, *J* = 2.0 Hz, H-6), 5.22 (1H, d, *J* = 12.0 Hz, H-2), 4.63 (1H, d, *J* = 12.0 Hz, H-3), 4.50 (1H, br s, H-1"), 3.07-4.02

(4H, m, H-2'', 3'', 4'',5 ''), 1.04 (3H, d, *J* = 6.2 Hz, rhamnosyl CH_3-6''); ^{13}C NMR (75 MHz, DMSO-d_6): δ 194.4 (C-4), 166.9 (C-7), 163.4 (C-5), 162.1 (C-8a), 145.8 (c-3'), 145.1 (C-4'), 126.9 (C-1'), 118.8 (C-6'), 115.3 (C-2'), 114.7 (C-5'), 101.0 (C-4a), 70.1 (C-3''), 68.9 (C-5''), 17.6 (rhamnosyl CH_3-6''); Mass: (EIMS, 70 eV): *m/z* 451.122 $[M+H]^+$.

Kaempferol 3-*O*-ß-D-glucopyranoside (**5**)
^{1}H NMR (300 MHz, DMSO-d_6): δ 12.60 (1H, s, OH-5), 10.40 (2H, br s, OH-7, 4'), 8.03 (2H, d, *J* = 8.9 Hz, H-2', 6'), 6.87 (2H, d, *J* = 8.9 Hz, H-3', 5'), 6.42 (1H, d, *J* = 2.0 Hz, H-8), 6.19 (1H, d, *J* = 2.0 Hz, H-6), 5.45 (1H, d, *J* = 7.3 Hz, H-1''), 2.90-3.57 (6H, m, H-2'', 3'', 4'',5 ''); ^{13}C NMR (75 MHz, DMSO-d_6): δ 177.5 (C-4), 164.3 (C-7), 161.2 (C-5), 160.0 (C-4'), 156.4 (C-8a), 156.2 (C-2), 133.2 (C-3), 130.9 (C-2', 6'), 120.9 (C-1'), 115.1 (C-3', 5'), 104.0 (C-4a), 100.9 (C-1''), 98.7 (C-6), 93.7 (C-8), 77.5 (C-3''), 76.4 (C-5''), 74.2 (C-2''), 69.9 (C-4''), 60.9 (C-6''); Mass: (EIMS, 70 eV): *m/z* 286 [M-glycosyl]$^+$.

Quercetin-3-*O*- L-rhamnoside (6)
Pale yellow amorphous powder, ^{1}H-NMR (300 MHz, DMSO-d_6): δ 0.88 (d, *J* = 6 Hz, CH_3), 5.19 (d, *J* = 1.5 Hz, H-1''), 6.16 (d, *J* = 2.2 Hz, H-6), 6.32 (d, *J* = 2.2 Hz, H-8), 6.94 (d, *J* = 8.2 Hz, H-5'), 7.23 (dd, *J* = 2.1, 8.1 Hz, H-6'), 7.35 (d, *J* = 2.2 Hz, H-2'), ^{13}CNMR (75 MHz, DMSO-d_6): δ 17.66 (C-6''), 70.21 (C-5''), 70.74 (C- 3''), 70.52 (C-2''), 71.36 (C-4''), 94.16 (C-8), 99.22 (C-6), 101.92 (C-1''), 104.18 (C-10), 114.98 (C-2'), 115.68 (C-5'), 120.78 (C-1'), 121.23 (C-6'), 134.24 (C-3), 145.44 (C-3'), 148.78 (C-4'), 156.66 (C-2), 157.25 (C-9), 161.36 (C-5), 165.59 (C-7), 177.74 (C-4). Mass: (EIMS, 70 eV): *m/z* 302 [M-glycosyl]$^+$.

Biological assay
Antibacterial studies
The isolated compounds were screened for their antibacterial activity against *Bacillus subtilis, Staphylocouccus aureus, Klebsiella pneumonia* and *Escherichia coli* (clinical isolate) bacterial strains by disc diffusion method[28,29]. A standard inoculums (1.2×10^7 c.f.u./ml 0.5 McFarland standards) were introduced on to the surface of sterile agar plates and a sterile glass spreader was used for even distribution of the inoculums. The disks measuring 6 mm in diameters were prepared from Whatman no.1 filter paper and sterilized by dry heat at 140 ^{0}C for 1 h. The sterile disks previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. Amoxicillin (30 µg) was used as positive control and the disk poured in DMSO was used as negative control and the test compounds were dissolved in DMSO at concentration of 100 and 50 µg/mL. The plates were inverted and incubated for 24 h at 37 ^{0}C. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition of zone of measured and compared with controls. The bacterial zone of inhibition values are given in **Table 1**.

Antifungal studies
The isolated compounds were screened for their antifungal activity against *Candida albicans* and *Aspergillus flavus* in DMSO by agar diffusion method[30]. Sabourauds agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting pH 5.7. Normal saline was used to make suspension of corresponding species. Twenty milliliters of agar media was poured into each Petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 ^{0}C for 1 h using an agar punch, wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37 ^{0}C for 3-4 days. The fungal activity of each compound was compared with Ketoconazole as a standard drug. Inhibition zone were measured and compared with the controls. The fungal zone of inhibition values are given in **Table 2**.

RESULT AND DISCUSSION
Acetone and chloroform extracts of *Cycas beddomei* were column chromatographed over silica gel which led to the isolation and identification of eight compounds which were conformed on the basis of ^{1}H-NMR, ^{13}C-NMR and mass spectral data. The ^{1}H-NMR spectrum of compound **2** showed three D_2O exchangeable signals at δ 12.11 (2H), 9.67 (1H) and 9.10 (1H) due to 3,5,7 and 4'-hydroxyl groups. It also showed the presence of *para* substituted phenyl group δ 8.14 (d, 2H), and 7.0 (d, 2H) and *meta* related C-6 and C-8 aromatic protons at δ 6.26 and 6.52 and the molecular ion at *m/z* 286. Thus the compound was characterized as kaempferol.
The conformation of the structure **3** was obtained from its ^{1}H-NMR spectrum which exhibited two D_2O exchangeable signals at δ 12.68 (1H), and 9.40 (2H) corresponding to a chelated hydroxyl at C-5 and two non chelated hydroxyls at 7 and 4'-positions, respectively. The presence two *meta* coupled doubled (*J* = 2.1 Hz) at δ 6.25 and 6.45 were attributed to at

C-6 and C-8 protons respectively. The four aromatic protons at ring B were shown up as typical A_2B_2 doublets *J* = 8.9 Hz at 7.84 and 7.0 were assigned to H-2', 6' and H-3', 5' respectively. An enomeric proton doublet at (*J* = 1.0 Hz) at 5.53 was attributed to ^{1}H of the rhamnosyl moiety. The presence of a three proton doublet at 0.89 (*J* = 6.0 Hz) indicated the rhamnosyl methyl group. The remaining sugar protons of the rhamnosyl moiety were resonated between 3.10-4.23.

The site of glycosylation at C-3 position in compound **3** was further evidenced by a comparison of its ^{13}C-NMR spectral data with its aglycone kaempferol[27]. The C-3 signal of compound **3** showed an upfield of δ 1.4 and the C-2 and C-4 signals showed downfield shifts of δ 9.6 and 9.8 and 1.8, respectively from the corresponding carbon resonances of kaempferol conforming the site of glycosylation at C-3 position. The configuration at the anomeric centre of the rhamnosyl moiety in compound **3** was determined to be α- from the presence of an anomeric carbon signal at 101.3 and also from the small coupling constant (*J* = 1.0 Hz) for the anomeric proton. The structure of compound **3** as kaempferol 3-*O*-*α*-*L*- rhamnopyranoside was further conformed by its EI Mass spectrum which exhibited the base peak at *m/z* 286 corresponding to the aglycone, kaempferol and the characteristic fragments at *m/z* 153 and 121.

Compound **4** showed a D_2O exchangeable proton downfield signal at δ 11.80 in the ^{1}H NMR spectrum of compound **4** supported the chelated hydroxyl at C-5 and another signal at δ 9.07 integrating for three protons indicated the presence of three more phenolic hydroxyls in compound **4**. The ^{1}H NMR spectrum of **4** exhibited the typical AB system due to C-2 And C-3 methine protons of dihydroflavonol at δ 5.22 (d, *J* = 12 Hz) and 4.63 (d, *J* = 12 Hz). A *trans*- orientation of C- ring methane protons was deduced from the large *J* value (12 Hz) which was typical of diaxial coupling. The presence of two meta coupled doublets (*J* = 2.0 Hz) at δ 5.86 and 5.88 were assigned to C-6 and C-8 protons, respectively of the three non-chelated hydroxyls in compound **4**, one of them was placed at C-7 as it gave positive sodium acetate shift. The remaining two phenolic hydroxyls should be present in ring. The presence of two characteristic fragments at m/z 152 $[A_1]^+$ and 123 $[B_4]^+$ in the EI mass spectrum of **4** were consistent with the presence of two hydroxyls in ring A and two hydroxyls in ring B, respectively. The three aromatic ring B appeared at δ 6.87 (1H) and 6.72 (2H). An anomeric proton doublets (*J* = 1.0 Hz) at δ 4.50, a three-proton doublets (*J* = 6.2 Hz) at δ 1.04 and a multiplet over the range 3.07-4.02 integrating for four protons indicated the presence of a rhamnosyl moiety in **4**. The configuration of anomeric centre of rhamnosyl moiety was determined to be α- from the presence of a carbon signal at δ 100.0, its ^{13}C NMR spectrum and also from the small coupling constant (*J* = 1.0 Hz) for anomeric proton signal of rhamnose in its ^{1}H NMR spectrum.

The structure **5** was deduced by the ^{1}H NMR spectral data which showed the presence of four aromatic protons of ring B as typical A_2B_2 doublets at δ 8.03 (2H, *J* = 8.9 Hz, H-2', 6') and 6.87 (2H, *J* = 8.9 Hz, H-3', 5'). A pair of *meta* coupled doublets at δ 6.19 and 6.42 were ascribed to H-6 and H-8, respectively. An anomeric proton doublet at δ 5.45 with a coupling constant of 7.3 Hz was ascribed to C-1'' proton of the β-glucosyl residue. A complex multiplet over the range δ 2.90-3.57 integrating for six protons were assigned to the remaining six protons of the glucose residue. Comparison of ^{13}C NMR spectrum of compound **5** was observed that the C-3 signal of **5** showed an upfield shift of δ 2.4, and C-2 and C-4 signals showed downfield shifts of δ 6.4 and 1.6, respectively from that of kaempferol showing the location of the glucose residue at C-3 position. Further evidence for the structure of **5** was obtained from its EI-Mass spectrum which showed the base peak at *m/z* 286 corresponding to its aglycone, kaempferol besides the characteristic fragments at *m/z* 153 and 121. Thus compound **5** was characterized as kaempferol 3-O-β-D-glucopyranoside. The structure of compounds **6** was confirmed as quercetin-3-O-L-rhamnoside by comparison of the spectral data to that of authentic sample of quercetin-3-O-L-rhamnoside.

Anti microbial activity

The isolated compounds (**1-6**) were screened for their *in-vitro* antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia* and *Escherichia coli* using Amoxicillin as standard by disc diffusion method (zone of inhibition). The test compounds were dissolved in dimethylsulfoxide (DMSO) at concentrations of 50 and 100 µg/mL. The antibacterial screening revealed that all the tested compounds showed good inhibition against various tested microbial strains compared to the standard drug. Along with the isolated compounds **4** and **6** were found to be more active against tested bacterial strains as compared to the standard. The enhanced antibacterial activity

of **1a, 2, 4** and **6** was due to the presence of hydroxyl groups and O-glycosyl residues in the flavones moiety.

The *in-vitro* antifungal activities of compounds (**1-6**) were determined by agar diffusion method. The results indicate that, among the tested compounds **2**, **3**, **4** and **6** were active against all tested fungal strains as compared with standard Ketoconazole. The **Table 1** and **Table 2** depict the antibacterial and anti-fungal screening results respectively.

CONCLUSION

The chromatographic separation of *C.beddomei* acetone and chloroform extracts were led to the isolation and characterization of eight known compounds and a significant antimicrobial activity was observed.

ACKNOWLEDGEMENTS

One of the authors N.Harikrishna grateful to MoES (*letter no MoES/11-MRDF/1/32/p/09-PC-III dated 18-05-2010*) for providing financial assistance to carry out research work.

Table 1: Antibacterial activity of compounds 1-6

Zone of inhibition measure in mm								
Isolated compounds	Gram positive				Gram negative			
	Bacillus subtilis		*Staphylococcus aureus*		*Klebsiella pneumonia*		*Escherichia coli*	
	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL
1a	14.5	11.0	13.0	10.5	16.0	11.5	13.5	12.5
1b	8.5	7.5	10.5	8.0	11.5	9.5	11.0	9.0
1c	9.0	8.5	7.0	8.5	9.5	6.0	8.0	5.5
2	13.0	10.5	11.5	10.0	14.0	12.0	13.0	11.5
3	8.5	7.0	8.0	6.5	9.0	7.0	6.5	5.0
4	12.0	11.5	13.0	11.5	13.5	11.0	15.0	12.0
5	9.0	7.5	8.5	7.0	6.5	4.0	8.5	5.5
6	13.5	12.5	11.0	10.5	16.0	12.0	14.5	11.5
Amoxicillin	17.5	13.0	15.5	12.5	19.5	15.5	18.0	14.5
Control (DMSO)	–	–	–	–	–	–	–	–

Table 2: Antifungal activity of compounds 1-6

Zone of inhibition measure in mm				
Isolated compounds	*Candida albicans*		*Aspergillus flavus*	
	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL
1a	10.5	9.5	9.5	7.0
1b	9.5	8.0	10.5	9.5
1c	8.0	10.5	8.5	8.0
2	17.5	12.5	16.0	12.0
3	13.0	11.0	13.0	10.5
4	15.5	12.5	12.0	11.0
5	9.0	7.5	8.5	6.5
6	12.5	13.0	14.5	12.0
Ketoconazole	18.5	14.0	20.5	16.0
Control (DMSO)	–	–	–	–

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