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NEW ANTIFUNGAL ALLELOCHEMICAL FROM SEEDS OF *HARDWICKIA BINATA ROXB*

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ABSTRACT
Chemical investigation of seeds of ***Hardwickia binata*** Roxb., resulted in the isolation of a new allelochemical 8, 3′-dihydroxy-5, 6, 7, 4′-tetramethoxyflavone-3′-O-β-D-xylopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-α-L-rhamnopyranoside (**1**) as well as four known compounds tectochrysin (**2**), 5, 7-dimethoxy flavone (**3**), campesterol (4) and quercetin-3-O-β-glucopyranoside (**5**). Structure of the new compound was elucidated by various colour reactions, spectral analysis and chemical degradations. Compound **1** showed significant antifungal activity.

KEYWORDS: ***Hardwickia binata*** Roxb., leguminosae, allelochemical, flavone glycoside, antifungal activity.

INTRODUCTION
Hardwickia binata Roxb.[1-4] belongs to family Leguminosae and subfamily caesalpiniaceae, which is commonly known as "**Anjan**" in hindi, "**Kamra**"in marathi and "**Indian Black wood**" in english. It is a huge deciduous ornamental tree with height in the range of 25-300 feet and is dark grey. It is found in Tropical south Asia, Bangladesh, Indonesia, Malasiya, Nepal and Pakistan. In India it is found naturally semi arid and arid regions of central, western and southern India. Bark is rich in tannins used as gums and resins. The resin obtained from the tree is used as diuretics. The gum of this tree is used in treatment of sexually transmitted diseases like chronic cystitis and gonorrhea. A decoction made from bark is used to treat piles, toxaemia, skin diseases and dysentery. It is used for folklore medicine for treatment of diarrhea, worms, indigestion and leprosy. It also produces an appetizer. Earlier workers [5-7] have reported various chemical constituents from this plant. In the present paper we report the isolation and structural elucidation of a new allelochemical 8, 3′-dihydroxy-5, 6, 7, 4′-tetramethoxyflavone-3′-O-β-D-xylopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-α-L-rhamnopyranoside (**1**) alongwith four known compounds tectochrysin (**2**), 5, 7-dimethoxy flavones (**3**), campesterol (4) and quercetin-3-O-β-glucopyranoside (**5**) from ethanolic extract of the seeds of this plant.

RESULTS AND DISCUSSION
Chemical examination of ethanolic extract of seeds of ***Hardwickia binata*** Roxb., yielded a new compound **1**. It has molecular formula, $C_{36}H_{46}O_{21}$, m. p. 178-188°C, $[M]^+$ 814 (FABMS). Positive results with Molisch and Shinoda tests[8] showed its flavonoidal glycosidic nature. The IR spectrum revealed the absorption at 3415 cm^{-1} and 1648 cm^{-1} indicating the presence of hydroxyl group and aromatic C=C stretching respectively. Absorption at 1033 cm^{-1} is characteristic glycosidic nature of the compund. UV spectrum of compound **1** showed the λ_{max} absorption at 340 and 238 nm suggestive of flavonoidal skeleton. In ^{1}H-NMR spectrum, four singlets at δ4.08, 3.94, 3.89 and 3.99 integrating each of three protons intensity revealed the presence of -OMe groups at C-5, C-6, C-7 and C-4′ positions, which is further evidenced by ^{13}C-NMR signals at 61.1, 62.7, 60.9 and 55.9. Formation of 3-hydroxy-4-methoxy benzoic acid on alkaline degradation confirmed the presence of -OH group at C-3′ and -OMe group at C-4′ position in aglycone. In ^{1}H-NMR spectrum, a singlet at δ6.56 was assigned as H-3. Doublets at δ7.38 and δ7.05 were assigned to H-2′ and H-5′ respectively. A doublet of doublet signal at δ7.54 with coupling constant 8.6 and 1.8 belongs to H-6′. The ^{13}C-NMR spectrum of compound **1** gave 36 signals. 17 carbon signals seen for the sugar moieties confirmed the presence of three monosaccharides one pentose and two hexose units. The remaining 19 carbon signals were due to the flavone aglycone. In ^{13}C-NMR a very downfield signal at δ182.7 belongs to carbonyl C-4. The downfield shift at δ176.4

suggested that compound **1** was a flavone glycoside with glycosylation at C-3′.

The anomeric proton signals at δ5.04 (1H, d, *J* 7.5Hz, H-1″), 4.86 (1H, d, *J* 6.8Hz, H-1‴) and 4.73 (1H, d, *J* 7.6Hz, H-1⁗) were assigned for H-1″, H-1‴ and H-1⁗ of L-rhamnose, D-glucose and D-xylose respectively. In ^{13}C-NMR the downfield shifted signals of C-4″(rha) at δ78.4 and C-4‴(glc) at δ77.6 inferring the glycosylation at C-4″-OH of rhamnose and C-4‴-OH of glucose respectively.

In the mass spectrum of the compound **1**, characteristic ion peaks at [M$^+$] m/z 814, 682, 520 and 374 were found by subsequent losses from the molecular ion of each molecule of D-xylose, D-glucose and L-rhamnose revealing D-xylose as terminal sugar and L-rhamnose was attached at C-3′ position of aglycone.

Acid hydrolysis of compound **1** with 10% ethanolic H_2SO_4 gave aglycone **1-A**, m.p. 182-184°C, m.f. $C_{19}H_{18}O_8$, [M]$^+$374 (EIMS). It was identified as 8, 3′-dihydroxy-5, 6, 7, 4′-tetramethoxyflavone by comparision of its spectral data with reported literature values.[9]

The aqueous hydrolysate after the removal of aglycone was neutralized with $BaCO_3$ and the $BaSO_4$ filtered off. The filtrate was concentrated under reduced pressure and subjected to paper chromatography examination and sugars were identified as D-glucose (R_f 0.18), D-xylose (R_f 0.28) and L-rhamnose (R_f 0.36)(Co-PC and Co-TLC).[10] Periodate oxidation of compound **1**, confirmed that all the sugars were present in the pyranose form.[11]

Permethylation [12] followed by acid hydrolysis of compound **1** yielded aglycone identified as 8, 3′-dihydroxy-5, 6, 7, 4′-tetramethoxyflavone showed that glycosylation was involved at C-3′ and methylated sugars were identified as 2, 3, 4-tri-O-methyl-D-xylose (R_G 0.94), 2, 3, 6-tri-O-methyl-D-glucose (R_G 0.83) and 2, 3-di-O-methyl-L-rhamnose (R_G 1.01) indicating that C-1⁗-OH of D-xylose was linked to C-4‴-OH of D-glucose, C-1‴-OH of D-glucose was linked to C-4″-OH of L-rhamnose and C-1″-OH of L-rhamnose was linked with C-3′ position of the aglycone. Therefore it was concluded that interlinkages (1→4) between D-xylose and D-glucose as well as between D-glucose and L-rhamnose. The linkages were further confirmed by spectral data of ^{13}C-NMR.

Enzymatic hydrolysis [13] of compound **1** with enzyme almond emulsion liberated D-xylose (R_f 0.28) followed by D-glucose (R_f 0.18) and proaglycone identified as 8, 3′-dihydroxy-5, 6, 7, 4′-tetramethoxyflavone-3′-O-α-L-rhamnopyranoside suggesting the presence of β-linkage between D-xylose and D-glucose as well as between D-glucose and proaglycone. Proaglycone on further hydrolysis with enzyme takadiastase liberated L-rhamnose (R_f 0.36) suggesting the presence of α-linkage between L-rhamnose and aglycone.

On the basis of above evidences, the structure of compound **1** was characterized as 8, 3′-dihydroxy-5, 6, 7, 4′-tetramethoxyflavone-3′-O-β-D-xylopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-α-L-rhamnopyranoside. Compound **2**, has m.p. 163-164°C, m.f. $C_{16}H_{12}O_4$, [M]$^+$ 268 (EIMS). It was characterized as **tectochrysin** by comparision of its spectral data with reported literature values.[14-15]

Compound **3**, has m.p. 156-157°C, m.f. $C_{17}H_{14}O_4$, [M]$^+$ 282 (EIMS). It was identified as **5, 7-dimethoxy flavone** by comparision of its spectral data with reported literature values.[16-17]

Compound **4**, has m.p. 158-159°C, m.f. $C_{28}H_{48}O$, [M]$^+$ 400 (EIMS). It was identified as **campesterol** by comparision of its spectral data with reported literature values.[18-19]

Compound **5**, has m.p. 228-230°C, m.f. $C_{21}H_{20}O_{12}$, [M]$^+$ 464 (EIMS). It was identified as quercetin-3-O-β-glucopyranoside by comparision of its spectral data with reported literature values.[20-22]

Compound **1** was screened for antifungal activity against various fungi. The results obtained in **Table III** showed that antifungal activity of compound **1** is fairly good against *Aspergillus niger* and *Candida albicans* even in very low concentrations.

EXPERIMENTAL

Plant material

The seeds of the plant were collected locally around sidhi region and were taxonomically authenticated by taxonomist, Department of Botany, Govt S G S College Sidhi (M.P.) India. A voucher specimen has been deposited in the Laboratory, Department of Chemistry of this college.

General experimental procedure

All of the melting points were determined on a thermoelectrical melting point apparatus and are uncorrected. The IR spectra were recorded in KBr disc on FT-IR spectrometer Shimadzu 8201 PC (4000-400 cm^{-1}). ^{1}H-NMR and ^{13}C-NMR spectra were recorded using solvent $CDCl_3$ and TMS as internal standard on Bruker DRX-300 spectrometer.

Extraction and isolation

Air dried powdered plant seeds (3kg) were extracted with 90% ethanol (50-55°C) in a Soxhlet apparatus for 74 hours. The ethanolic extract was further exhaustively partitioned with chloroform, ethyl acetate, acetone and methanol. The methanol soluble fraction was further concentrated under reduced pressure to yield brown viscous mass (3.65g), which was subjected to TLC examination using nBAW (4:1:5) as eluent and I_2

vapours as visualizing agent. It gave five spots indicating it to be a mixture of five compounds **1**, **2, 3, 4** and **5**. These compounds were separated by TLC and purified by column chromatography over silica gel using $CHCl_3$: MeOH (4:8) as eluent and studied separately.

Study of compound 1

It was crystalysed from acetone to yield 1.30g. It has m.p. 178-188°C , m.f. $C_{36}H_{46}O_{21}$, $[M]^+$ 814 (FABMS); found(%): C 53.93, H 8.78, O 37.27 calcd.(%) for m.f. $C_{36}H_{46}O_{21}$: C 53.07, H 5.65, O 41.28; UV: λ_{max} nm: (MeOH) 340, 262, 238; IR: ν_{max}^{KBr} (cm^{-1}), 3415, 2924, 2859, 1742, 1648, 1610, 1572, 1510, 1475, 1434, 1371, 1280, 1223, 1033, 964, 838, 816. For ^{1}H-NMR and ^{13}C-NMR data see tables I and II respectively.

Table I: ^{1}H-NMR (300 MHz, $CDCl_3$) of compound 1.

Position	δ_H(ppm)	Position	δ_H(ppm)
3	6.56 (1H, s)	6″	0.97 (3H, *J* 6.2Hz, Me-6″)
5	4.08 (3H, s, 5-OCH_3)	glu1‴	4.86 (1H, d, *J* 6.8Hz)
6	3.94 (3H, s, 6-OCH_3)	2‴, 3‴, 4‴, 5‴	3.23-3.40 (4H, m)
7	3.89 (3H, s, 7-OCH_3)	6_a‴	3.49 (1H, dd, *J* 12.0, 5.3Hz)
2′	7.38 (1H, d, *J* 1.8 Hz	6_b‴	3.62(1H, dd, *J* 12.0, 5.3Hz)
4′	3.99 (3H, s, 4′-OCH_3)	xyl 1⁗	4.73 (1H, d, *J* 7.6 Hz)
5′	7.05(1H, d, *J* 8.6 Hz)	2⁗	3.31 (1H, dd, *J* 9.2, 7.2Hz)
6′	7.54 (1H, dd, *J* 1.8, 8.6 Hz)	3⁗, 4⁗	3.52 (2H, m)
rha1″	5.04 (1H, d, *J* 7.5Hz)	5_a⁗	3.26(1H, dd, *J*13.2, 10.2Hz)
2″, 3″, 4″, 5″	3.08-3.65 (4H, m)	5_b⁗	3.66(1H, m)

Table II: ^{13}C-NMR (125 MHz, $CDCl_3$) of compound 1.

C	δ(ppm)	C	δ(ppm)
2	163.9	4′-OCH_3	55.9
3	108.2	1″	101.02
4	182.7	2″	71.6
5	152.1	3″	72.3
6	136.2	4″	78.4
7	131.9	5″	68.3
8	106.0	6″-CH_3	17.89
9	148.5	1‴	102.4
10	96.1	2‴	74.5
1′	122.9	3‴	76.4
2′	104.1	4‴	77.6
3′	176.4	5‴	75.8
4′	146.2	6‴	62.7
5′	114.9	1⁗	103.7
6′	121.0	2⁗	73.9
5-OCH_3	61.1	3⁗	76.8
6-OCH_3	62.7	4⁗	70.4
7-OCH_3	60.9	5⁗	66.6

Acid hydrolysis of compound 1

Compound **1** (450 mg) was dissolved in ethanol (20 ml) and refluxed with 25 ml of H_2SO_4 on water bath for 7 hr. The reaction mixture was concentrated and allowed to cool and residue was extracted with diethyl ether (Et_2O). The ether layer was washed with water and evaporated to dryness. The residue was subjected to column chromatography over silica gel column using $CHCl_3$: MeOH (4:8) to give compound **1-A**, identified as 8, 3′-dihydroxy-5, 6, 7, 4′-tetramethoxyflavone by comparison of its spectral data with reported literature values. The aqueous hydrolysate was neutralized with $BaCO_3$ and the $BaSO_4$ filtered off. The filtrate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) solvent and aniline hydrogen phthalate as spraying reagent, showed the presence of D-xylose (R_f 0.28), D-glucose (R_f 0.18) and L-rhamnose (R_f 0.36), (Co-PC and Co-TLC).

Study of compound 1-A

It has m.f. $C_{19}H_{18}O_8$, m.p. 186-188°C, $[M]^+$ 374 (EIMS); found(%): C 60.82, H 4.78, O 34.40, calcd.(%) for m.f. $C_{19}H_{18}O_8$, C 60.96, H 4.82, O 34.22; UV: λ_{max} nm: (MeOH) 343, 264, 240; IR: ν_{max}^{KBr} (cm^{-1}), 3418, 2926, 2861, 1745, 1650, 1607, 1575, 1513, 1478, 1436, 1370, 1282, 1226, 1035, 967, 840, 818; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 6.56 (1H, s, H-3), 7.38 (1H, d, *J* 1.8 Hz, H-2′), 7.05(1H, d, *J* 8.6 Hz, H-5′), 7.54 (1H, dd, *J* 1.8, 8.6 Hz, H-6′), 4.08 (3H, s, 5-OCH_3), 3.94 (3H, s, 6-OCH_3), 3.89 (3H, s, 7-OCH_3), 3.99 (3H, s, 4′-OCH_3). ^{13}C-NMR (125 MHz, $CDCl_3$): δ(ppm) 164.2 (C-2), 108.4 (C-3), 183.2 (C-4), 152.7 (C-5), 136.7(C-6), 132.6 (C-7), 106.6 (C-8), 148.9 (C-9), 95.8 (C-10), 123.3 (C-1′), 103.6 (C-2′), 135.5 (C-3′), 145.8 (C-4′), 115.6 (C-5′), 120.6 (C-6′), 61.6 (5-OCH_3), 62.4 (6-OCH_3), 61.5 (7-OCH_3), 56.2 (4′-OCH_3).

Permethylation of compound 1

Compound **1** (35mg) was refluxed with MeI (10ml) and Ag_2O (25ml) in DMF (25mg) for four days. The reaction mixture was filtered and washed with DMF. The filtrate was concentrated under reduced pressure and treated with $CHCl_3$ (15ml) and washed with water. After removal of solvent a syrupy mass was obtained which was hydrolyzed with 8% ethanolic H_2SO_4 (8ml) for 7-8 hrs, to give aglycone, identified as 8, 3′-dihydroxy-5, 6, 7, 4′-tetramethoxyflavone. The aqeous hydrolysate after the removal of aglycone was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to paper chromatography examination on

Whatmann filter paper No.1 using n-butanol:ethanol:water (5:1:4) solvent and aniline hydrogen phthalate as spraying agent. The methylated sugars were identified as 2, 3, 4-tri-O-methyl-D-xylose (R_G 0.94), 2, 3, 6-tri-O-methyl-D-glucose (R_G 0.83) and 2, 3-di-O-methyl-L-rhamnose (R_G 1.01).

Enzymatic hydrolysis of compound 1

Compound **1** (20 mg) was dissolved in MeOH (15 ml) and hydrolysed with equal volume of almond emulsion enzyme. The reaction mixture was allowed to stay at room temperature for 40hrs and filtered. The proaglycone and hydrolysate were studied separately. The hydrolysate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) as solvent and aniline hydrogen phthalate as spraying reagent, which showed the presence of sugars D-xylose (R_f 0.28) and D-glucose (R_f 0.18). The proaglycone was dissolved in MeOH (25ml) and further hydrolysed with equal volume of takadiastase enzyme at room temperature as usual procedure yielded aglycone and sugar was identified as L-rhamnose (R_f 0.36).

Study of compound 2

It has m.f. $C_{16}H_{12}O_4$, m.p. 163-164°C, $[M]^+$ 268 (EIMS); found(%), C 71.60, H 4.42, O 23.98, calcd.(%) for m.f. $C_{16}H_{12}O_4$, C 71.64, H 4.48, O 23.88; UV: λ_{max} nm: (MeOH) 259, 310; IR: ν_{max}^{KBr} (cm^{-1}), 3102-2810, 1659, 797, 761, 680, 633. ^{1}H-NMR (300 MHz, $CDCl_3$) : δ(ppm) 6.68 (1H, s, H-3), 6.42 (1H, d, *J* 2.1Hz, H-6), 6.52 (1H, d, *J* 2.1Hz, H-8), 7.88 (2H, d, *J* 7.8Hz, H-2′, H-6′), 7.55 (3H, m, H-3′, H-4′, H-5′), 3.90 (3H, s, 7-OCH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): δ(ppm) 165.1(C-2), 105.6 (C-3), 182.3 (C-4), 162.4 (C-5), 98.3 (C-6), 165.4 (C-7), 92.5 (C-8), 165.7 (C-9), 131.6 (C-1′), 126.4 (C-2′,C-6′), 129.3 (C-3′,C-5′), 131.8 (C-4′).

Study of compound 3

It has m.f. $C_{17}H_{14}O_4$, m.p. 156-157°C, $[M]^+$ 282 (EIMS); found(%), C 72.40, H 4.88, O 22.72, calcd.(%) for m.f. $C_{17}H_{14}O_4$, C 72.34, H 4.96, O 22.70; UV: λ_{max} nm: (MeOH) 264, 305; IR:ν_{max}^{KBr} (cm^{-1}), 2923, 1662, 1616, 1602, 1450, 1124. ^{1}H-NMR (300 MHz, $CDCl_3$) : δ(ppm) 3.91 (3H, s, 7-OCH_3), 3.95 (3H, s, 5-OCH_3), 6.56 (1H, d, *J* 2.0Hz, H-6), 6.36 (1H, d, *J* 2.0Hz, H-8), 6.67(1H, s, H-3), 7.48 (3H, m, H-3′, H-4′, H-5′), 7.85 (2H, dd, *J* 8.0Hz, 3.5Hz, H-2′, H-6′). ^{13}C-NMR (75 MHz, $CDCl_3$): δ(ppm) 159.7 (C-2), 100.2 (C-3), 176.6 (C-4), 160.8 (C-5), 92.7 (C-6), 161.2 (C-7), 96.3 (C-8), 164.2 (C-9), 109.3 (C-10), 131.6 (C-1′), 125.7 (C-2′, C-6′), 128.6 (C-3′, C-5′), 131.3 (C-4′).

Study of compound 4

It has m.f. $C_{28}H_{48}O$, m.p. 158-159°C, $[M]^+$ 400 (EIMS); found(%), C 83.40, H 12.32, O 4.28, calcd.(%) for m.f. $C_{28}H_{48}O$, C 84.00, H 12.00, O 4.00; UV: λ_{max} nm: (MeOH) 202, 250 ; IR: ν_{max}^{KBr} (cm^{-1}), 3472, 3320, 2918, 2851, 1462, 1375, 1236, 1096, 1040, 682. ^{1}H-NMR (300 MHz, $CDCl_3$) : δ(ppm) 5.34 (1H, m, H-6), 3.34 (1H, tdd, OH-3), 0.66 (3H, s, CH_3-18), 1.15 (3H, s, CH_3-19), 1.03 (3H, s, CH_3-21), 0.81 (3H, s, CH_3-26), 0.79 (3H, s, CH_3-27), 0.83 (3H, s, CH_3-28). ^{13}C-NMR (75 MHz, $CDCl_3$): δ(ppm) 11.94 (C-18), 11.99 (C-29), 18.78 (C-21), 18.87 (C-27), 19.24 (C-19), 19.53 (C-28), 21.14 (C-11), 23.18 (C-26), 24.39 (C-15), 26.22 (C-23), 28.32 (C-16), 29.27 (C-25), 31.66 (C-2), 31.96 (C-7), 32.08 (C-8),32.49 (C-22), 34.00 (C-20), 36.23 (C-10), 36.54 (C-1), 37.3 (C-14), 39.88 (C-12), 42.36 (C-13), 45.93 (C-24), 50.23 (C-9), 56.13 (C-17), 56.87 (C-4), 77.44 (C-3), 121.69 (C-6), 140.88 (C-5).

Study of compound 5

It has m.f. $C_{21}H_{20}O_{12}$, m.p. 228-230°C, $[M]^+$ 464 (EIMS); found(%), C 54.38, H 4.42, O 41.20, calcd.(%) for m.f. $C_{21}H_{20}O_{12}$, C 54.31, H 4.31, O 41.38; UV: λ_{max} nm: (MeOH) 258, 358; IR:ν_{max}^{KBr} (cm^{-1}), 3580, 3446, 3340, 3362. ^{1}H-NMR (300 MHz, $CDCl_3$) : δ(ppm) 6.18 (1H, d, *J* 2.1Hz, H-6), 6.36 (1H, d, *J* 2.1Hz, H-8), 7.68 (1H, d *J* 2.1Hz, H-2′) , 6.87 (1H, d *J* 8.6 Hz, H-5′), 7.56 (1H, dd *J* 8.6Hz, 2.1Hz, H-6′), 5.25 (1H, d, *J* 7.8Hz, H-1″), 3.46 (1H, t, *J* 9.2Hz, H-2″), 3.33 (1H, t, *J* 8.7Hz, H-3″), 3.45 (1H, d, *J* 9.6Hz, H-4″), 3.22 (1H, m, H-5″), 3.70 (2H, dd, *J* 2.1Hz, 11.6Hz, H-6″). ^{13}C-NMR (75 MHz, $CDCl_3$): δ(ppm) 158.5 (C-2), 135.3 (C-3), 179.8 (C-4), 163.4 (C-5), 100.7 (C-6), 166.5 (C-7), 94.5(C-8), 159.7 (C-9), 105.0 (C-10), 123.1 (C-1′), 1116.7 (C-2′), 145.6 (C-3′),149.2 (C-4′),117.3 (C-5′), 123.4 (C-6′), 104.5 (C-1″), 75.9 (C-2″), 78.4 (C-3″), 72.0 (C-4″), 77.7 (C-5″), 68.2 (C-6″).

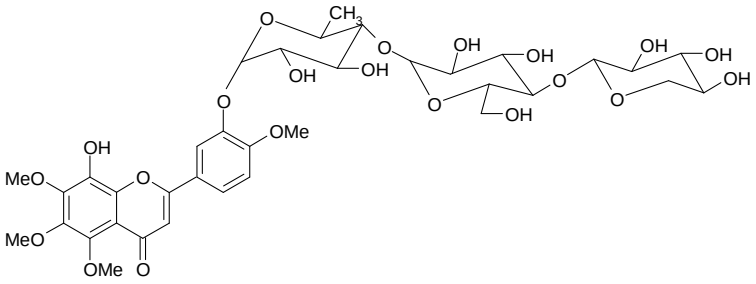
Antifungal activity of compound 1

The antifungal activity of compound **1** was measured by Paper Disc Diffusion method[23] using PDA (Potato Dextrose Agar) with 4% agar for the preparation of plates and incubated with spores and mycelium suspension of fungi obtained from one week old culture. The diameters of zone of inhibition were measured at 28±1°C after 45 h. The results are recorded in **Table III**.

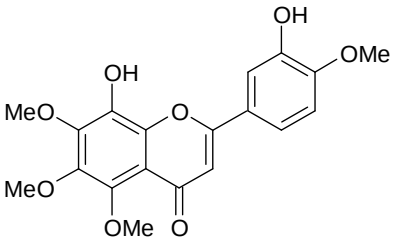
Table III: Antifungal activity of compound 1.

	Fungal species	Diameters of zone of inhibition (mm)* Concentration of compound 1 (%)					Std.**
		100	80	60	40	20	
	Candida albicans	13.8	8.5	6.3	4.3	3.2	20.8
	Mucor indicus	10.2	6.1	2.5	-	-	20.5
	Rhizopus oryzae	9.5	5.2	3.3	-	-	15.8
	Aspergillus niger	9.7	7.6	5.8	3.4	2.3	22.4

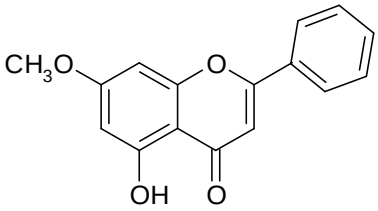
*The zone of inhibition (mm) taken as average of four determination direction.
**Ketocozole (100 mg/mL) used as standard antifungal agent.



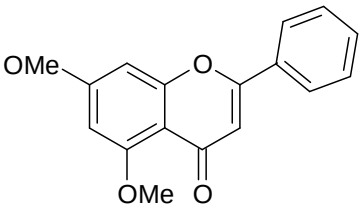
Compound 1



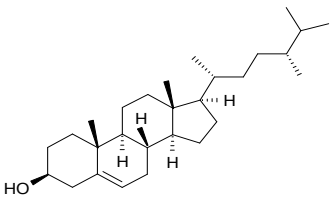
Compound 1A



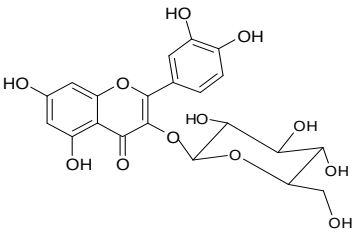
Compound 2



Compound 3



Compound 4



Compound 5

CONCLUSION

Thus on the basis of above evidences the structure of compound **1** was established as 8, 3′-dihydroxy-5, 6, 7, 4′-tetramethoxyflavone-3′-O-β-D-xylopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-α-L-rhamnopyranoside, along with four known compounds from ethanolic extract of the seeds of ***Hardwickia binata*** Roxb. Compound **1** showed significant antifungal activity against various fungi. Hence, compound **1** can be used as a potent antifungal agent.

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