

## SUPPLEMENTARY MATERIAL

### Phytochemical constituents, antimicrobial and antioxidant activities of Kumaun Himalayan Hoop pine bark extract

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#### Abstract

The chemical composition of the methanol extract obtained from *Araucaria cunninghamii* Sweet. bark was determined by GC-MS analysis with its antioxidant and antibacterial potential, for the first time. A total of 73 compounds were identified and the extract was strongly characterised by Viridiflorene; Androstan-17-one, 3-ethyl-3-hydroxy-, (5 $\alpha$ )-; 8-Isopropenyl-1,3,3,7-tetramethyl-bicyclo[5.1.0]oct-5-en-2-one; 3,6,9-Triethyl-3,6,9-trimethyl-tetracyclo[6.1.0.0~2,4~.0~5,7~]-nonane; Longifolenaldehyde; (-)-Caryophyllen-(II); 2-hydroxymethyl-5-furfural; Methyl commate B; 3-Ethyl-3-hydroxyandrostan-17-one; 3-Hydroxybenzoic acid as the main components (55.39%). *A. cunninghamii* extract presents IC<sub>50</sub>, 53.52  $\mu$ g/mL and 65.29  $\mu$ g/mL using DPPH and H<sub>2</sub>O<sub>2</sub> scavenging assay, respectively. The antibacterial activity of the extract was evaluated for eight microorganisms showed that the extract had a remarkable inhibitory potential with a mean zone diameter of inhibition ranging from 09 to 21 mm. The methanol extract of *A. cunninghamii* showed significant antibacterial activity against *X. campestris* (21 mm, ZOI) with MIC and MBC values 15.6 and 31.25  $\mu$ g/mL, respectively.

**Keywords:** Antibacterial activity, antioxidant activity, *Araucaria cunninghamii*, GC-MS analysis, plant extract

## **Experimental Section**

### ***Collection of plant material***

The internal bark of *Araucaria cunninghamii* was collected in the month of May from Nainital, Kumaun Himalaya, India and identified with the help of available literature and plant taxonomist (Prof. Y.P.S. Pangtey) of the Botany department, Kumaun University Nainital. A voucher specimen was deposited in the Herbarium of Botanical Survey of India (BSI), Northern Regional Centre 192, Kaulagarh Road, Dehradun, Uttarakhand for further reference (BSI-Accession no. 117255).

### ***Extraction procedure***

The collected bark material of the plant was dried at room temperature ( $23 \pm 2^\circ \text{C}$ ). The dried material was powdered in an electric grinder. To prepare the stock solution 50 g of this powder was placed in a 500 mL conical flask mixed with 200 mL of methanol solvent (w/v, 50g/200 mL). The mouth of flasks are tightly plugged with non-absorbent cotton and tightly wrapped with aluminum foil to prevent evaporation. The mixture was shaken on a rotary incubator shaker at 190-220 rpm for 24 h at room temperature  $37^\circ \text{C}$ . The mixture was filtered through Whatman filter paper no.1 and filtrate collected separately in a clean beaker. The extract was evaporated, using steam bath to dryness at  $30^\circ \text{C}$ . The dry extract was weighed and kept in sterile sample bottles and stored in the refrigerator at  $4^\circ \text{C}$  for further use.

### ***Chemical characterization via GC-MS analysis:***

Detection and quantification of the presence of secondary metabolites in methanol extract were carried out by gas chromatography coupled with mass spectrometry (Shimadzu QP2010 Plus) equipped with an Rtx- 5 MS capillary column (0.25 mm film thickness, 0.25 mm internal diameter, and 30 m in length). The oven temperature was set at  $100^\circ \text{C}$  for 2 min, then increased to  $250^\circ \text{C}$  with a rate of  $5^\circ \text{C}$  per minute, and finally to  $280^\circ \text{C}$  with a rate of  $10^\circ \text{C}$  per minute. One  $\mu\text{L}$  of each sample was injected into the column in split mode (split ratio 10) with helium as the carrier gas with a flow rate of 1.21 mL per minute. The presence of distinctive peak fragmentation patterns for various metabolites was detected by an MS detector in full scan mode. The identification of metabolites was confirmed by comparing the spectral data of peaks with the corresponding standard mass spectra from the library database [National Institute of Standards and Technology library (NIST) and Wiley 8].

### ***Antibacterial activity of the plant extract***

#### ***Microorganism used***

Five (Gram-positive and Gram-negative) bacteria (*Bacillus subtilis* MTCC No. 121, *Escherichia coli* MTCC No. 40, *Agrobacterium tumefaciens* MTCC No. 609, procured from Institute of Microbial Technology Chandigarh, India and [\*Ralstonia solanacearum\* ITCC No. BH0007](#), [\*Xanthomonas campestris\* ITCC No. BD0006](#), [\*Xanthomonas oryzae\* ITCC No. PI0012](#) obtained from [Indian Agriculture Research Institute, New Delhi, India](#) on the other hand *Xanthomonas phaseoli* and *Erwinia chrysanthemi* obtained from Plant Pathology Department, G. B. Pant University, Pantnagar, India, were used in this investigation. Pathogens obtained from respective stock cultures were inoculated (1 % v/v) into nutrient agar broth followed by incubation at 37° C (for 18 h) to activate cultures.

#### ***Screening of antibacterial activity of plant extract***

Antibacterial activity of *A. cunninghamii* bark extract against selected microorganisms was carried out using the disc diffusion method (Bauer et al., 1966). Nutrient agar plates (90 mm size) were prepared and cool down at room temperature (20± 2°C). Test bacterial inoculums containing 10<sup>6</sup> CFU/mL of test bacteria were spread uniformly. A small sterile swab was dipped into 24h old test culture of bacteria and was inoculated by streaking the swab over the entire agar surface. The process was repeated by streaking the swab 2 or more times rotating the plates approximately 60° each time to ensure even distribution of inoculums. Each bacterial culture was inoculated on three plates as replicates. The stock solution of 1000µg/mL concentration of the test sample was prepared into a 10% v/v aqueous dimethyl sulfoxide solution. The sterile filter paper disc (5 mm) loaded with 20µl of extract were placed on the surface of the bacteria seeded agar plates at equidistance and it was allowed to diffuse for 5 min then these plates were incubated at 37± 1°C for 24 h of incubation in the bacterial incubator. Gentamycin and kanamycin (30 mcg each) were placed into agar plates used as positive controls and 10% v/v aqueous dimethyl sulfoxide solution was also used as the negative control. After 24 h of incubation, the diameter was observed for inhibition zone (measured in mm including disc size). All tests were performed in triplicates and observed values of ZOI are expressed as mean value with the standard error of means (SEM).

#### ***Determination of minimum inhibitory concentrations (MIC's)***

MIC is defined as the lowest concentration of the antimicrobial agent that inhibits the microbial growth after 24 h. of incubation. MIC tests were conducted only for those

microorganisms which have more sensitivity ( $ZOI \geq 10$  mm) at 1000  $\mu\text{g/mL}$  concentration using the disk diffusion method and evaluate their efficiency in controlling bacterial strains causing plant pathogenic diseases. MIC and MBC were performed at seven concentrations of extract (500, 250, 125, 62.5, 31.25, 15.625 and 7.8  $\mu\text{g/mL}$ ) following two fold serial dilution technique (Zaidi et al., 2009), and loaded their requisite amount over sterilized filter paper discs (6 mm in diameter). Nutrient agar was poured into sterile Petri dishes and seeded with bacterial suspensions of the pathogenic strains. The loaded filter paper discs with different concentrations of the extract were placed on the Nutrient agar plates. The plates were incubated at 35°C for 24 h. The inhibition zones were measured by Verniercaliper and recorded against the concentrations of the plant extract.

#### *Determination of minimum bactericidal concentrations (MBC's)*

Streaks were taken from the two lowest concentrations of the plant extract plates exhibiting invisible growth (from inhibition zone of MIC plates) and subcultures onto sterile nutrient agar (NA) plates. The plates were incubated at 35°C for 24 h. then examined for bacterial growth in corresponding to plant extract concentration. MBC was taken as the concentration of plant extract that did not exhibit any bacterial growth on the freshly inoculated agar plates.

#### *Determination of antioxidant activity*

##### *Radical Scavenging activity by the DPPH Assay*

The antioxidant activity of methanol extract of *A. cunninghamii* bark was assessed on the basis of its scavenging effect against the DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical. DPPH radical scavenging ability was evaluated based on the method given by Xie et al. (2010) with some modifications. A fresh solution of DPPH (0.1mM) was prepared in methanol and 2 mL of this solution was mixed with 2 mL of the extract at different concentrations (10 -100  $\mu\text{g/mL}$ ). The mixture of 2mL DPPH in 2mL methanol was used as control. The reaction mixture was incubated in dark for 30 minutes at room temperature and analyzed spectrophotometrically at 517 nm against the blank. Ascorbic acid was used as the positive control. The free radical scavenging potential (%) of the plant extract was calculated with the formula given by Blois (1958).

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of test Sample}}{\text{Absorbance of Control}} \times 100$$

The IC<sub>50</sub> value of the test sample and standard was calculated from the graph of inhibition percentage plotted against concentration.

#### *Hydrogen peroxide scavenging activity*

Hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 mL) of different concentrations of extract were transferred into the test tubes and their volumes were made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4). After the addition of 0.6 mL hydrogen peroxide solution, tubes were vortexed and absorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against a blank (Ruch et al., 1989). The abilities to scavenge the hydrogen peroxide were calculated using the following equation:

$$\text{Hydrogen peroxide scavenging activity} = \frac{1 - \text{absorbance of sample}}{\text{absorbance of sample}} \times 100$$

#### *FRAP Antioxidant Assay*

Ferric reducing antioxidant power (FRAP) assay was performed by the using method of Cai et al. (2004), with minor modification. The reductive potential of *A. cunninghamii* was determined based on the chemical reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup>. 10µl of sample extract was taken and make it up to 100µl with double distilled (d.d.) water and was mixed with 1.5mL of freshly prepared and pre-warmed (37°C) FRAP reagent and kept at 37°C for 10 minutes. Absorbance was taken at 593 nm. The standard was prepared by using ascorbic acid. For control absorbance of FRAP reagent (300 mM acetate buffer, pH = 3.6, 10 mM tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 20 mM FeCl<sub>3</sub>.6H<sub>2</sub>O in the ratio of 10:1:1) was taken without adding sample extract. Results were expressed in mg ascorbic acid equivalent (AAE) per g dry weight of the sample extract.

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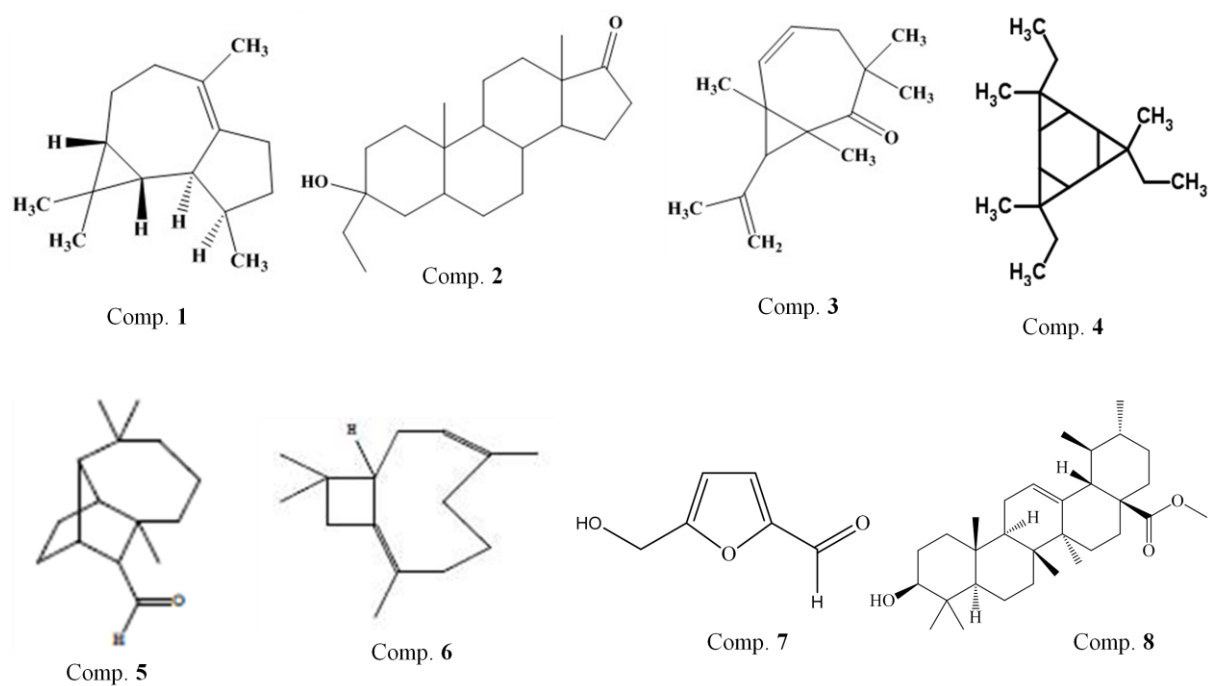
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**Table S1:** Compounds identified in the methanol extract of *A. cunninghamii* bark

S. No.	Compound Name	Retention Time	Area %	Molecular formula	Retention index	Molecular weight (g/mol)
1	Cyclobutanecarboxylic acid, hexyl ester	6.556	0.45	C <sub>11</sub> H <sub>20</sub> O <sub>2</sub>	1304	184
2	Tetrahydrocyclopenta[1,3]dioxin-4-one	7.513	0.65	C <sub>7</sub> H <sub>10</sub> O <sub>3</sub>	1196	142
3	1,3,5-Triazine-2,4,6-triamine	8.845	0.44	C <sub>3</sub> H <sub>6</sub> N <sub>6</sub>	1538	126
4	Pyrimidine-4,6-diol, 5-methyl-	9.376	0.23	C <sub>5</sub> H <sub>6</sub> N <sub>2</sub> O <sub>2</sub>	1222	126
5	4H-1, 3, 2-Dioxazine-2-acetic acid, dihydro- $\alpha$ - $\alpha$ ,4-trimethyl-, methyl ester	10.502	1.22	C <sub>9</sub> H <sub>17</sub> NO <sub>4</sub>	1195	203
6	Catechol	11.792	0.66	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>	1122	110
7	2-hydroxymethyl-5-furfural	12.525	3.32	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	1267	126
8	Trimethylsilyl ester of 3-methyl-furan-2-carboxylic acid	14.422	0.27	C <sub>9</sub> H <sub>14</sub> O <sub>3</sub> Si	1420	198
9	2-Propylphenol	15.797	0.09	C <sub>9</sub> H <sub>12</sub> O	1213	136
10	3,4-Dimethoxyphenol	16.977	0.32	C <sub>8</sub> H <sub>10</sub> O <sub>3</sub>	1383	154
11	4-hydroxybenzoic acid, methyl ester	17.608	0.15	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>	1410	152
12	3-Hydroxybenzoic acid	18.508	2.54	C <sub>7</sub> H <sub>6</sub> O <sub>3</sub>	1417	138
13	Nonanedioic acid, dimethyl ester	19.436	0.09	C <sub>11</sub> H <sub>20</sub> O <sub>4</sub>	1449	216
14	Vanillic acid	19.791	0.14	C <sub>8</sub> H <sub>8</sub> O <sub>4</sub>	1560	168
15	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	21.160	0.13	C <sub>15</sub> H <sub>24</sub> O	1690	220
16	3-Butyn-2-one, 4-[3,3-dimethyl-2-(1-methylethyl)oxiranyl]-	21.497	1.45	C <sub>11</sub> H <sub>16</sub> O <sub>2</sub>	1204	180
17	Selina-6-en-4-ol	21.801	0.34	C <sub>15</sub> H <sub>26</sub> O	1593	222
18	Methyl homovanillate	22.843	0.18	C <sub>10</sub> H <sub>12</sub> O <sub>4</sub>	1540	196
19	Longifolenaldehyde	23.060	0.41	C <sub>15</sub> H <sub>24</sub> O	1581	220
20	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol	23.406	0.54	C <sub>15</sub> H <sub>24</sub> O	1745	220
21	3-Ethyl-3-hydroxyandrostane-17-one	23.857	2.85	C <sub>21</sub> H <sub>34</sub> O <sub>2</sub>	2251	318
22	Glaucyl alcohol	24.347	0.19	C <sub>15</sub> H <sub>24</sub> O	1732	220
23	Mome inositol	24.696	0.95	C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>	1714	194
24	Methyl palmitate	26.402	1.57	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	1927	270
25	Androstan-17-one, 3-ethyl-3-hydroxy-, (5. $\alpha$ .)-	26.747	0.21	C <sub>21</sub> H <sub>34</sub> O <sub>2</sub>	2609	318
26	(-)-Spathulenol	26.858	2.20	C <sub>15</sub> H <sub>24</sub> O	1536	220
27	l-(+)-Ascorbic acid 2,6-dihexadecanoate	26.997	1.22	C <sub>38</sub> H <sub>68</sub> O <sub>8</sub>	4765	652
28	6-[1-(Hydroxymethyl)vinyl]-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro--2-naphthalenol	27.640	0.55	C <sub>15</sub> H <sub>24</sub> O <sub>2</sub>	1933	236
29	Bicyclo[4.4.0]dec-2-en-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)-	27.990	2.16	C <sub>15</sub> H <sub>24</sub> O <sub>2</sub>	1904	236
30	3. $\alpha$ .,7. $\beta$ .-Dihydroxy-5. $\beta$ .,6. $\beta$ .-epoxycholestane	28.407	0.16	C <sub>27</sub> H <sub>46</sub> O <sub>3</sub>	2797	418
31	Linoleic acid,methyl ester	29.077	2.37	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	2093	294
32	(Z,Z)-6,9-cis-3,4-epoxy-nonadecadiene	29.170	2.30	C <sub>19</sub> H <sub>34</sub> O	2108	278
33	(E)-phytol	29.400	0.18	C <sub>20</sub> H <sub>40</sub> O	1949	296
34	Leinoleic acid	29.665	0.90	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	2183	280
35	Dichloroacetic acid, tridec-2-ynyl ester	29.761	1.13	C <sub>15</sub> H <sub>24</sub> Cl <sub>2</sub> O <sub>2</sub>	2042	306

36	Tetraneurin- $\alpha$ -diol	30.116	0.50	C <sub>15</sub> H <sub>20</sub> O <sub>5</sub>	1704	280
37	$\gamma$ -himachalene	30.245	0.73	C <sub>15</sub> H <sub>24</sub>	1499	204
38	1,3A-Ethano-3ah-indene, 1,2,3,4,7,7a-hexahydro-1,4,4,5-tetramethyl- or $\alpha$ -clovene	30.428	0.16	C <sub>15</sub> H <sub>24</sub>	1454	204
39	geranyl- $\alpha$ -terpinene	30.596	0.14	C <sub>20</sub> H <sub>32</sub>	1962	272
40	Octanoic acid, [1-methyl-3-(2,6,6-trimethylcyclohex-1-enyl) propylidene] hydrazide	30.803	0.42	C <sub>11</sub> H <sub>13</sub> DO <sub>2</sub>	2702	179
41	Methyl (Z)-5,11,14,17-eicosatetraenoate	31.800	0.19	C <sub>21</sub> H <sub>34</sub> O <sub>2</sub>	2308	318
42	$\alpha$ -Bulnesene	31.903	0.97	C <sub>15</sub> H <sub>24</sub>	1513	204
43	8-Isopropenyl-1,3,3,7-tetramethyl-bicyclo[5.1.0]oct-5-en-2-one	32.348	6.09	C <sub>15</sub> H <sub>22</sub> O	1524	218
44	Viridiflorene	32.700	15.59	C <sub>15</sub> H <sub>24</sub>	1512	204
45	Androstan-17-one, 3-ethyl-3-hydroxy-, (5. $\alpha$ .)-	33.179	8.14	C <sub>21</sub> H <sub>34</sub> O <sub>2</sub>	2499	318
46	T-2 tetraol	33.785	0.88	C <sub>15</sub> H <sub>22</sub> O <sub>6</sub>	2331	298
47	Grandiflorenic acid	34.106	0.19	C <sub>20</sub> H <sub>28</sub> O <sub>2</sub>	2156	300
48	1-Phenan	34.282	0.19	C <sub>21</sub> H <sub>32</sub> O <sub>2</sub>	2211	316
49	Dihydrotestosterone propionate	34.430	0.10	C <sub>22</sub> H <sub>34</sub> O <sub>3</sub>	2367	346
50	Germacrene B	34.638	0.42	C <sub>15</sub> H <sub>24</sub>	1481	204
51	Methyl commate B	34.827	3.50	C <sub>31</sub> H <sub>50</sub> O <sub>3</sub>	3560	470
52	Bicyclo[10.6.0]octadeca-1(12),15-dien	35.083	0.40	C <sub>18</sub> H <sub>30</sub>	2082	246
53	Methyl (20s,22e)-3. $\beta$ -acetoxy-5. $\alpha$ -chol-22-enate	35.806	1.00	C <sub>27</sub> H <sub>42</sub> O <sub>4</sub>	3299	430
54	Abietic acid	36.134	0.51	C <sub>20</sub> H <sub>30</sub> O <sub>2</sub>	2265	302
55	Longifolenaldehyde	36.479	4.85	C <sub>15</sub> H <sub>24</sub> O	1581	220
56	Tetracyclo[6.1.0.0(2,4).0(5,7)]nonane, 3,6,9-triethyl-3,6,9-trimethyl-	37.363	2.04	C <sub>18</sub> H <sub>30</sub>	1365	246
57	Methyl copalate	37.673	0.84	C <sub>21</sub> H <sub>34</sub> O <sub>2</sub>	2186	318
58	$\alpha$ -cedrane	37.959	1.67	C <sub>15</sub> H <sub>26</sub>	1441	206
59	(-)-caryophyllen-(II)	38.447	3.65	C <sub>15</sub> H <sub>24</sub>	1454	204
60	17. $\beta$ -Hydroxy-6-oxo-4,5-secoandrostan-4-oic acid methyl ester	38.696	2.06	C <sub>20</sub> H <sub>32</sub> O <sub>4</sub>	2496	336
61	Alloaromadendrene oxide-(2)	38.905	0.61	C <sub>15</sub> H <sub>24</sub> O	1462	220
62	3,6,9-Triethyl-3,6,9-trimethyltetracyclo[6.1.0.0~2,4~.0~5,7~]-nonane	39.562	4.86	C <sub>18</sub> H <sub>30</sub>	1365	246
63	Longifolenbromid-i	39.785	0.90	C <sub>15</sub> H <sub>23</sub> Br	1656	282
64	Humulane-1,6-dien-3-ol	40.129	0.26	C <sub>15</sub> H <sub>26</sub> O	1757	222
65	Shonanin	42.128	0.17	C <sub>20</sub> H <sub>24</sub> O <sub>5</sub>	3336	344
66	3a-Methoxy-9b-lanosta-7,24-dien-26,23-olide	44.757	0.77	C <sub>31</sub> H <sub>48</sub> O <sub>3</sub>	3158	468
67	2-Propenoic acid, 3-(3-hydroxy-4-methoxyphenyl)-	47.332	0.29	C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	1476	194
68	Campesterin	48.093	0.41	C <sub>28</sub> H <sub>48</sub> O	3131	400
69	Colchiceinamide	48.737	0.33	C <sub>21</sub> H <sub>24</sub> N <sub>2</sub> O <sub>5</sub>	3324	384
70	$\beta$ -sitosterol	50.316	2.39	C <sub>29</sub> H <sub>50</sub> O	3220	414
71	Cycloartane-3. $\beta$ .,25-diol (campesterol)	51.371	0.46	C <sub>30</sub> H <sub>52</sub> O <sub>2</sub>	2923	444
72	4,4-Dimethylcholest-5-enol	51.955	0.41	C <sub>29</sub> H <sub>50</sub> O	2731	414
73	Sitostenone	54.123	0.33	C <sub>29</sub> H <sub>48</sub> O	3458	412



**Figure S1**-Chemical structures of identified major compounds from the bark extract of *A. cunninghamii*. **Comp.1:** Viridiflorene; **Comp. 2:** Androstan-17-one, 3-ethyl-3-hydroxy-, (5 $\alpha$ )-; **Comp. 3:** 8-Isopropenyl-1,3,3,7-tetramethyl-bicyclo[5.1.0]oct-5-en-2-one; **Comp. 4:** 3,6,9-Triethyl-3,6,9-trimethyltetracyclo[6.1.0.0~2,4~.0~5,7~]; **Comp. 5:** Longifolenaldehyde; **Comp. 6:** (-)-Caryophyllen-11; **Comp. 7:** 2-hydroxymethyl-5-furfural; **Comp. 8:** Methyl commate B



**Table S2** - Antibacterial activity of bark extract of *A. cunninghamii* and its minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

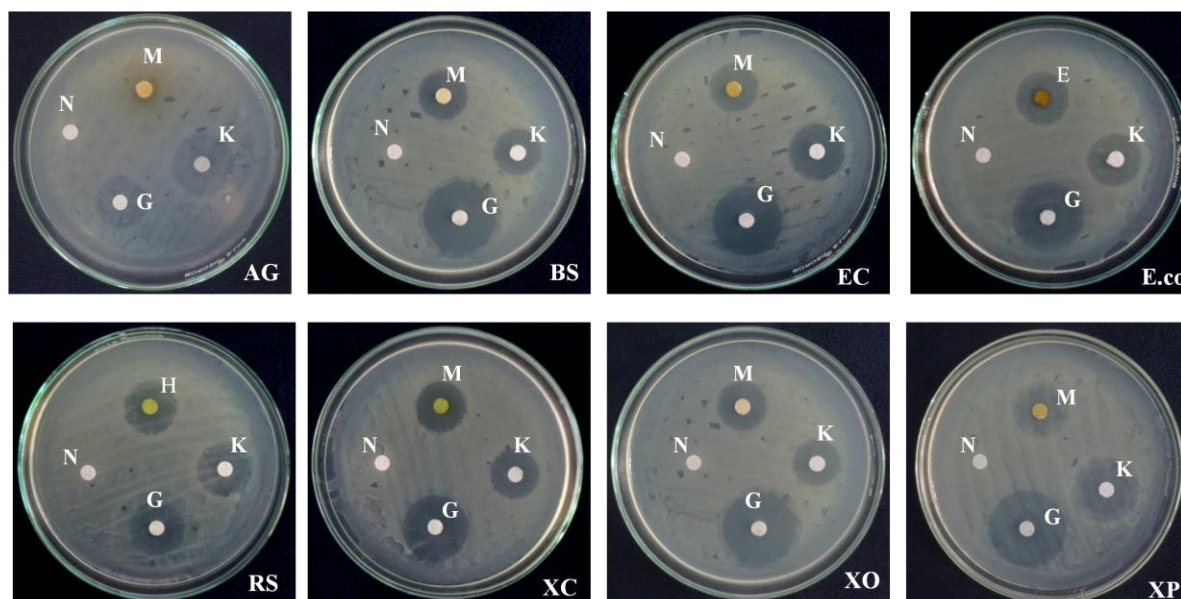
Microorganisms	Plant extract		Standard drugs			
	<i>A. cunninghamii</i> extract		Kanamycin		Gentamycin	
	Zone Inhibition (mm)	MIC/MBC (µg/mL)	Zone Inhibition (mm)	MIC/MBC (µg/mL)	Zone Inhibition (mm)	MIC/MBC (µg/mL)
<i>A. tumefaciens</i>	09±0.6	nt/nt	18±0.7	3.9/15.6	24±0.9	0.48/3.9
<i>B. subtilis</i>	13±1.4	500/na	19±0.0	3.9/7.8	23±1.6	1.9/3.9
<i>E. chrysanthemi</i>	12±1.2	500/500	16±0.0	7.8/15.6	25±0.7	0.48/1.9
<i>E. coli</i>	15±0.6	250/500	20±0.7	1.9/7.8	26±0.6	1.9/1.9
<i>R. solanacearum</i>	18±0.6	31.25/62.5	20±1.6	7.8/15.6	22±1.2	3.9/7.8
<i>X. campestris</i>	21±1.7	15.6/31.25	22±0.9	3.9/7.8	26±1.8	0.48/3.9
<i>X. oryzae</i>	19±1.1	31.25/62.5	21±0.6	1.9/7.8	24±1.7	1.9/3.9
<i>X. phaseoli</i>	10±0.3	na/na	20±0.3	3.9/15.6	25±0.6	3.9/7.8
Mean activity	14.6±1.3		19.5±1.7		24.4±1.1	

\*All the values are mean ± Standard Error of Mean of three determinations, na- not active, nt-not tested

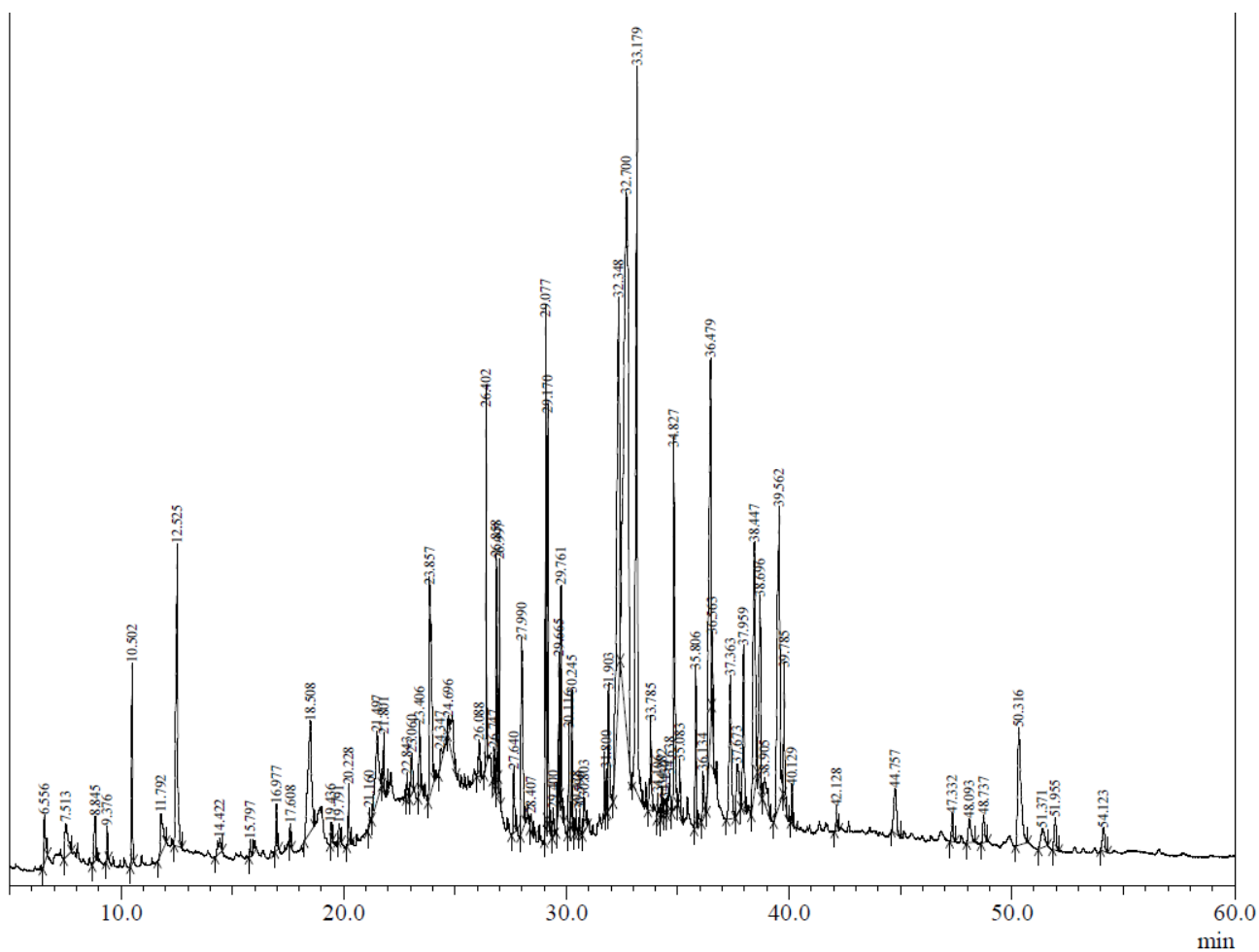
**Table S3** - Antioxidant activity of methanol extract of *A. cunninghamii* bark

Plant extracts	IC <sub>50</sub> value (µg/mL) of radical scavenging		*FRAP activity (mg AAE/g of dry extract)
	DPPH scavenging	Hydrogen peroxide scavenging	
Methanol	53.52±2.4	65.29±3.1	52.15±2.1
Ascorbic acid	19.84±0.7	12.56±1.5	

\*FRAP- Ferric reducing antioxidant power, mg Ascorbic acid equivalent (AAE)/g of dry extract



**Figure S2-** Antibacterial activity of *A. cunninghamii* bark methanol extract against eight pathogenic bacteria, M - methanol extract, AG- *A. tumefaciens*, BS- *B. subtilis*, EC- *E. chrysanthemi*, E.co- *E. coli*, RS- *R. solanacearum*, XC- *X. campestris*, XO- *X. oryzae*, XP- *X. phaseoli*, K- Kenamycin, G- Gentamycin (positive controls), N- negative control (solvent).



**Figure S3:** GC-MS Chromatogram of methanol extract of *A. cunninghamii* bark