

# Potential Bioactive Compounds Isolated from *Boesenbergia rotunda* as Antioxidant and Antimicrobial Agents

ABOUT AUTHORS

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## Authors

## INTRODUCTION

The plant of Zingiberaceae family spread in South Asia and Southeast Asia, consisting of 47 genera and approximately 1000 species.<sup>1,2</sup> The rhizome of this plant have a high commercial value because it contains volatile oil and other important compounds of enormous medicinal values and food processing.<sup>3</sup> *Kaempferia* is a genus, and belongs to family of Zingiberaceae. This plant grows in Southeast Asia, India, Sri Lanka, Indonesia, and Southern China. *Boesenbergia rotunda* L. is synonym with *Kaempferia pandurata* ROXB. The local name in Indonesia is “Temu kunci”, this plant is a common edible ingredient in many Asian countries. In Indonesia, *B. rotunda* is typically used to prepare “jamu,” a popular traditional tonic for women after childbirth as well as a beauty aid for teenage girls and to prevent leukorrhea.<sup>4</sup>

Some essential oils extracted from *B. rotunda* rhizome are identified as for example geranyl formate, geranyl propionate, geraniol, neral, myrcene, isoborneol,  $\beta$ -pinene, neryl acetate, geranial,  $\beta$ -thujaplicin (E, E)- $\alpha$ -farnesene, borneol, tricyclene, terpinen-4-ol, terpinolene, myristicin, allo ocimene,  $\alpha$ -thujene, (Z)- $\beta$ -ocimene, sabinene, (E)- $\beta$ -ocimene, (Z)-nerolidol, cis-linalool oxide, 3-carene,  $\delta$ -elemene, (Z)- $\beta$ -farnesene,  $\gamma$ -elemene, and  $\beta$ -elemen.<sup>5</sup> Essential oil of *B. rotunda* showed antifungal properties against *Aspergillus niger*, *A. fumigatus* and *Mucor*. The extract of *Boesenbergia pandurata* very effectively kills pathogenic bacteria *C. albicans*<sup>5,6</sup> *in vitro*. The polyphenols compounds includes such as quercetin, kaempferol, naringin, hesperidin, caffeic acid, *p*-coumaric acid, and chlorogenic acid.<sup>4</sup> Many researchers reported of the chemical constituents of *B. rotunda* to have activities as anticancer.<sup>7,8</sup> and antioxidant.<sup>9</sup> A particular compound of panduratin isolated from *B. pandurata* showed cytotoxic against pancreatic PANC-1 cancer cell under nutrient deprived condition.<sup>10</sup> This paper reports our continued investigation of some compounds

isolated from *B. rotunda* and its biological activity as antioxidant and antimicrobial agents.

## MATERIALS AND METHODS

### Apparatus and Reagents

UV and IR spectra were measured with Varian Cary 100 Conc and Shimadzu 8300 FTIR, respectively.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded with Jeol JNM A-5000 spectrometers, operating at 500.0 MHz  $^1\text{H}$  and 125.0 MHz  $^{13}\text{C}$  using residual and deuterated solvent peaks as internal standards. Evaporator Buchi Rotavapor R-114, vacuum liquid chromatography (VLC) was carried out using Si-gel Merck 60 GF254 (230–400 mesh), column chromatography using Si-gel Merck 60 (200–400 mesh), and TLC analysis on precoated Si gel plates Merck Kieselgel 60 F254 0.25 mm, 20 x 20 cm, spectronic 20, and analytical balance were used in this work. Ethanol, methanol, hexane, ethyl acetate, acetone, 2,2-diphenyl-1-picrylhydrazyl (DPPH, Aldrich), ascorbic acid (Aldrich), chloramphenicol, Mueller-Hinton agar, paperdisk, and aquadest were used in this work without further purification.

### Plant Materials

Samples of the rhizome of *B. rotunda* were collected in December 2015 from the Beringharjo market, Yogyakarta, Indonesia. The plant was identified by the staff at the Faculty of Biology, Gadjah Mada University, Indonesia and a voucher specimen (BR-01-2016) was deposited at the Organic Laboratory, Department of Chemistry Education, Universitas Negeri Yogyakarta, Indonesia.

### Microorganism

Pathogenic bacterial isolates of *Escherichia coli* ATCC-11229, *Staphylococcus aureus* ATCC-25923, and *Staphylococcus epidermidis* FNCC-0048 were obtained from the Microbiology laboratory, Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Yogyakarta, Indonesia, while *Streptococcus mutans* was obtained from Dentistry laboratory, Gadjah Mada University, Indonesia. The microorganism were sub cultured and stored in a semisolid medium (Mueller Hinton agar plates) at 4°C until needed.

### Isolation and Structural Identification

The milled dried rhizome of *B. rotunda* (5 kg) was macerated by ethanol at 24 h for three times. The filtrate was separated by filtration and evaporated to dry using vacuum evaporator and yielded brown residue for about 147.6 g. The ethanol extract of *B. rotunda* (100 g) was partitioned within three times using n-hexane, chloroform, and ethyl acetate, respectively. Each fraction was evaporated to dryness under vacuum to yield brown residue chloroform fraction (50 g), and ethyl acetate fractions (30 g), while the hexane fraction was found to be an oil.

The ethyl acetate fraction (30 g) was fractionated in the vacuum liquid chromatography (VLC) (silica gel GF 60, 250 g;  $\phi$ : 10 cm, t = 10 cm), using n-hexane, n-hexane-ethyl acetate (9:1; 8:2; 6:4; 5:5; 4:6; and 6:4), ethyl acetate, acetone, and methanol in order of increasing polarity as eluent to give twenty fractions. These fractions were combined based on the same TLC profiles. Furthermore, each group was purified by column chromatography gravitation using Si-gel Merck 60 (200–400 mesh,  $\phi$ : 1.5 cm, t = 15 cm) and eluted with hexane-ethyl acetate (6:4) as solvent. From the results of this separation it can be obtained three pure compounds, compound-1 (125 mg); compound-2 (350 mg) and compound-3 (200 mg). The compounds were identified the molecular structure based on UV, IR, NMR ( $^1\text{H}$  and  $^{13}\text{C}$  in one and two dimensions).

## Antioxidant Activity Test

Antioxidant activity was analyzed by DPPH (2, 2-diphenyl-1-picrylhydrazyl) method. The method was adopted with slight modification of Hanumantharaju.<sup>11</sup> DPPH was used as the source of free radical. About 5 ml of the sample (in various concentration: 100-3.125 µg/mL) and positive control (ascorbic acid in various concentration: 6.25-0.390 µg/mL) were mixed with 5 ml of methanolic solution of DPPH (0.12 mM) and kept in the dark bottle at room temperature for 30 min. The DPPH scavenging activity was determined using spectronic 20 (Genesys) at 516 nm against DPPH solution as control. The samples were tested in triplicates. The antioxidant activity was calculated as percentage of DPPH that was decreased in comparison with the control (blank) and the inhibition activity could be calculated to determine the IC<sub>50</sub>.

## Antimicrobial screening

Screening of antimicrobial activity of the crude extract samples and three isolated compounds was done by using agar diffusion method. The method was adopted with slight modification of Bisnu.<sup>12</sup> Four organisms of *Escherichia coli* ATCC-11229, *Staphylococcus aureus* ATCC-25923, *Staphylococcus epidermidis* FNCC-0048, and *Streptococcus mutans* were used in this study to determine the antimicrobial activity of the crude extracts and isolated compounds. In disk- diffusion method, nutrient media was used as a culture media and the cavities were made aseptically over the bacterial culture on nutrient agar plates using borer and filled with standards of chloramfenicol as positive control, while DMSO (dimethyl sulfoxide) was used as solvent and also as a negative control.

The paper disk was immersed in the solution of each sample for five minute and then placed on the media, and incubated at 37°C for 6 to 24 h, with observations for every 6 h. On every six-h observation after incubation, the zone of inhibition around the disks was measured in millimeter scale. A five serial dilution (0.5; 5; 50; 250; and 500 µg/mL) was carried out for each sample. All experiments were performed in triplicate. The MIC (Minimum Inhibitory Concentrations) value was determined using broth dilution method.

## RESULTS AND DISCUSSION

From the dried and milled rhizome of *B. rotunda* (5 Kg), three compounds were isolated. Compound-1 was isolated from ethyl acetate fraction, while from the chloroform fraction it was obtained two compounds, compound-2 and compound-3. The structure identification of the compounds as corresponding UV, IR and NMR spectra obtained the following data.

Compound-1, 2',4'-dihydroxy-6-methoxychalcone, was obtained as light yellow solid form. The UV (in methanolic solvent)  $\lambda$  : 215; 342 nm. The IR (KBr pellet)  $\nu$  : 3454; 1627; 1540; 1337, and 1225 cm<sup>-1</sup>. The <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) :  $\delta$  3.98 (3H, s); 6.08 (1H, d, J=2.16 Hz); 7.77 (1H, d, J=15.7 Hz); 8.00 (1H, d, J=15.7 Hz); 7.73 (2H, d, J= 6.89 Hz); 7.43 (3H, m); 7.44 (3H, m); 7.45 (3H, m); 7.73 (2H, d, J=6.89); 9.60 (2H, s); 9.61 (2H, s); 9.62 (2H, s); 6.0 (2H, d, J=2.16) ppm. The <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$ 135.55; 128.31; 128.94; 130.07; 128.34; 128.31; 127.58; 141.75; 192;23; 91.38; 163.48; 55.54; 105.29; 168.41; 96.06 ppm.

Compound-2, 5-Hydroxy-7-methoxy-flavanone was obtained as a colorless crystal. The UV (in methanolic solvent)  $\lambda$  : 213; 287 nm. The IR (KBr pellet)  $\nu$  : 3444; 1645; 1621; 1581; 1381; and 1158 cm<sup>-1</sup>. The <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) :  $\delta$  3.08 (1H, dd, 2.8; 12.0 ); 2.84 (1H, d, 2.8); 3.81 (3H, s); 5.43 (1H, d, 12.0); 6.04 (1H, br s); 6.06 (1 H, br s); 7.42 (2H, brs); 7.43 (3H, br s); and 12.03 (1H, s, OH) ppm. The <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  43.5; 55.85; 77.45; 94.43; 95.3; 103.3; 126.3 (3C); 129.0 (2C); 138.54; 163.14; 164.3; 168.3; 195.93 ppm.

### Table 1

**<sup>1</sup>H NMR, and <sup>13</sup>C NMR data of compound-1, -2, and -3.**

No	Compound-1		Compound-2		Compound-3	
No	$\delta$ C ppm	$\Delta$ H ( $\Sigma$ H;m;J Hz)	$\delta$ C ppm	$\Delta$ H ( $\Sigma$ H;m;J Hz)	$\delta$ C ppm	$\Delta$ H ( $\Sigma$ H; m; J Hz)
1	135.55	-	-	-	-	-
2	128.31	$\delta$ 7.73(2H;d;6.89)	77.45	5.43 (1H; d;12.0)	79.47	5.56 (1H, dd, 2.9; 12.6)
3	128.94	$\delta$ 7.45 (3H, m)	43.50	3.08(1H;dd; 2.8; 12.0; 2.84 (1H, d, 2.8)	43.63	2.82 (1H, dd, 2.9; 12.6); 3.18 (1H, dd, 2.9; 12.6)
4	130.07	$\delta$ 7.44 (3H, m)	195.93		196.85	
5	128.34	$\delta$ 7.43 (3H, m)	164.30	-	165.33	-
6	128.31	$\delta$ 7.73 (2H; d;6.89)	95.30	6.04 (1H, br s)	96.60	5.98 (1H, d, 8.0)
7	127.58	$\delta$ 8.00 (1H, d,15.7)	168.30	-	164.19	-
8	141.75	$\delta$ 7.77 (1H, d,15.7)	94.43	6.06 (1 H, br s)	95.91	6.01 (1H, br s)
9	192.23	-	163.14	-	167.38	-
10	91.38	$\delta$ 6.08 (1H, d, 2.16)	103.30	-	103.29	-
1'	163.48	-	138.54	-	140.06	-
2'	55.54	$\delta$ 3.98 (3H, s)	126.30	7.43 (1 H, br s)	127.32	7.56 (1 H, d, 8.0)
3'	105.29	-	129.00	7.42 (1H, brs)	129.45	7.45 (1H, t,8.0; 8.0)
4'	168.41	-	126.30	7.43 (1 H, br s)	129.51	7.42 (1H, t, 8.0; 8.0)
5'	-	$\delta$ 9.60 (2H, s)	129.00	7.42 (1H, brs)	129.45	7.45 (1H, t,8.0; 8.0)
6'	96.06	$\delta$ 6.00 (2H, d, 2.16)	126.30	7.43 (1 H, br s)	127.32	7.56 (1 H, d, 8.0)
5-OH	165.18	-	-	12.03 (1H, s)	-	12.16 (1H, br s)
6'-OH	-	$\delta$ 9.60 (2H, s)	-	-	-	-
7-OCH <sub>3</sub>	-	-	55.85	3.81 (3H, s)	-	-
7-OH	-	-	-	-	-	9.63(1H, br s)

Compound-3, 5,7-Dihydroxyflavanone was obtained as a yellow needle shaped crystals. UV (in methanolic solvent)  $\lambda$  : 210; 288 nm. The IR (KBr pellet)  $\nu$  : 3444; 1631; 1583; 1488; 1302; 1168; 1089 cm<sup>-1</sup>. The <sup>1</sup>H NMR (500 MHz, Acetone-d<sub>6</sub>) :  $\delta$  2.82 (1H, dd, 2.9; 12.6); 3.18 (1H, dd, 2.9; 12.6); 5.56 (1H, dd, 2.9; 12.6); 5.98 (1H, d, 2.5); 6.01 (1H, br s); 7.42 (1H, t, 8.0; 8.0); 7.45 (2 H, t, 8.0; 8.0); 7.56 (2H, d, 8.0); 9.63 (1 H, br s, OH); 12.16 (1H, br s, OH) ppm. The <sup>13</sup>C NMR (125 MHz, Acetone-d<sub>6</sub>):  $\delta$  43.63; 79.47; 95.91; 96.96; 103.29; 127.32 (2C); 129.45 (2C); 129.51; 140.06; 164.19; 165.33; 167.38; 196.85 ppm. The whole data of NMR are listed in [Table 1](#).

The UV spectrum of compound-1 indicated the presence of a chromophore of C=C conjugated. The IR (KBr pellet) spectrum exhibited hydroxyl group (3454 cm<sup>-1</sup>), carbonyl group (1627 cm<sup>-1</sup>), C=C aromatic (1540; 1488 cm<sup>-1</sup>), and C-O-C group (1225 cm<sup>-1</sup>). The analysis of <sup>1</sup>H-NMR spectral data of compound-1 showed signals corresponding to protons. The presence of two aromatic proton signals indicated monosubstituted benzene rings at  $\delta$  7.73 (2H, d, J = 6.89 Hz, H<sub>2</sub>, H<sub>6</sub>) and  $\delta$  7.45 (3H, m, H<sub>3</sub>, H<sub>4</sub>, H<sub>5</sub>) ppm. The presence of aromatic proton signals at  $\delta$  6.08 (1H, d, J = 2.16 Hz, H<sub>1</sub>) and  $\delta$  6.00 (1H, d, J = 2.16 Hz, H<sub>5</sub>) ppm indicated meta disubstituted benzene ring. The hydroxyl proton signal at  $\delta$  9.60 ppm (2H, s, H<sub>4</sub>'-OH; H<sub>6</sub>'-OH) showed a 4,6-dihydroxy-tetrasubstituted benzene ring. One proton signal of methoxyl group was likely observed at  $\delta$  3.98 ppm (3H, s, H<sub>2</sub>'-OMe). The two alkene proton signals were appeared at  $\delta$  8.00 (1H, d, J = 15.7 Hz, H<sub>7</sub>) and 7.77 (1H, d, J = 15.7 Hz, H<sub>8</sub>) ppm. The <sup>13</sup>C-NMR

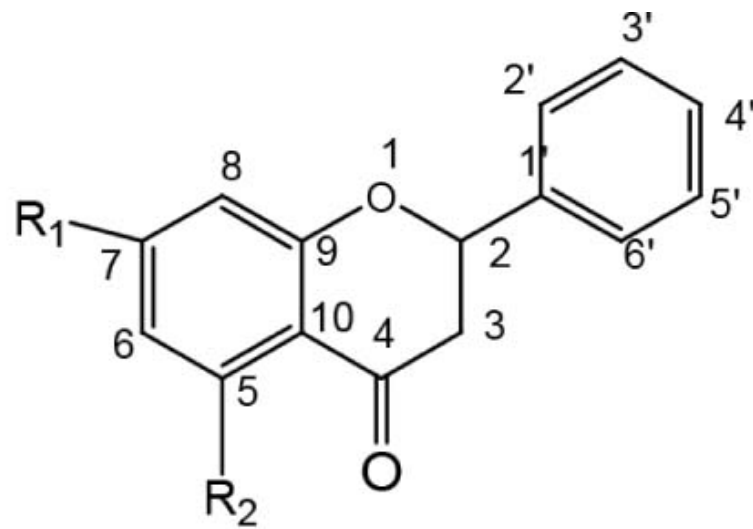
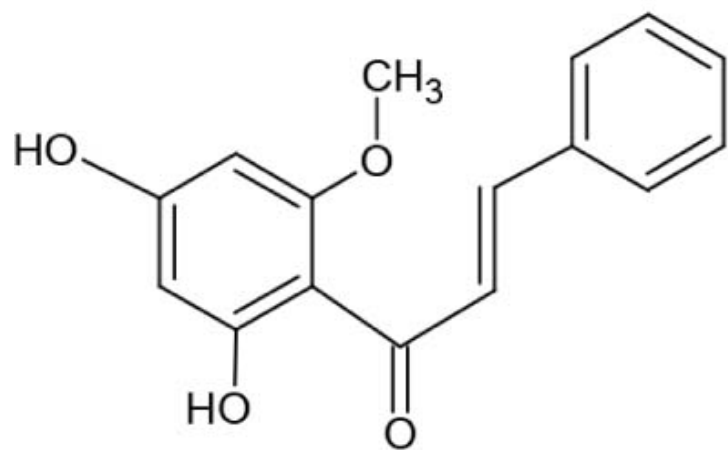
spectral data of compound-1 might have 13 types of carbon atoms. The  $^{13}\text{C}$  NMR spectrum showed the signals in the area of aromatic carbon chemical shift at  $\delta$  128.31 (C2, C6), 128.94 (C3, C5), 130.07 (C4) and 135.55 (C7) ppm. The chemical shift of  $\delta$  128.31 (C2, C6) and 128.94 (C3, C5) ppm were to be a signal to the integration of the two carbon, it means there are two identical carbon atoms. The spectrum was also emerging carbon signal of oxyaryl, the three signals were contained in the chemical shift area of  $\delta$  163.48 (C2'), 165.18 (C6') and 168.11 (C4') ppm. Methoxyl carbon signal appeared on the area of  $\delta$  55.54 (C2'-OMe) ppm. Carbon signal at  $\delta$  192.23 ppm chemical shift area (C9) showed signals for carbon carbonyl group. Two carbon signals at chemical shift of  $\delta$  91.38 ppm (C1') and  $\delta$  96.06 ppm (C5') indicated that both included in the aromatic ring is affected by the hydroxy group contained around carbon atom. The signal at 105.29 (C3') ppm indicated aromatic carbon signals. There are two carbon signals indicating the carbon alkene at  $\delta$  127.58 (C7) and 141.58 (C8) ppm. The connection between protons and their corresponding carbons was established by HMQC. Further support for the structural analysis was obtained from HMBC measurement. These results suggested that compound-1 was a chalcon with substituted methoxyl and hydroxyl groups. Further evidence for the structure assigned to compound-1 came from the comparison of their spectral data with those reported in the literature.<sup>13</sup> Therefore, it may be concluded that compound-1 is 2', 4'-dihydroxy-6-methoxychalcone (cardamonin).

The compound-2,5-hydroxy-7-methoxyflavanone (pinostrobin) and compound-3, 5,7-hydroxy-dihydroxy flavanone (pinocembrin) can be isolated from *Kaempferia rotunda*.<sup>14</sup> and from the genus *Bosenbergia*. The both compounds are a major component, so in this study was obtained from the fraction of ethyl acetate and chloroform. This compound is a major component in the rhizome of *B. rotunda* plant, and previously this compound has been obtained as a crystalline solid in the ethanol extract of rhizome *B. rotunda*.<sup>15</sup> The structures of isolated compound-1, -2, and -3 are show in [Figure 1](#).

Antioxidant activity assay for the samples were indentified using DPPH method. Antioxidant activity test by DPPH method is based on the reaction of DPPH radical attacked by antioxidant compounds through the oxygen atom transfer mechanism, which produces DPPH-H molecule non-radical stable form.<sup>16</sup> In this research an activity test has been conducted as DPPH radical scavenger from ethanol extract of *B. rotunda*, isolated compounds, and ascorbic acid (positive control) at various concentrations with in three times measurements (triple). The data of percentage of activity at various concentration were used to determine the value of  $\text{IC}_{50}$  (that is the concentration of sample having inhibition activity of 50%) from each sample following linear regression equation. The inhibition activity ( $\text{IC}_{50}$ ) of ethanol extract and isolated compounds from *B. rotunda* are presented in [Table 2](#).

#### Figure 1

The structure of the three isolated compounds from ethanol extract of *B. rotunda*



(2)  $R_1 = \text{OCH}_3$ ;  $R_2 = \text{OH}$

(3)  $R_1 = \text{OH}$ ;  $R_2 = \text{OH}$

**Table 2**

**The Inhibition activity ( $\text{IC}_{50}$ ) of extract, isolated compounds of *B. rotunda* and positive control.**

Sample	IC $\mu\text{g/mL}$	Note
Ethanol extract of <i>B. rotunda</i>	92.64	active
2',4'-dihydroxy-6-methoxychalcone (1)	46.66	active
5-hydroxy-7-methoxyflavanone (2)	62.84	active
5, 7-dihydroxyflavanone (3)	62.66	Active
Ascorbic acid (Positive control)	3.77	Very active

**Table 3**

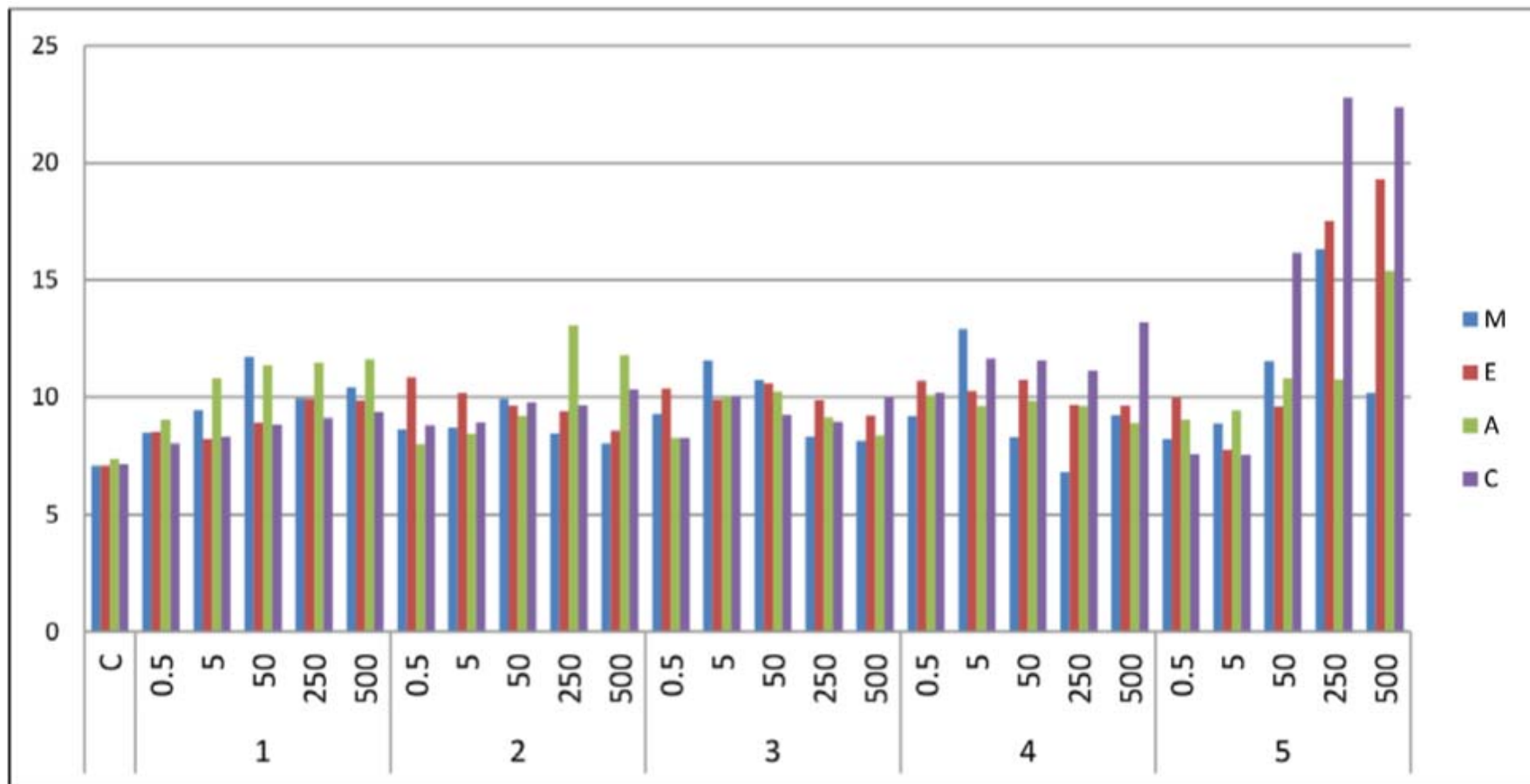
**Zones of inhibitions as shown by ethanolic plant extract of *B. rotunda* and isolated compounds at different concentrations against selected microorganisms.**

No	sample	Concentration ( $\mu\text{g/mL}$ )	Diameter Zona of Inhibition (Mean $\pm$ SD) mm after 6 h incubations
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			<b><i>Streptococcus mutans</i></b>	<b><i>Staphylococcus epidermidis</i> FNCC-0048</b>	<b><i>Staphylococcus aureus</i> ATCC-25923</b>	<b><i>Escherichia coli</i> ATCC- 11229</b>
1	DMSO	-	7.08±0.42	7.08±0.32	7.38±1.06	7.16±0.19
		0.5	8.50±0.41	8.54±0.72	9.06±0.44	8.03±0.44
		5	9.46±0.56	8.21±0.39	10.82±1.72	8.33±0.48
		50	11.73±1.13	8.92±0.43	11.38±0.88	8.83±0.76
		250	9.96±0.43	9.93±0.68	11.46±2.65	9.11±0.30
		500	10.44±1.00	9.87±0.41	11.63±0.86	9.36±0.36
2	2',4'-dihydroxy-6-methoxy-chalcone (compound-1)	0.5	8.62±0.55	10.86±0.49	8.02±0.62	8.81±0.47
		5	8.73±0.72	10.18±0.35	8.43±0.79	8.94±0.27
		50	9.94±1.37	9.64±0.26	9.20±0.79	9.78±0.54
		250	8.45±1.16	9.40±0.53	13.08±0.75	9.66±0.24
		500	8.03±0.44	8.57±0.69	11.81±0.97	10.34±0.77
3	5-hydroxy-7-methoxyflavanone (compound-2)	0.5	9.63±0.73	10.37±0.23	8.27±0.54	8.27±0.54
		5	11.58±2.11	9.91±0.64	10.03±0.49	10.07±2.38
		50	10.75±0.66	10.60±0.26	10.24±0.26	9.26±1.07
		250	8.33±0.95	9.90±0.86	9.13±0.81	8.96±0.64
		500	8.15±1.01	9.22±0.97	8.36±0.48	10.03±0.60
4	5, 7-dihydroxy-flavanone (compound-3)	0.5	9.20±0.75	10.70±0.62	10.08±0.76	10.20±0.72
		5	12.91±2.15	10.26±0.54	9.62±0.58	11.66±0.76
		50	8.31±0.26	10.75±0.26	9.86±0.88	11.59±1.23
		250	6.83±0.87	9.69±0.83	9.62±0.60	11.13±1.08
		500	9.23±0.73	9.64±0.86	8.90±0.37	13.20±0.76
5	Chloramphenicol (positive control)	0.5	8.21±0.46	9.97±0.95	9.06±0.66	7.58±0.52
		5	8.89±0.88	7.77±0.51	9.44±0.88	7.54±0.45
		50	11.54±0.75	9.61±1.15	10.82±1.20	16.16±0.49
		250	16.33±2.45	17.51±1.23	10.77±0.62	22.80±0.99
		500	10.19±0.49	19.30±3.45	15.41±3.08	22.38±1.97

**Figure 2**

The graph of inhibition activity of each sample.



C= DMSO; 1=Ethanol extract of *B. rotunda*; 2=2',4'-dihydroxy-6-methoxy-chalcone (1); 3=5-hydroxy-7-methoxyflavanone (2); 4=5, 7- dihydroxy-flavanone (3); 5=Chloramphenicol (positive control)

M= *Streptococcus mutans*; E= *Staphylococcus epidermidis* FNCC-0048; A= *Staphylococcus aureus* ATCC-25923; C= *Escherichia coli* ATCC-11229

These results indicated that ethanol extract of *B. rotunda* and isolated compounds exhibited significant radical scavenging activity with  $IC_{50}$ , being lower than 100  $\mu\text{g/mL}$ . Ascorbic acid is an antioxidant compound that is widely known, showed very high antioxidant activity and is used as a positive control as practiced by Biswas.<sup>17</sup> Some studies show many secondary metabolites derived from plants are antioxidants, especially phenolic compounds such as flavonoid and polyphenol. The chemical activity of a number of antioxidants depends on a number of factors, including the stability and reactivity. Primary antioxidants forming a bond with the radical after hydrogen removal is more important than any other factor. Phenol compounds include a number of compounds generally have aromatic rings with one or more hydroxyl groups.<sup>18</sup> Screening of antimicrobial activity of the crude extract samples and three isolated compounds was done by using agar disk-diffusion method activities against pathogenic



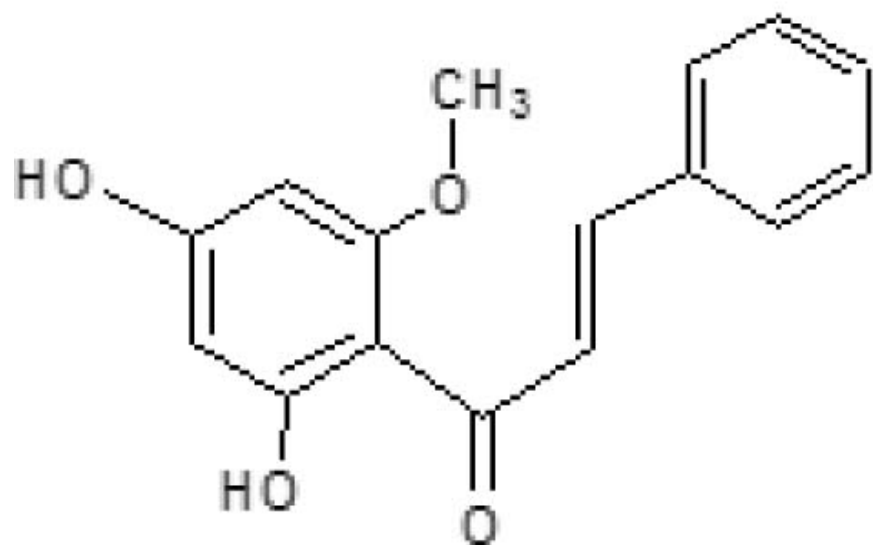
bacteria i.e. *Escherichia coli* ATCC-11229, *Staphylococcus aureus* ATCC-25923, *Staphylococcus epidermidis* FNCC-0048, and *Streptococcus*. The zones of inhibitions for each of compounds against pathogenic bacteria are presented in [Table 3](#). Furthermore, to clarify the inhibition activity of each sample the corresponding graph should be performed as in [Figure 2](#).

In this study, the ethanol extract from *B. rotunda* and isolated compounds showed inhibition activity against four types of bacteria used, namely *Streptococcus mutans*, *Staphylococcus epidermidis* FNCC-0048, *Staphylococcus aureus* ATCC-25923, and *Escherichia coli* ATCC-11229 with moderate inhibition criteria.<sup>19</sup> However, these data indicated that the ethanol extract of *B. rotunda* showed the highest inhibition zone in *Streptococcus mutans* bacteria at concentration of 50 µg/mL; 2', 4'-dihydroxy-6-methoxy-chalcone showed the highest inhibitory activity against *Staphylococcus aureus* ATCC-25923 bacteria at a concentration of 250 µg/mL; 5-hydroxy-7-methoxyflavanone showed the highest inhibitory activity against *Streptococcus mutans* bacteria at concentrations of 5 µg/mL; and 5, 7- dihydroxyflavanone showed the highest inhibitory activity against *Escherichia coli* ATCC-11229 bacteria at a concentration of 500 µg /mL. The results of previous studies showed that each compound showed antimicrobial activity with different mechanism. They can inhibit cell wall synthesis, cause energy depletion by accumulating in cell membranes, disrupt the permeability of cell membranes, caused membrane disturbances, modify cellular constituents, cell damage or cell mutations.<sup>20</sup>

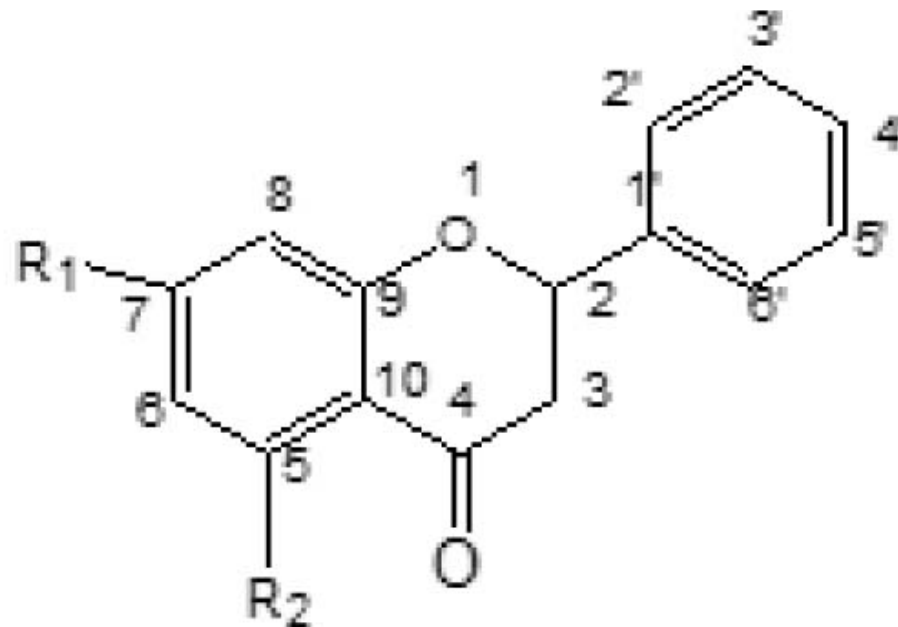
## CONCLUSION

From these results it can be concluded that the ethanol extract of *B. rotunda* rhizome can be isolated the three flavanones, namely 2', 4'-dihydroxy-6-methoxychalcone (compound-1), 5-hydroxy-7-methoxyflavanone (compound-2), and 5, 7-dihydroxyflavanone (compound-3). The ethanol extract of *B. rotunda* rhizomes and the isolated compounds showed significant antioxidant activity by DPPH and antibacterial activity against *Escherichia coli* ATCC-11229, *Staphylococcus aureus* ATCC-25923, *Staphylococcus epidermidis* FNCC-0048, and *Streptococcus mutans*. Thus *B. rotunda* rhizomes can be developed as a natural antioxidant and antibacterial agents.

## GRAPHICAL ABSTRACT



(1)



(2)  $R_1 = \text{OCH}_3$ ;  $R_2 = \text{OH}$

(3)  $R_1 = \text{OH}$ ;  $R_2 = \text{OH}$

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**Prof. Dr. Sri Atun:** Lecturer in the Department of Chemistry Education, Faculty of Mathematics and Natural Science, Universitas Negeri Yogyakarta, in the field of Natural Products and Biomedicine Chemistry.



**Dr. Sri Handayani:** Lecturer in the Department of Chemistry Education, Faculty of Mathematics and Natural Science, Universitas Negeri Yogyakarta, in the field of synthesis organic



**Anna Rakhmawati:** M.Sc Lecturer in the Department of Biology Education, Faculty of Mathematics and Natural Science, Universitas Negeri Yogyakarta, in the field of Microbiology

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