

## Bioactive compounds from the edible mushroom *Cortinarius caperatus*

著者	Ridwan Arif Yanuar, Wu Jing, Choi Jae-Hoon, Hirai Hirofumi, Kawagishi Hirokazu
journal or publication title	Mycoscience
volume	59
number	2
page range	172-175
year	2018-03
出版者	Elsevier
権利	(C)2017. This manuscript version is made available under the CC-BY-NC-ND 4.0 license <a href="http://creativecommons.org/licenses/by-nc-nd/4.0/">http://creativecommons.org/licenses/by-nc-nd/4.0/</a>
URL	<a href="http://hdl.handle.net/10297/00025847">http://hdl.handle.net/10297/00025847</a>

doi: 10.1016/j.myc.2017.08.015

## Note

### Bioactive compounds from the edible mushroom *Cortinarius caperatus*

Arif Yanuar Ridwan <sup>a</sup>, Jing Wu <sup>b</sup>, Jae-Hoon Choi <sup>a,b</sup>, Hirofumi Hirai <sup>a,b</sup>, Hirokazu Kawagishi <sup>a, b, c,\*</sup>

<sup>a</sup> Graduate School of Integrated Science and Technology, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

<sup>b</sup> Research Institute of Green Science and Technology, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

<sup>c</sup> Graduate School of Science and Technology, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

\* Correspondence author:

H. Kawagishi

Tel: +81-54-238-4885

Fax: +81-54-238-4885

E-mail: kawagishi.hirokazu@shizuoka.ac.jp

Text: 7 pages; figures: 2

## Abstract

Six compounds, 5-(1-hydroxyethyl)dihydrofuran-2(3*H*)-one (1), 4-ethoxy-4-oxobutanoic acid (2), 4-ketnonanoic acid (3), *S*-acetyl-*N*-glycyl cysteamine (4), methyl benzoate (5) and *p*-hydroxybenzoic acid (6) were isolated from the fruiting bodies of *Cortinarius caperatus*. Compounds 1, 3, 5, and 6 inhibited growth of *Flammulina velutipes* mycelia. Compounds 2, 3, 5, and 6 exhibited growth regulatory activities toward rice seedlings, while compounds 3, 5, and 6 regulated the growth of lettuce. Compound 4 was first isolated from a natural source. In addition, the activity of compound 6 against rice was compared with those of its analogs.

## Keywords

Mycelia growth regulation; Plant growth regulation; Structural identification

Higher fungi have been recognized to produce secondary metabolites with a variety of biological activities. In recent years, our group has been searching for metabolites of mushroom-forming fungi with specialized activities, such as growth regulation toward plants and fungi. For example, erinaceolactones A to C from the culture broth of *Hericium erinaceus* and melleolide and armillarikin from *Armillaria* sp. were reported to regulate the growth of lettuce and mycelia of *Coprinopsis cinerea* and *Flammulina velutipes* (Kobori et al. 2015; Wu et al. 2015). Recently, we also reported five compounds from the edible mushroom *Russula vinosa*, and two compounds from the fruiting bodies of *Leccinum extremiorientale* that regulated the growth of lettuce, and three compounds that inhibited the root growth of bentgrass from the fairy ring-forming fungus *Lepista sordida* (Ito et al. 2017a,b; Matsuzaki et al. 2016). In our continuing attempt to search for bioactive compounds from mycelia and fruiting bodies of higher fungi, we revealed that extracts from *Cortinarius caperatus* regulated the growth of rice seedlings, lettuce, and mycelium of *F. velutipes*.

*Cortinarius caperatus* is an edible mushroom and widely grows in the temperate zone of the northern hemisphere (Falandysz 2014). This species has been reported to produce an antiviral compound, RC-183, toward herpes simplex virus (Piraino and Brandt 1999). However, further studies on secondary metabolites and the biological activities of this mushroom have not been carried out.

In this article, we describe the isolation, structural identification, and biological activities of the compounds.

### ***Extraction and isolation***

Fresh fruiting bodies of *C. caperatus* (38.4 kg) were collected at Narusawa village, Yamanashi Prefecture, in Japan. The crushed fruiting bodies were extracted with ethanol (EtOH) and then acetone. The solutions were combined, concentrated under reduced pressure, and partitioned between *n*-hexane and water, ethyl acetate (EtOAc) and water, and *n*-butanol and water, successively. The EtOAc soluble part (14.9 g) was subjected to silica gel column chromatography (Silica gel 60N, Kanto Chemical, Tokyo, Japan); 100% CH<sub>2</sub>Cl<sub>2</sub> (fraction 1), 90% (fractions 2 to 3), 70% (fractions 4 to 6), 50% (fractions 8 to 11) CH<sub>2</sub>Cl<sub>2</sub>/acetone; 80% (fractions 12 to 15), 60% (fractions 16 to 18) CH<sub>2</sub>Cl<sub>2</sub>/methanol (MeOH), 100% MeOH (fraction 19) to obtain 19 fractions (fractions 1 to 19). Fraction 10 (701 mg) was fractionated by ODS gel flash chromatography (60%, 70%, 80%, 90%, 100% MeOH) to afford 12 fractions (fractions 10-1 to 10-12). HPLC separations were performed with a Jasco Gulliver system. Fraction 10-2 (48.7 mg) was separated by reverse-phase HPLC (Develosil C30-UG-5, Nomura Chemical, Seto, Japan; 40% MeOH) to obtain compounds 2 (9.9 mg) and 6 (10.7 mg), respectively. Fraction 10-2-2 (16.2 mg) was further purified by reverse-phase HPLC (Cosmosil PBr, Nacalai Tesque, Kyoto, Japan; 40% MeOH) to afford compound 1 (7.8 mg). Fraction 8 was subjected to ODS gel flash chromatography (60%, 70%, 80%, 90%, 100% MeOH) to obtain 10 fractions (fractions 8-1 to 8-10). Fraction 8-2 (44.4 mg) was further fractionated by reverse-phase HPLC (InertSustain Phenyl, GL Sciences, Tokyo, Japan; 40% MeOH) to yield compound 5 (6.2 mg). Fraction 8-3 (41.0 mg) was purified by reverse-phase HPLC (InertSustain Phenylhexyl, GL Sciences, Tokyo, Japan; 40% MeOH) to afford compound 3 (9.1 mg). Fraction 11 (620 mg) was separated by silica gel MPLC using (90%,

80%, 70%, CHCl<sub>3</sub>/MeOH, 100% MeOH) to give 9 fractions (fractions 11-1 to 11-9). Fraction 11-3 (27.7 mg) was further fractionated by normal-phase HPLC (Cosmosil 5SLII, Nacalai Tesque, Kyoto, Japan; 90% CHCl<sub>3</sub>/MeOH) to obtain compound 4 (1.5 mg).

<sup>1</sup>H NMR spectra (one- and two-dimensional) were recorded on a Jeol lambda-500 spectrometer at 500 MHz, and <sup>13</sup>C NMR spectra were recorded on the same instrument at 125 MHz (Jeol, Tokyo, Japan). ESIMS spectra were measured on a JMS-T100LC mass spectrometer (Jeol, Tokyo, Japan). IR spectra were recorded on a FT/IR-4100 (Jasco, Tokyo, Japan).

We isolated six compounds from the fruiting bodies of *C. caperatus*.

Compound 1: colorless oil; ESIMS *m/z* 153 [M+Na]<sup>+</sup>; IR (neat): 3419 cm<sup>-1</sup>; [α]<sub>D</sub><sup>27</sup> = +6 (*c* = 0.7, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (in CD<sub>3</sub>OD) δ<sub>H</sub>: 1.16 (d, *J* = 6.4), 2.20 (m), 2.53 (dd, *J* = 7.9, 8.8), 3.92 (m), 4.40 (m), 7.30; <sup>13</sup>C-NMR, δ<sub>C</sub>: 18.7, 22.6, 29.4, 68.8, 85.6, 180.3.

Compound 2: pale yellow oil; ESIMS *m/z* 145 [M-H]<sup>-</sup>; <sup>1</sup>H-NMR (in CD<sub>3</sub>OD) δ<sub>H</sub>: 1.24 (t, *J* = 8.0), 2.58 (s), 4.13 (m), 4.89 (s); <sup>13</sup>C-NMR δ<sub>C</sub>: 14.5, 29.8, 30.1, 61.7, 174.3.

Compound 3: pale yellow oil; ESIMS *m/z* 171 [M-H]<sup>-</sup>; <sup>1</sup>H-NMR (in CD<sub>3</sub>OD) δ<sub>H</sub>: 0.9 (t, *J* = 7.0), 1.28 (m), 1.32 (m), 1.56 (q, *J* = 7.3), 2.47 (t, *J* = 7.3), 2.51 (t, *J* = 6.7), 2.72 (t, *J* = 6.7); <sup>13</sup>C-NMR δ<sub>C</sub>: 14.3, 23.5, 24.5, 28.8, 32.5, 38.0, 43.4, 176.6, 212.0.

Compound 4: pale yellow amorphous; ESIMS *m/z* 172 [M+Na]<sup>+</sup>; IR (neat): 3584 cm<sup>-1</sup>; [α]<sub>D</sub><sup>22</sup> = +4.7 (*c* = 0.22, MeOH); <sup>1</sup>H-NMR (in CD<sub>3</sub>OD) δ<sub>H</sub>: 1.90 (s), 2.63 (s), 2.86 (ddd, *J* = 13.4, 6.1, 5.8), 3.00 (ddd, *J* = 13.1, 6.1, 5.8), 3.51 (m), 3.55 (m); <sup>13</sup>C-NMR δ<sub>C</sub>: 22.4, 34.6, 38.4, 54.8, 173.6.

Compound 5: white powder; ESIMS *m/z* 135 [M-H]<sup>-</sup>; <sup>1</sup>H-NMR (in CD<sub>3</sub>OD) δ<sub>H</sub>: 4.8 (s), 6.9 (d), 7.3 (s), 7.6 (d); <sup>13</sup>C-NMR δ<sub>C</sub>: 42.0, 127.9, 129.4, 130.3, 136.1, 175.6.

Compound 6: white amorphous; ESIMS *m/z* 137 [M-H]<sup>-</sup>; <sup>1</sup>H-NMR (in CD<sub>3</sub>OD) δ<sub>H</sub>: 4.9 (s), 6.8 (d), 7.9 (d); <sup>13</sup>C-NMR δ<sub>C</sub>: 116.0, 122.8, 132.9, 163.3, 170.1.

All the compounds have been previously reported and their structures were identified by interpretation of spectroscopic data (Fig. 1). Compound 1 was identified as 5-(1-hydroxyethyl)dihydrofuran-2(3*H*)-one. The stereochemistry of the compound remains undetermined. Compound 2 was identified as 4-ethoxy-4-oxobutanoic acid, which has been previously isolated from *Saccharomyces cerevisiae* (Vigentini et al. 2016). This compound also has been found as an antioxidant (Pereira et al. 2017). Compound 3, 4-ketnonanoic acid, has been isolated from tobacco (Leffingwell et al. 1972). The biological activity of this compound has not been reported yet. Although compound 2 was detected by GC-MS in the fruiting bodies of *Dictyophora indusiata*, this is the first isolation of compounds 2 and 3 from mushrooms (Chao et al. 2014). Compound 4 was identified as *S*-acetyl-*N*-glycyl cysteamine. This compound has been synthesized as a radioprotectant in the treatment of tumour, however, this is the first isolation from a natural source (Maurizis et al. 1988, 1989). Compound 5 was identified as methyl benzoate and has been isolated from *Agaricus subrufucens* (Chen and Wu 1984). Methyl benzoate has been previously reported as antioxidant and insect attractant for *Euglossa cybela* (Schiestl and Roubik 2003). Compound 6 was identified as *p*-hydroxybenzoic acid. This compound has been isolated from *Ganoderma lucidum* and exhibited antimicrobial and demelanizing activity (Heleno et al.

2013).

### ***Mycelial growth regulatory activity***

The mycelia of *Flammulina velutipes* were placed onto the center of a potato dextrose agar (PDA) plate. Meanwhile, each test compound solutions were poured onto autoclaved paper disks (Advantec  $\phi$  8 mm) and then air-dried. Each air-dried paper disk containing 10  $\mu\text{mol}$ /paper disk of the compounds were placed directly onto the incubated plate ( $n = 3$ ). Plates were further incubated at room temperature for 1 wk. After the incubation, the activity was evaluated by observation of the inhibition zones.

All the compounds except for compound 4 were evaluated for growth regulating activity against a fungus *F. velutipes* (Supplementary Fig. S1). All the tested compounds except for compound 2 inhibited growth of the mycelia at 10  $\mu\text{mol}$ /paper disk. Among them, compound 1 showed the strongest activity.

### ***Plant growth regulatory activity***

Lettuce seeds (*Lactuca sativa* L. cv. Great Lakes 366, Takii Co., Ltd., Tokyo, Japan) were put on filter paper (Advantec No. 2,  $\phi$  55 mm; Toyo Roshi Kaisha, Ltd., Tokyo, Japan), soaked in distilled water in a Petri dish ( $\phi$  60  $\times$  20 mm) and incubated in a growth chamber under dark at 25 °C for 1 d. Each sample was dissolved in 1 mL of methanol (1,  $10^{-1}$ ,  $10^{-2}$  and  $10^{-3}$   $\mu\text{mol}/\text{mL}$ ) and then poured onto filter paper ( $\phi$  55 mm) in a Petri dish ( $\phi$  60  $\times$  20 mm). After the solvent was air-dried, 1 mL of distilled water was poured on the sample-loaded paper or intact filter paper (control). The pre-incubated lettuces ( $n = 9$  in each petri dish) were transferred onto the filter paper and incubated in a growth chamber under dark at 25 °C for 3 d. The elongations were measured using a ruler.

Rice seeds (*Oryza sativa* L. cv. Nipponbare) were sterilized in ethanol for 5 minutes and then 1% sodium hypochlorite for 30 min on a plastic container (18.5  $\times$  14.5  $\times$  4.5 cm). The seeds were washed completely in sterile water and germinated for 2 d at 30 °C with intensive light. The seedlings ( $n = 5$ ) were planted onto glass jars ( $\phi$  5.5  $\times$  10 cm) containing 500  $\mu\text{L}$  test samples (10, 100, 1000  $\mu\text{M}$ ) and 5 mL distilled water. For the comparison of *p*-hydroxybenzoic acid and its analogues, different concentrations (0.01, 0.1, 0.5, 1, and 5 mM) were applied ( $n = 15$ ). Afterward incubated for 5 d at 30 °C with intensive light. The elongations were measured using a ruler and digimatic caliper (Mitutoyo, Kawasaki, Japan).

We examined the plant regulatory activity using lettuce, in the assay, 2,4-dichlorophenoxyacetic acid was also used as a positive control, which inhibited lettuce growth dose-dependently (Supplementary Fig. S2). Compounds 5 and 6 inhibited the growth of the root at 1  $\mu\text{mol}$ /paper. As for the hypocotyl, compounds 3 and 5 showed inhibition at  $10^{-3}$  and 1  $\mu\text{mol}$ /paper, respectively. While compounds 5 and 6 promoted the plant at  $10^{-2}$  and  $10^{-1}$   $\mu\text{mol}$ /paper, respectively. Compound 4 was not tested due to the insufficient amount.

Compounds 1–6 were examined for growth regulatory activity toward rice (Supplementary Fig. S3). The positive control, 2,4-dichlorophenoxyacetic acid, inhibited the plant significantly. Compounds 2 and 6 promoted the growth of the root at 1mM. Compound

3 promoted the root growth at 100  $\mu$ M, however, inhibited the root and shoot growth at 1 mM. As for the shoot, compound 5 inhibited the growth of the shoot at 1 mM. Compound 6 has been previously reported as a wheat allelochemical (Cheng and Cheng 2015).

Since compound 6, *p*-hydroxybenzoic acid, showed the strongest promotion activity against rice among the compounds tested, we compared the activity with those of its commercially available analogues, *m*-hydroxybenzoic acid (7), *o*-hydroxybenzoic acid (8), benzoic acid (9), methyl *p*-hydroxybenzoate (10), methyl *m*-hydroxybenzoate (11), methyl *o*-hydroxybenzoate (12), and methyl benzoate (5). As shown in Fig. 2, compounds 6 and 7 promoted the root growth at 1 mM and 5 mM. Meanwhile, compounds 8 and 12 showed promotion activity at 0.1 mM. In addition, compounds 10 and 11 have the optimum concentration for promotion of the growth at 0.5 mM. Compounds 9 and 5 showed no significant effect on the growth. As for the shoot, compound 6 showed promotion activity at 0.1, 0.5, and 1 mM. In this assay, it is very difficult to discuss the structure-activity relationship. However, it is allowed us to conclude that at least *p*-hydroxybenzoic acid (6) isolated from the mushroom in this study was the strongest growth promoter and hydroxy group attached to the benzene ring tends to increase the promotive activity. The previous study with the similar bioassay toward dwarf rice (Tan-ginbozu rice) revealed that compounds with no hydroxy substitution had no effect or are slightly inhibitory for the root growth. *Ortho*-substituted compounds were highly inhibitory, *meta*-substituted compounds were less inhibitory, and *para*-substituted compounds stimulated the root growth. In addition, the presence of methoxy group exhibited root growth inhibitory activity (Ueda 1989).

Plant and mycelial growth regulatory activities of the obtained compounds have not been reported previously. Due to structures of compounds and variety of activities, it might be difficult to explain the structure-activity relationships or mechanisms among them in the plant and mycelial growth regulatory activity. However, our results will provide advantageous information for the further study on fungal bioactive compounds specifically for agricultural usage.

## Disclosure

The authors declare no conflict of interest. All the experiments undertaken in this study comply with the current laws of Japan.

## REFERENCES

- Chao D, Hai-tian F, Peng G, Zhun H, Jing-hua C, 2014. Chemical composition and antimicrobial activity of the extracts with ethyl ether and ethyl acetate from *Dictyophora indusiata*. *Science and Technology of Food Industry* 35: 128–134.
- Chen CC, Wu CM, 1984. Volatile components of mushroom (*Agaricus subrufecens*). *Journal of Food Science* 49: 1208–1209; <http://dx.doi.org/10.1111/j.1365-2621.1984.tb10433.x>.
- Cheng F, Cheng Z, 2015. Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Frontiers in Plant Science* 1020: 1–16; <http://dx.doi.org/10.3389/fpls.2015.01020>.
- Falandysz J, 2014. Ecotoxicology and environmental safety distribution of mercury in gypsy

- Cortinarius caperatus* mushrooms from several populations: an efficient accumulator species and estimated intake of element. *Ecotoxicology and Environmental Safety* 110: 68–72; <http://dx.doi.org/10.1016/j.ecoenv.2014.08.018>.
- Heleno SA, Ferreira ICFR, Esteves AP, Ciric A, Glamoclija J, Martins A, Sokovic M, Queiroz MJRP, 2013. Antimicrobial and demelanizing activity of *Ganoderma lucidum* extract, *p*-hydroxybenzoic and cinnamic acids and their synthetic acetylated glucuronide methyl esters. *Food and Chemical Toxicology* 58: 95–100; <http://dx.doi.org/10.1016/j.fct.2013.04.025>.
- Ito A, Choi JH, Wu J, Ozawa N, Hirai H, Kawagishi H, 2017a. Plant growth regulators from the edible mushroom *Leccinum extremiorientale*. *Mycoscience*. In press.
- Ito A, Choi JH, Wu J, Tanaka H, Hirai H, Kawagishi H, 2017b. Plant growth inhibitors from the culture broth of fairy ring-forming fungus *Lepista sordida*. *Mycoscience*. In press.
- Kobori H, Sekiya A, Suzuki T, Choi JH, Hirai H, Kawagishi H, 2015. Bioactive sesquiterpene aryl esters from the culture broth of *Armillaria* sp. *Journal of Natural Product* 78: 163–167; <http://dx.doi.org/10.1021/np500322t>.
- Leffingwell JC, Young HJ, Bernasek E, 1972. Tobacco flavoring for smoking products. In: Leffingwell JC, Young HJ, Bernasek E (eds), *Evaluation of specific flavoring materials*. R. J. Reynolds Tobacco Company. Winston-Salem, pp 10–13.
- Matsuzaki N, Wu J, Kawaide M, Choi JH, Hirai H, Kawagishi H, 2016. Plant growth regulatory compounds from the mushroom *Russula vinosa*. *Mycoscience* 57: 404–407; <http://dx.doi.org/10.1016/j.myc.2016.07.001>.
- Maurizis JC, Madelmont JC, Godeneche D, Moreau MF, Oiry J, Imbach J, Veyre A, 1988. Metabolism of a new radioprotector; *S*-acetyl-*N*-glycyl cysteamine. I. Absorption, distribution and excretion metabolites in mice bearing EMT6 tumours. *Xenobiotica* 10: 1119–1128; <http://dx.doi.org/10.3109/00498258809042234>.
- Maurizis JC, Madelmont JC, Godeneche D, Moreau MF, Oiry J, Imbach J, Veyre A, Meyniel G, 1989. Metabolism of a new radioprotector; *S*-acetyl-*N*-glycyl cysteamine. II. Main tissue metabolites in mice bearing EMT6 tumours. *Xenobiotica* 19: 833–842; <http://dx.doi.org/10.3109/00498258909043144>.
- Pereira V, Santos M, Cacho J, Marques JC, 2017. Assessment of the development of browning, antioxidant activity and volatile organic compounds in thermally processed sugar model wines. *LWT-Food Science and Technology* 75: 719–726; <http://dx.doi.org/10.1016/j.lwt.2016.10.022>.
- Piraino F, Brandt CR, 1999. Isolation and partial characterization of an antiviral, RC-183, from the edible mushroom *Rozites caperata*. *Antiviral Research* 43: 67–78; [http://dx.doi.org/10.1016/S0166-3542\(99\)00035-2](http://dx.doi.org/10.1016/S0166-3542(99)00035-2).
- Schiestl FP, Roubik DW, 2003. Odor compound detection in male euglossine bees. *Journal of Chemical Ecology* 29: 253–257; <http://dx.doi.org/10.1023/A:1021932131526>.
- Ueda J, 1989. Promotive effect of capillarol and related compounds on root growth. *Physiologia Plantarum* 76: 42–46.
- Vigentini I, Maghradze D, Petrozziello M, Bonello F, Mezzapella V, Valdetara F, Failla O, Foschino R, 2016. Indigenous georgian wine-associated yeasts and grape cultivars to edit the wine quality in a precision oenology perspective. *Frontiers of Microbiology* 7: 1–13; <http://dx.doi.org/10.3389/fmicb.2016.00352>.

Wu J, Tokunaga T, Kondo M, Ishigami K, Tokuyama S, Suzuki T, Choi JH, Hirai H, Kawagishi H, 2015. Erinaceolactones A to C, from the culture broth of *Hericium erinaceus*. *Journal of Natural Products* 78: 155–158; <http://dx.doi.org/10.1021/np500623s>.

## Figure legends

Fig. 1– Structures of compounds 1–6.

Fig. 2– Effect of *p*-hydroxybenzoic acid and its analogues on the growth of rice seedlings. Results are the mean  $\pm$  standard deviation (n = 15). [ \*p < 0.05, \*\*p < 0.01 (growth inhibition); +p < 0.05, ++p < 0.01 (growth promotion)].

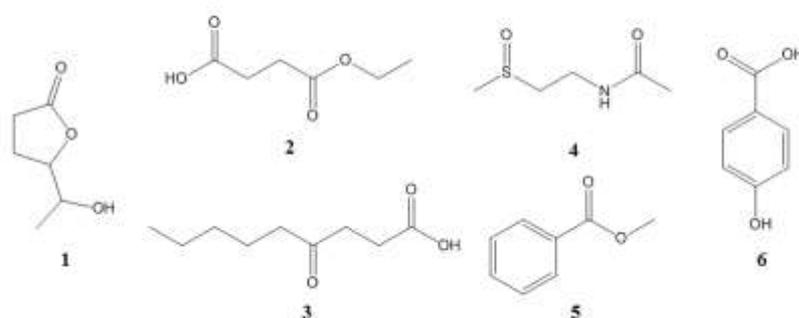


Figure 1. Ridwan *et al.*

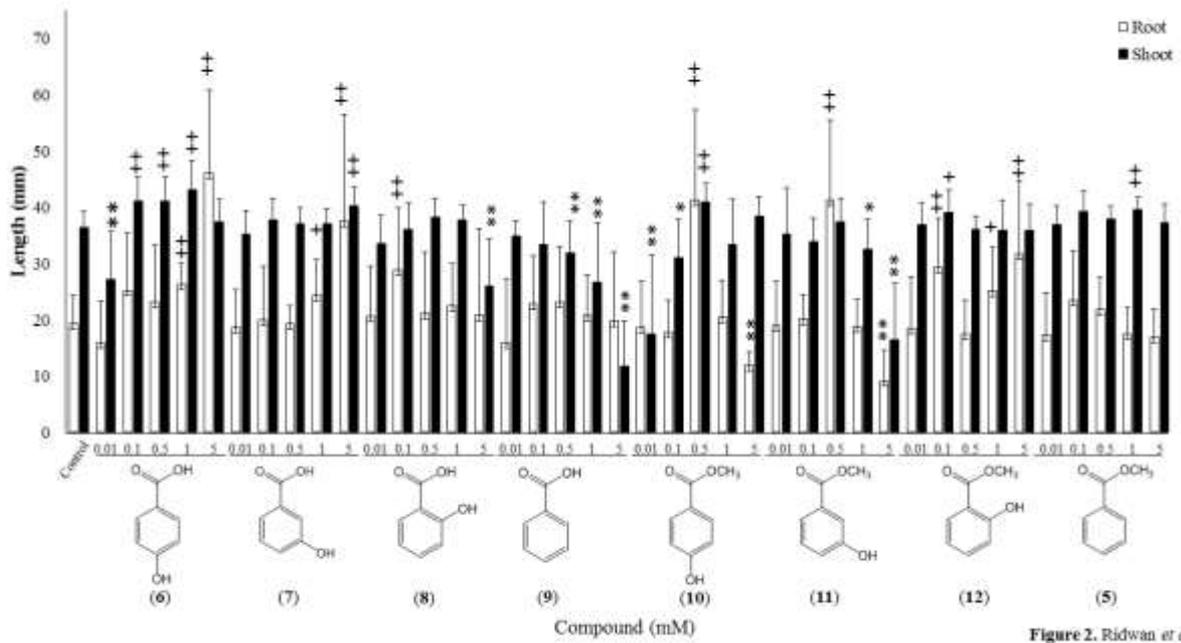


Figure 2. Ridwan *et al.*