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Effect of Certain Plant Crude Extracts on the Growth of Upland Rice (*Oryza sativa* L.)

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Abstract

Test of crude extracts of several plant species was conducted in a screen house to determine the best plant species and the most effective concentration of extract for increasing growth of upland rice (*Oryza sativa* L.) cultivated in Ultisol. The test was done using *Nested Design*. First factor was sources of extracts: 1) leaves of cassava, 2) leaves of *G. linearis*, 3) *C. asiatica*, 4) stem bark of *A. scholaris*, and 5) fruit pericarp of mangosteen. Second factor was extract concentrations: 0 (control), 25, 50, and 100 mg/L. Applications of crude extract of mangosteen fruit pericarp at concentration 50 mg/L increased fresh weight of roots with the highest average weight 48.33 g, while the control was 13.33 g. However, plant height given extract of mangosteen (50 mg/L) was lower (74.90 cm) compared to the one in control (87.70 cm). On lower (25 mg/L) and higher (100 mg/L) concentrations of mangosteen pericarp extracts, fresh weight of roots tended to be lower than in control. Application of crude extract of *C. asiatica* at concentration 100 mg/L showed higher plant height with average 79.30 cm than in control 69.60 cm. Application of crude extract of mangosteen fruit pericarp showed the increasing of plant height from two to three weeks after planting, and crude extract of *C. asiatica* increased the plant height in third weeks after planting.

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Introduction

Indonesian government has targeted to reach rice (*Oryza sativa* L.) production 81.97 million tons in 2019 with the increase 2.3 % every year. To achieve the target, Directorate General of Food Crops is programming to increase rice production, productivity, and yield quality of food crops (Directorate General of Food Crops, 2014). In increasing rice production there is an obstacle due to reduced areas of rice field because of changes in land use function. Therefore, it needs agriculture extensification using suboptimal land dominating 88.6% of Indonesia areas with type of Ultisol soil (Utomo, 2002).

Upland rice is suitable to be planted in Ultisol soil because it grows in dry land. Ultisol is not fertile soil, but when managed well it will become a potential agricultural lands (Gofar and Marsi, 2013). Several ways

are available to overcome the physical, chemical, and biological problems of ultisol soil such as applications of organic materials, lime, and biostimulant. Biostimulants have been developed to use the increasing of growth and yield plants (Calvo et al., 2014). According to Du Jardin (2008), one of sources of biostimulants is plant secondary metabolite. Plant extracts contain bioactive compounds capable of improving nutrient efficiency and increasing tolerance to biotic and abiotic stress (Bulgari et al., (2015), such as triterpenoid saponin (Andresen and Cedergreen, 2010), flavonoid (Prabhu et al., 2010) and alkaloid (Aniszewski, 2007).

Groups of secondary metabolites potentially developed as biostimulants are mostly found in tropical plants like in leaves of cassava (*Manihot esculenta*) and fern (*Gleichenia linearis*) containing flavonoid (Bakhtiar et al., 1994), fruit pericarp of mangosteen (*Garcinia mangostana*) containing xanton (phenolic) (Orozco and Failla, 2013), stem bark of *Alstonia scholaris* containing alkaloid (Marliana and Ismail, 2011), and *Centella asiatica* containing terpenoid (Singh et al., 2012).

Application of secondary metabolites to promote plant growth depends on group of compounds and concentrations on growth stages (Abdalla, 2013; Ertani et al., 2015). Here, we examined the effects of crude extracts of several plants on the growth of upland rice.

Materials and methods

The effects of crude plant extracts on vegetative growth of upland rice were studied in screen house in experimental field of University of Andalas, Indonesia from May until Agust 2016. The trial was arranged by *Nested Design* in two factors. First factor was the sources of extract: 1) Leaves of cassava, 2) fern leaves, 3) *C. asiatica*, 4) stem bark of *A. scholaris*, and 5) fruit pericarp of mangosteen. Second factor was concentrations of extracts: 1) water (control), 25, 50, and 100 mg/L. The experiment done with three replications where each plant was sprayed by 25 ml diluted extract.

Preparing crude extract

Crude extracts of cassava and fern leaves were prepared by boiling method (Bakhtiar et al., 1994) and those of pegagan, fruit pericarp of mangosteen and stem bark of *A. scholaris* by macerated with methanol (Singh et al., 2012; Orozco and Failla, 2013).

Planting and maintenance

Three seeds of rice, UNSOED 1 variety (Faculty of Agriculture, University of Soedirman, Puwokerto, Indonesia) were sowed in a poly bag (40×50 cm) containing fined Ultisol soil. Soil was mixed with manure (4:1). Poly bags were arranged with distance 25×25 cm. The following fertilizers were given two times: 15 days after planting NPK was applied at 200 kg/ha and urea at 100kg/ha; and 30 days after planting NPK was applied at 100 kg /ha. Plants were watered in the morning when it did not rain. Weeding was carried out every week.

Application of crude extract

Crude extract was first diluted in organic solvent, *Dymethyl sufoxide* (DMSO), then in 1 liter water. Extracts were applied ±25 ml for each plant by spraying leaves evenly at 14 days after seedling. Spraying was conducted in the morning at the time relative humidity close to saturation.

Phytochemical analysis

Phytochemical analysis of crude extracts was done qualitatively and for the best extract it was completed with test of thin layer cromathograp (TLC). Phytochemical analyzing for secondary metabolite such as flavonoid, terpenoid, steroid, alkaloid, fenolic, and saponin was carried out using standard phytochemicals method according to Harborne (1973) and Trease and Evans (1983).

Justification of purified extract content using TLC for flavonoid group was done using stationary phase with Silica Gel 60 F_{254} and mobile phase with *n* butanol : etyl acetate : water (3 : 1 : 1). For terpenoid, silica Gel 60 F_{254} was used at stationary phase with chloroform : metanol (4 : 1) at mobile phase.

Data analysis

Effects of plant crude extracts on rice growth were examined using ANOVA and continued with DNMRT at 5% confident level.

Results and discussion

Applications of several plant crude extracts on upland rice plants affected plant height and fresh weight of

roots in eighth weeks after planting, but number of tillers, dry fresh of roots, fresh weight and dry weight of shoot were not affected (Table 1). The crude extract concentrations of mangosteen fruit pericarp affected plant height and fresh weight of roots, and the crude extract concentrations of *C. asiatica* affected plant height, while other crude extracts are not significant. The growth respond of upland rice to five plant crude extracts showed a different as reported by Zakiah et al. (2017), vegetative growth of soybean was inhibited by a crude extract of mangosteen fruit pericarp but it will be promoting by a crude extract of *C. asiatica*.

Table 1. Effect of crude extract of several plants to growth of upland rice in eight weeks after planting.

Treatment		Plant height (cm)	No. of bud	Fresh weight of root (g)	Dry weight of root (g)	Fresh weight of shoot (g)	Dry weight of shoot (g)
Extracts	**Concentrations (mg/l)**						
Cassava leaf	Control	75.73 a	17.00 ns	30.67 a	12.69 ns	54.75 ns	12.69 ns
	25	72.00 a	20.00 ns	50.00 a	17.32 ns	77.67 ns	17.32 ns
	50	67.76 a	16.67 ns	28.33 a	15.82 ns	65.00 ns	15.83 ns
	100	76.13 a	17.67 ns	50.00 a	17.40 ns	61.67 ns	17.40 ns
G. linearis leaf	Control	72.50 a	17.00 ns	18.33 a	16.42 ns	61.67 ns	16.42 ns
	25	64.90 a	14.67 ns	23.33 a	12.38 ns	53.33 ns	12.38 ns
	50	61.70 a	11.00 ns	8.33 a	8.60 ns	40.00 ns	8.60 ns
	100	70.60 a	14.33 ns	11.67 a	10.29 ns	45.00 ns	10.29 ns
C. asiatica	Control	69.60 ab	17.00 ns	16.67 a	11.40 ns	46.67 ns	11.40 ns
	25	68.47 b	15.67 ns	11.67 a	13.88 ns	63.33 ns	13.88 ns
	50	74.43 ab	18.67 ns	15.00 a	15.89 ns	66.67 ns	15.89 ns
	100	79.30 a	19.33 ns	21.67 a	17.22 ns	70.00 ns	17.22 ns
Stem bark of *A. scholaris*	Control	77.30 a	21.33 ns	28.33 a	19.24 ns	88.33 ns	19.24 ns
	25	80.60 a	12.33 ns	11.67 a	9.61 ns	53.33 ns	9.61 ns
	50	83.56 a	9.67 ns	10.00 a	10.90 ns	53.33 ns	10.90 ns
	100	88.50 a	11.33 ns	11.67 a	12.69 ns	71.67 ns	12.69 ns
Fruit pericarp of mangosteen	Control	87.70 a	12.67 ns	13.33 b	13.49 ns	68.33 ns	13.49 ns
	25	88.67 a	16.33 ns	26.67 ab	19.10 ns	86.67 ns	19.10 ns
	50	74.90 b	22.00 ns	48.33 a	19.21 ns	91.67 ns	19.21 ns
	100	84.83 a	14.33 ns	20.00 ab	13.37 ns	63.33 ns	13.37 ns

Numbers followed by same character in same coloumn for each extract is not significant with DNMRT 5% (ns = non-significant).

The highest fresh weight of roots (48.33 g) was obtained from treatment with crude extract of mangosteen fruit pericarp given concentration 50 mg/L, while the one in control was only 13.33 g. However, the same treatment, 50 mg/L mangosteen fruit pericarp extract resulted in the lowest plant height (74.90 cm), while the control showed higher plant height (87.70 cm). Crude extract of *C. asiatica* at 100 mg/L increased plant height with average 79.30 cm which was higher than control, 69.60 cm.

Crude extract of *Senna alata* sprayed at concentration 75% could increase plant height and leaf width of *Celosia argentea*, but dry weight was lower than control (Agbagwa et al. 2003). The treatment with crude extract (acetone solvent) of *Agapanthus caulescens* at 5 mg/ml was effective in increasing germination percentage and emergence of common bean and coffee (Masangwa et al., 2017).

Fresh weight of roots in treatment of crude extract of mangosteen fruit pericarp at concentration 25 mg/L and 100 mg/L tended to be lower, 26.67 g and 20.00 g compared to weight given concentration at 50 mg/L, 48.33 g. However, plant height was higher at concentration 25 mg/L and 100 mg/L, 88.67 cm and 84.83 g than at concentration 50 mg/L, 74.90 cm. These results indicate that there is linearity in concentration of compounds contained in crude extract in affecting plant growth.

Crude extract of *Vitex negundo* promoted germination and growth of green gram (*Vigna radiata*) and black gram (*Vigna mungo*) at low concentration but it acts as inhibitor at higher concentration (Kavitha et al., 2012). Water extract of alfalfa (*Medicago sativa*) leaves at concentration 50% increased seedling roots length of beet (*Beta vulgaris*) but it decreased at higher concentration (Shikur, 2015).

Differences in effects of crude extract of five plant species related to secondary metabolite contents (Table 2). Crude extract of mangosteen fruit pericarp contains terpenoid, and phenolics. Crude extract of *C. asiatica* contains steroid, terpenoid, and phenolics. Terpenoid and steroid in both above extracts have been known to increase plant growth, while combination of flavonoid, alkaloid, and phenolics role as growth inhibitor.

Table 2. Phytochemical analysis of crude extract of several plants.

Crude extract	Components					
	Flavonoid	Steroid	Terpenoid	Alkaloid	Polar phenolic	Saponin
Cassava *leaf*	+	-	+	-	+	-
G. linearis leaf	+	-	+	-	-	+
C. asiatica	-	+	+	-	+	-
Stem bark *of A. scholaris*	-	+	+	+	+	+
Fruit pericarp of mangosteen	-	-	+	-	+	-

The component is (+) : detectable; while (-) : Undetected

Terpenoid shows activity to promote germination and growth of rice at low concentration (Saha et al., 2010). Steroid functions as important hormone on plant growth, like brassinolide or brassinosteroid which is more bioactive than plant steroid which promotes growth (Bishop and Koncz, 2002). Steroid derivative of pentacyclic triterpene is able to stimulate growth and biosynthesis of radish chlorophyll (Geuns, 1978). Application of 0.05 ppm epibrassinolide is able to stimulate mytotic and growth of onion root cap cells (Howell et al. 2007). Brassinosteroid is able to increase root formation and growth of stem cuts of coleus, *Plectranthus forskohlii* (Swamy and Rao, 2010).

Inhibition effect is complex and involves interaction between some groups of compound like phenolic, flavonoid, terpenoid and alkaloid (Kavitha et al., 2012). Terpenoid and alkaloid from ethanol extract of leaves of *Solanum nigrum* have inhibition effect on germination and length of radicles of *Pisum sativum* (Girija and Gowri, 2008). Phenolic compound and terpenoid from *Gmelina arborea* extract function as allelopathy on black gram (*Vigna mungo*) and green gram (*Vigna radiata*) (Shankar et al., 2009). Alkaloid, terpenes, flavonoid and saponin from water extract of roots and leaves of *Costus speciosa* Koen and *Justicia adhatoda* inhibited germination and growth of sprouts of wheat and pea (Devkota and Sharma, 2014). Alkaloid, saponin, flavonoid and terpenoid in leaves extract of *Tagetes minuta*, and *T. minuta* inhibit germination, root growth and shoot, and decrease fresh and dry weight of weeds (Sadia et al., 2015).

Application of crude extract of mangosteen fruit pericarp at concentration 25 mg/L and 50 mg/L, showed the increasing of plant height from two to three weeks after planting (Fig. 1), and crude extract of *C. asiatica* at concentration 50 mg/L and 100 mg/L increased the plant height in third week after planting (Fig. 2).

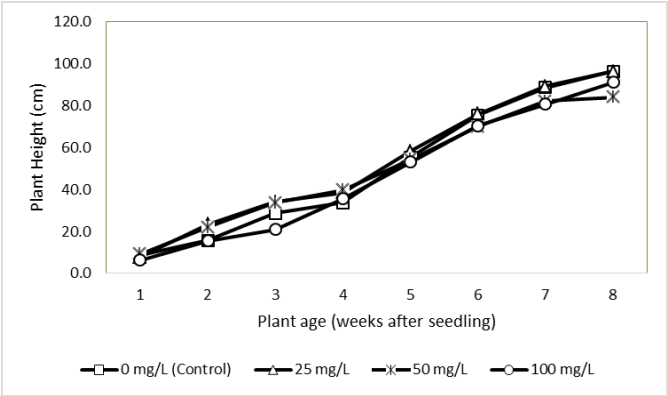

Fig. 1: Plant height of upland rice first to eighth week resulted by application of crude extract of fruit pericarp of mangosteen.

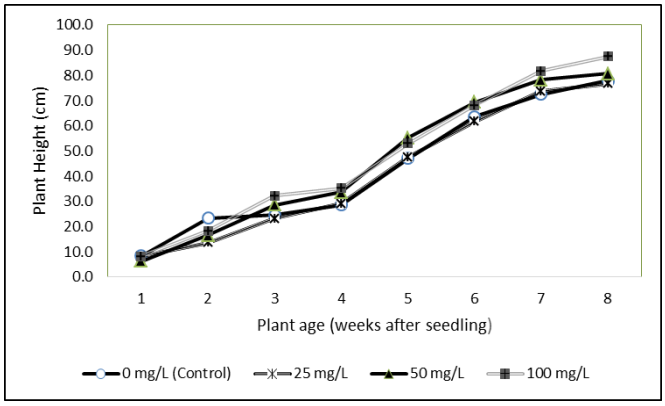

Fig. 2: Plant height of upland rice first to eighth week resulted by application of crude extract of *C. asiatica.*

From fourth to eighth week, plant height still growing but it tends to be the same between the treatments. Therefore, application crude extracts of mangosteen fruit pericarp and *C. asiatica* able to promote the plant height

until two weeks. This research will recommend twice applications of the crude extracts, second and fourth weeks after planting in respectively.

Further studies are needed to evaluate the potential of crude extract of mangosteen fruit pericarp at concentration 50 mg/L in increasing plant height and fresh weight of roots, and crude extract of *C. asiatica* at concentration 100 mg/L in increasing plant height. Further studies are also needed to trace purified terpenoid compound from crude extract of mangosteen fruit pericarp and *C. asiatica*, and to examine their bioactivity in promoting growth of upland rice.

Conclusion

Crude extracts of mangosteen fruit pericarp and *C. asiatica* have a potential to increase the vegetative growth of upland rice. Application of crude extract of mangosteen fruit pericarp at concentration 50 mg/l significantly increased fresh weight of roots of rice but decreased plant height. Application of *C. asiatica* at concentration 100 mg/l could increase rice plant height. Application of crude extract of mangosteen fruit pericarp and *C. asiatica* is able to promote the plant height until third week.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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