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Optimal extraction solvents use for extraction of *Thunbergia laurifolia* Linn. leaves and its mode of action on weed control

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Abstract *Thunbergia laurifolia* Lindl. leaves were extracted by various ratios (0, 25, 50, 75 and 100%) of ethanol in water as solvents for crude extraction. The highest crude extract yield was obtained by using 25% ethanol solvent with three time extractions. The inhibitory effect of each extract on seed germination and seedling growth of *Amaranthus gracilis* was investigated. The results indicated that the 100% ethanol extract expressed the best inhibitory effect. At concentration of 1250 ppm, the 100% ethanol extract completely inhibited seed germination of *A. gracilis*. The 100% ethanol extract also inhibited α-amylase activities in *A. gracilis* seed during seed germination. However, the extract does not show effect on seed imbibition. The inhibition of induction of α-amylase increased with increasing concentration of the 100% ethanol extract.

Keywords: *Thubergia laurifolia*, Allelopathy, Solvent, Weed control

Introduction

The competition of weeds with crop plants is responsible for a huge loss in the plant productivity. Yield losses of crops due to weeds are higher than yield losses compared to any other agricultural pests (Jabran *et al.*, 2015). Weeds are the cause by 34% of major crop yield loss, and the potential crop yield loss without control was estimated by 43%, on worldwide (Oerke, 2006). In North America, from 2007 to 2013, without weed management method, soybean and corn yield losses due to weeds probably was up to 52%. In the seven years, decrease of value of soybean and corn approximately $16 and $28 billion annually, respectively (Soltani *et al.*, 2016; 2017). Several reports indicated that crop yield loss caused by weeds such as in rice by 10-100%, maize by 25-93%, and wheat by 10 - 60% (Rao *et al.*, 2014; Jabran and Chauhan, 2015; Yaduraju *et al.*, 2015). Crop can be lost completely without weed control (Bastiaans and Kropff, 2017). Furthermore, using synthetic

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herbicides, the most prevalent method for controlling weed nowadays, often leave negative consequences for the environment and human health. Synthetic herbicides can cause a negative effect on human health, from skin rashes to death. Moreover, the inappropriate utilized of herbicides also contributes to evolution of herbicide-resistant weeds. The rise of herbicide-resistant of weeds is one of great challenges to crop system (Yang *et al.,* 2017). Therefore, need to find alternative means to overcome those problems.

The previous reports showed that the ability of allelopathy for weed control (Islam *et al.,* 2018). Allelopathic plants express their allelopathic activity by releasing allelochemical compounds. Products from allelopathic compounds may help to reduce the use of synthetic compounds on weed control. Importantly, it's harmless to environment and safer for human health compared with the use of synthesis compounds. Further, it can be applied in combination with other methods to achieve integrated weed management (Jabran *et al.,* 2015). Numerous plants are showed to possess allelopathic activity, such as *Aglaia odorata* Lour. (Laosinwattana *et al.,* 2009), *Acacia concinna* (Willd.) DC (Boonmee and Kato-Noguchi, 2017), *Sesamum indicum* (Hussain *et al.,* 2017). Kato-Noguchi *et al.* (2016) reported that rocaglaol-isolated from *A. odorata* strongly inhibited the growth of *Lepidum sativum* and *Echinochloa crus-galli*. Although most plants have allelopathic effective for weed control, however, the need for finding reliable allelopathic crops to control weeds is important.

Extraction yields and allelopathic effect not only depend on the extraction method but also depend on the solvent used for extraction. The most suitable solvents are mixtures of aqueous with acetone, ethanol, ethyl acetate and methanol. (Sun and Ho, 2005). Ethanol has been known as a good solvent for extraction of polyphenols and is safe for human.

In this study extracts were obtained from dried leaf of *T. laurifolia* using various ratio ethanol in water solvents. Inhibitory effects of the extracts were evaluated on seed germination and seedling growth of *A. gracilis seeds.* Mode of action of the extracts also demonstrated base on imbibition and α-amylase activity of *A. gracilis* seeds during seed germination period.

Materials and methods

Preparation of plant materials

The mature and healthy leaves of *T. laurifolia* were harvested from the plants in experimental field at King Mongkut's Institute of Technology Ladkrabang and these leaves were cleaned from dust and soil by running tap

water, after that dried-up in a hot-air oven at 45^{o}C for 3 days and then ground to small pieces using an electrical blender.

Effect of various ethanol ratios on the crude extraction yields

The experiment was performed with according to the method described by Teerarak (2012). 20 gram of *T. laurifolia* dried leaves were soaked in each 180 ml of various ethanol raio solvents (25, 50, 75 and 100% ethanol in distilled water) at room temperature, excepted distilled water treatment was placed at 8℃ for 3 days. After 3 days, the solutions were filtered through 2 layers of cheesecloth and re-filtered through Whatman no. 93 filter paper. Following filtration, the solution was dried up by a rotary evaporator (Buchi R215, Switzerland), under a partial vacuum at 45^{o}C until the weight of crude extract was constant. Then each residue was re-extracted 2 times with the same extraction solvent and the same conditions of the first extraction procedure as well. The crude extract of the extraction first time, second time and third time were pooled. Extraction yield was compared among the different solvents.

Bioassay effect of various ethanol ratio extracts from T. laurifolia on germination and seedling growth of A. gracilis

For this study, the crude extracts (0, 25, 50, 75, 100% ethanol) were prepared by dissolving 1 g of the extract in 100 ml distilled water (100% ethanol crude extract dissolving in ethanol), to have a stock solution of 1% concentration. The extracts were prepared to obtain serial concentration of 625, 1,250, 2,500, 5,000 and 10,000 ppm from stock extracts.

The crude extracts of *T. laurifolia* were tested for their effect on seed germination of *A. gracilis*. 5 ml of each concentration (625, 1,250, 2,500, 5,000 and 10,000 ppm) were added to petri dish (9 cm diameter) containing germination paper, and then 20 testing seeds were placed on the germination paper. The petri dishes that have only distilled water was used as control treatments. All of treatments were replicated four times in a completely randomized design (CRD). The germination was deemed to have occurred only after the radicle had protruded beyond the seed coat by at least 2 mm at seven days after treated. All petri dishes were covered and placed at room temperature. After seven days treated, germination percentage, shoot, and root length were recorded in all of treatments.

Seed imbibition and α-amylase activity bioassay

Effect on seed imbibition

To investigate the imbibition of seeds, a method according to Turk and Tawaha (2003) was used. Four replicates of 100 healthy seeds of *A. gracilis* were weighed and recorded as starting seed weight (W1). These seeds were separately germinated in crude extracts of *T. laurifolia* and distilled water as control (according to above treatment). After imbibition period, seed weights were recorded as final seed weight (W2) for each treatment and exposure time. Seed imbibition percentage of the seeds was calculated from following the equation:

Water uptake (%) = [(W2-W1)/W1] x 100

Bioassay of α-amylase activity

The method according to Bernfeld (1955) and Sadasivam (1996) was used to investigate activity of α-amylase of the seeds. After measuring imbibition, the seeds (100 seeds of *A. gracilis*. for one determination) were homogenized with 4 ml ice-cold solution of 0.1M $CalCl_2$ and then centrifuged at 10,000 rpm for 20 minutes at 4^oC. Supernatant was used as enzyme extract. The α-amylase activity was then assayed by measuring rate of generation of reducing sugar from soluble starch. 1 ml of supernatant was mixed with 1 ml of 1% soluble starch in acetate buffer solution at pH 5.5. After that, the assay medium was incubated for 15 minutes at 37^oC. Next, 1 ml of DNS reagent (40 mM 3.5 dinitrosalicylic acid, 0.4 N NaOH and 1M K-Na tartrate) was added, and immediately heated in a boiling water bath for 5 minutes. The mixture was cooled under running tap water. The intensity of color was measured as absorption at 560 nm by a spectrophotometer. The experiment was replicated four times in a completely randomized design (CRD). A standard graph was prepared using maltose, and the amount of α-amylase present in sample was calculated from standard curve and expressed as μmol maltose/min/g (fresh weight).

Results

Effect of various ethanol ratio solvents on the crude extraction yields and inhibitory effect of the extracts

Solvent selection is one of the most important factors for obtaining extracts with a mount of crude yields and strong bioactivities. In this study, the extraction solvents significantly affected on crude yield of *T. laurifolia* leaf

extracts (Figure 1). The results showed that, after 3 times of extraction, the extraction yield of 25% ethanol was the highest (4.31 g/g DW), following by the 50% ethanol (4.07 g/g DW), the 75% ethanol (3.54g/ g DW), the water (3.47 g/g DW) and the extraction yield of 100% ethanol was lowest (1.62 g/g DW).

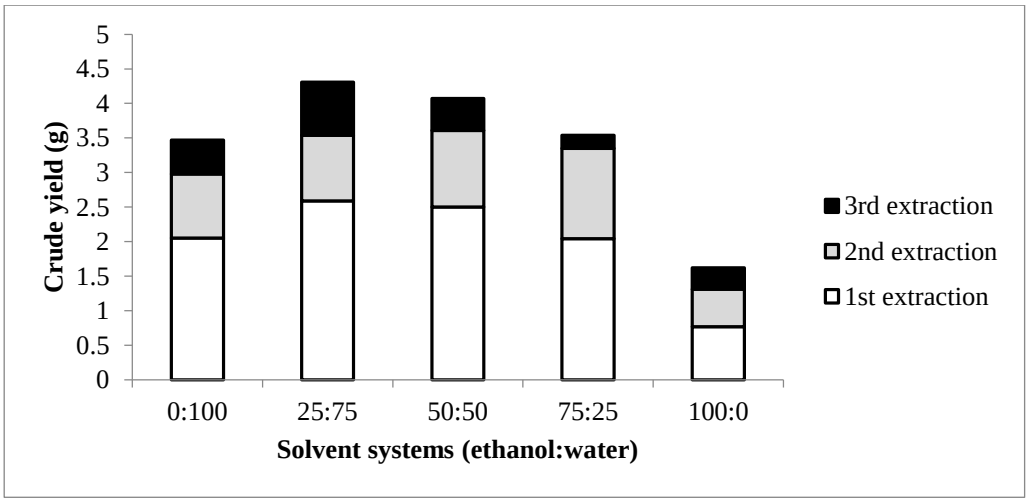

Figure 1. The effect of various ethanol ratios on crude extract yields from *T. laurifolia* dried leaves

Bioassay effect of different ethanol ratio extracts from T. laurifolia on seed germination and seedling growth of A. gracilis

The effect of crude extract from *T. laurifolia* dried leaves on germination and seedling growth of *A. gracilis* showed in Figure 2. The results revealed that the extracts obtained from different ethanol ratio significantly affected on seed germination and seedling growth of *A. gracilis*. The inhibitory effect also increased with the increase of concentration of the testing extracts. The extracts of 75 and 100% ethanol in water completely inhibited seed germination, shoot length and root length, at concentration of 2,500 ppm. Moreover, the extracts from all ratios completely inhibited seed germination, at concentration of 10,000 ppm. The 100% ethanol extract expressed the strongest inhibitory effect on seed germination of *A. gracilis* followed by the 75% ethanol, 50% ethanol, 25% ethanol and the water extracts (inhibited 100, 84.29, 78.57, 42.86 and 35.71%, respectively), at concentration of 1250 ppm.

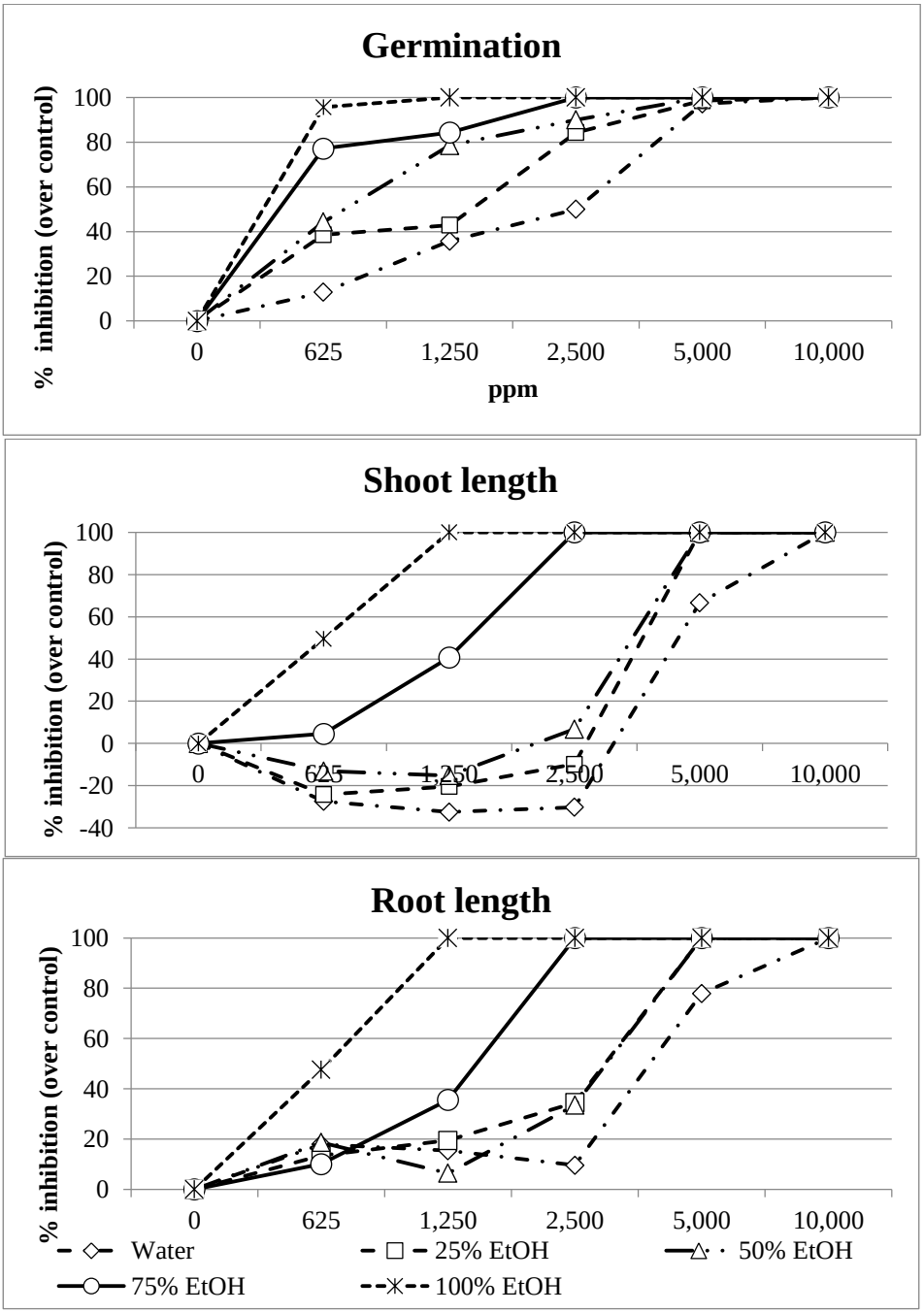

Figure 2. Inhibitory effect of crude extracts obtained by various ratios of ethanol in water from *T. laurifolia* dried leaves and concentrations of the extracts (625, 1,250, 2,500, 5,000 and 10,000 ppm) on germination and seedling growth of *A. gracilis*

Seed imbibitions and α-amylase activity bioassay

Percentage of imbibition and α-amylase experiments were studied to understand the mechanism of inhibition on *A. gracilis* seeds. Figure 3 exhibited the percentage of imbibition at different imbibition periods and concentration of the 100% ethanol extract. The results exposed that the percentage of imbibition increased by prolonging the imbibition period, at the same concentration. In the control seeds, the percentage of imbibition at different imbibition periods of 12, 18 and 24 hours were 18.52, 23.50 and 28.67%, respectively.

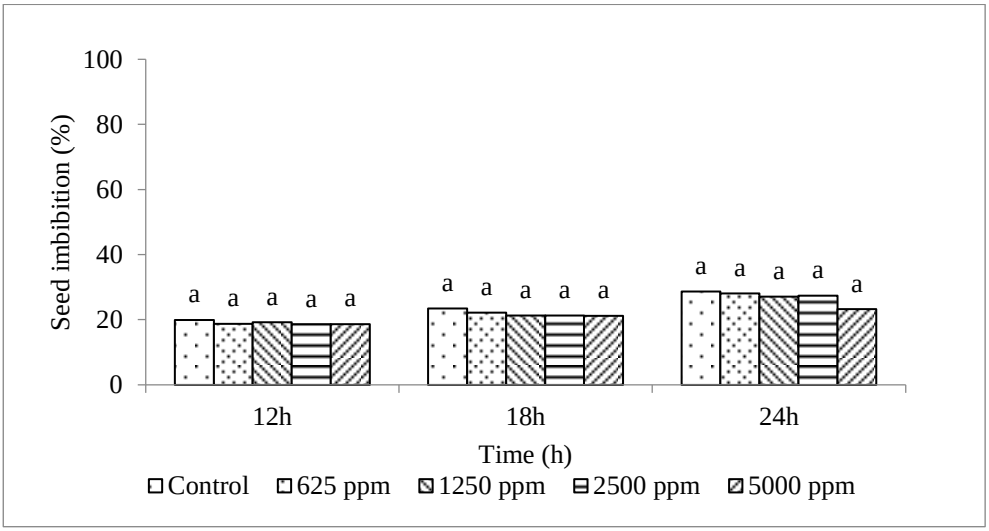

Figure 3. Effects of the 100% ethanol extract from *T. laurifolia* on imbibition of *A. gracilis* seeds at different imbibition periods. Different letters are significance differences ($p<0.05$)

During seed germination, α-amylase plays an important role in the breakdown starch and proteins, which provide the energy for the growth of roots and shoots. The α-amylase activity of *A. gracilis* seeds was tested and the results are shown in Figure 8. The results indicated that increasing the concentration of the extract leads to a significant increase of the inhibition on α-amylase activity of *A. gracilis* seeds. Moreover, the α-amylase activity also increased by prolonging the imbibition period, under the same concentration. For the imbibition periods of 12 and 24 hours, the inhibitory effect on α-amylase activity at each concentration is significantly different. At the same imbibition period, the α-amylase activity was strongest inhibited at the concentration of 5000 ppm.

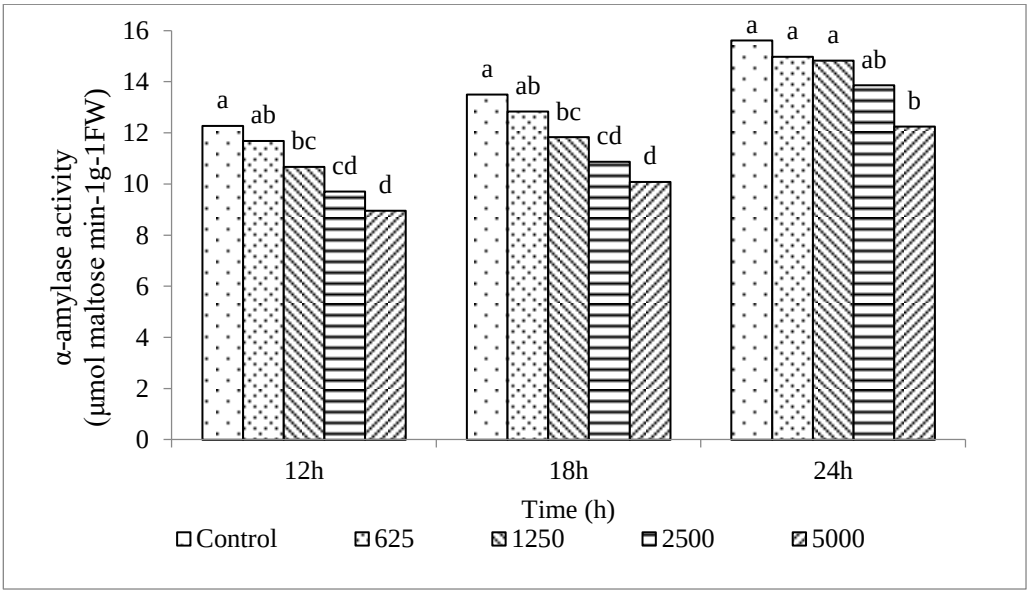

Figure 4. Effects of the 100% ethanol extract from *T. laurifolia* on α-amylase activity of *A. gracilis* seeds at different imbibition periods. Different letters are significance differences ($p<0.05$)

Discussion

In the present study, the results showed that crude extract yields from *T. laurifolia* depending on ethanol ratio of solvents. The extraction yields of various solvents decreased in the following order: 25% ethanol > 50% ethanol > 75% ethanol > 0% ethanol > 100% ethanol. This finding was in an agreement with the study of Wichittrakarn *et al*. (2011), who reported that the greatest recovery was achieved by using ethanol at 25%. The results could be explained by several factors such as composition of each particular plant, differences in the solubility of extractive from *T. laurifolia* and their polarity.

The results exhibited that all of extracts from *T. laurifolia* inhibited seed germination and seedling growth of *A. gracilis* seeds. In contrast with crude extract yields, the extract obtained by 100% ethanol solvent showed the highest inhibitory effect. The variation in inhibition of germination and seedling growth of the extracts in different solvents may be attributed to the different polarity of the solvents. Different chemicals were dissolved in different polar solvents that led to the variability of the extracts of same plant in different ethanol ratio solvents. This result was supported by the study of Wichittrakarn (2011) who studied the optimal extraction solvent for extraction of *Tagetes erecta* Linn. and

found that 75% ethanol in water demonstrated the highest inhibitory effect. The difference may be due to allelochemicals present in the plants are different.

T. laurifolia extracts could inhibited seed germination and seedling growth of *A. gracilis A. gracilis* by inhibiting α-amylase activity of the seeds. The results found in this study are consistent with those of (Kato-Noguchi and Mac ás, 2008), who suggested that 6-methoxy-2-benxozazolinone may inhibit the seed germination by inhibiting the induction of α-amylase activity. However, under the same imbibition period, no significant differences in imbibition among all concentrations that were observed. It may explain that the extract from *T. laurifolia* inhibits seed germination and seedling growth of *A. gracilis* not by inhibiting the imbibition of the seeds. This finding are inconsistent with the study of (Teerarak *et al.*, 2012), who reported that the wettable powder formulation of crude extract from *Jasminum officinale* f. var. *grandiflorum* (Linn.) Kob. inhibited the imbibition of *Echinochloa crus-galli* seeds. The difference could be due to allelochemical compounds exist inside plants are different.

Conclusion

The present study evaluated crude extract yields of five different ethanol ratios in water of *T. laurifolia.* The crude extract yield was obtained by the 25% ethanol solvent is the highest. However, the 100% ethanol extract exhibited the strongest inhibitory effect on seed germination and seedling growth of *A. gracilis* seeds. The extracts from *T. laurifolia* inhibited *A.gracilis* seeds may by inhibited the induction of α-amylase in these seeds. Therefore, *T. laurifolia* leaves may be considered as a source for natural herbicides.

Acknowledgements

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