Abstract. The present study was aimed to evaluate the phytotoxic activity of crude leaf extract from Dilleniaceae family on seed germination of goosegrass (E. indica) under different concentrations, ranging from 0-10 mg/mL. The total phenolic content (TPC) was studied and found to be about 209 mg GAE/g extract. Crude extract was significantly inhibitory towards germination of E.indica seeds. Concentration of 10 mg/mL extract demonstrated high germination inhibition rate at 90 % of goosegrass seeds as compared to the control. The bioassay results illustrated that the extract markedly inhibited the germination of goosegrass seeds which may require further investigation for the potential use in crop protection.

1. Introduction
The repetitive use of synthetic herbicides has resulted in serious environmental impacts and human health as well as the emergence of herbicide resistant biotypes [1]. In recent years, natural product received considerable attention to minimize and tackle the issues associated with commercial herbicides [2][3]. It can be attained via living organisms (fungi and bacteria), minerals or plant extracts. In this regard, plants are one of the richest sources of novel phytotoxic compounds to be utilized as natural herbicides. The secondary metabolites (allelochemicals) present in the plants may offer unique modes of action in controlling the weeds. Thus, this study offered a preliminary screening of plant species from Dilleniaceae family in inhibiting the seed germination of goosegrass (E. indica). Goosegrass has been well known as one of the most problematic weeds in the world due to its high reproductivity and good adaptability to changing environments. The heavy reliance of herbicide on goosegrass has resulted in evolution of resistance to several modes of actions and herbicides such as ACCase inhibitors and glyphosate herbicides [4]. This study aims to determine the total phenolic content (TPC) of plant extract and its phytotoxic effects towards seed germination of goosegrass.

2. Materials and methods

2.1 Plant materials and chemical reagents
The fresh adult leaves from Dilleniaceae family were collected around Universiti Teknologi PETRONAS campus. They were washed thoroughly and left to dry. Then, the dried leaves were ground into powder and stored until further use. Seeds of goosegrass were collected in Universiti Teknologi PETRONAS campus. The healthy and uniform seeds were selected, and surface sterilized with 2% sodium hypochlorite for a few minutes. Then, the seeds were rinsed several times with distilled water, air-dried and stored until use. The germination of seeds was randomly checked and was ~85-95%. Chemicals and reagents used were AR grade sourced from Sigma-Aldrich and Merck.

2.2 Preparation of plant extract
Dried leaves powders were extracted using organic solvent at ratio 1:10. The mixture was then agitated for 24 h. Then, the mixture was filtered using muslin cloth to remove large solids, followed by Whatman No.1 filter paper to obtain the filtrate. The filtrate was concentrated under reduced pressure by rotary vacuum evaporator (BUCHI Rotavapor R-215, Switzerland).

2.3 Determination of total phenolic content
The total phenolic content (TPC) of crude leaf extract was identified using Folin-Ciocalteu method with gallic acid as standard. Standard curve (y = 0.0011x + 0.0295, R² = 0.998) was plotted using different concentrations of gallic acid, ranging from 25-400 µg/mL. 1 mL of diluted gallic acid was mixed with 5 mL of Folin-Ciocalteu reagent and 4 mL of 7.5% sodium carbonate solution. The mixture was left for 30 minutes and measured at 765 nm spectrometrically. For plant extract, 1 mg/mL concentration was used. The result was expressed as mg of gallic acid equivalent (GAE) per g of extract.

2.4 Seed germination bioassay
Goosegrass seeds were used to test the germination response to different concentrations (ranging from 0 to 10 mg/mL) of crude leaf extract from Dilleniaceae family. Completely randomized design (CRD) with four replicates was adopted in the bioassay. Distilled water was served as a parallel control. Briefly, 20 seeds of goosegrass were distributed evenly in petri dishes on a double layers of Whatman No. 1 filter paper wetted with 5 mL of respective concentrations of crude extracts. Then, the petri dishes were sealed and placed in an incubator at 27 °C in the dark for one week. Number of germinated seeds was counted after 7 days. Final germination percentage (GP) were calculated based on Equation (1) was determined.

\[ GP = \frac{n}{N} \times 100 \]  

where,
\( n \) is Number of germinated seeds
\( N \) is Total number of seeds

2.5 Statistical analysis
The result was analysed using one-way ANOVA, followed by comparison of mean values using post hoc Tukey’s test at p ≤ 0.05. All statistical analyses were performed using SPSS software ver. 23.0.

3. Result and discussion
The TPC of plant extract was determined from a linear gallic acid standard curve (y= 0.0011x + 0.0295, R²= 0.998) which has an average of 209 ±1.04 mg GAE/g extract. At low concentration, the inhibitory effect of plant extract was not profound as shown in figure 1. As the concentration of extract increases, the seed germination rate was reduced significantly (p < 0.05). At 10 mg/mL concentration, inhibitory rate of about 90% could be attained. The trend is showing levelling-off beyond the concentration of 10 mg/mL. This might be due to the different sensitivity of plant seeds and selective permeability of seed coats which may protect the inhibitory activity of phytotoxic extract. The inhibitory effects are complex and might result from combined actions induced by the phenolic and other chemical groups present in the extract. Various phenolic compounds including ferulic acid, caffeic acid, p-hydroxybenzoic and cinnamic acids have been shown to exert phytotoxic activity on
These compounds might interact and hinder the physiological and biochemical processes such as hormonal stability, ion uptake water balance, respiration, protein synthesis, chlorophyll generation, photosynthetic and mitochondrial functions in the seeds.

Figure 1. Germination rate of goosegrass seeds under different concentration of plant extract. Means labelled with distinct letters in a column are significantly different (p < 0.05).

4. Conclusion
Sustainable weed management has been one of the most challenging tasks in crop production. Repetitive use of herbicides induces adverse impacts to the environment and human health as well as emergence of herbicide resistant weed biotypes. In view of this, plant-based natural herbicides could act as a greener alternative to commercial herbicides due to their safe nature and high biodegradability. Based on this study, the TPC of plant extract was around 209 mg GAE/g extract. It exhibited high inhibition (~90%) towards goosegrass seed germination. Further study is necessary to isolate and characterize the phytotoxic compounds present in the plant extract which are effective for weed growth inhibition.
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