Journal of Science and Technology, Vol. 10 No. 4 (2018) p. 1-7

Effect of Light Intensity on the Total Flavonoid and Total Phenolic Contents of *Mikania Micrantha* and *Tridax Procumbens*

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Received 15 September 2018; accepted 1 December 2018; available online 30 December 2018
DOI: https://10.30880/jst.2018.10.04.001

Abstract: Flavonoid and phenolics are secondary metabolites produced by plants in response to harsh environmental conditions. Light is one of the most important factor that affects their production. *Mikania micrantha* and *Tridax procumbens* are plants belonging to *Asteraceae* family, and they have bioactivity due to their flavonoid and phenolic contents. The objective of the research is to determine the effect of varying light intensities on the total flavonoid and total phenolic contents of *M. micrantha* and *T. procumbens* using three solvents (ethanol, methanol and water). Total flavonoid contents was determined based on the aluminium chloride colorimetry method while total phenolic contents was determined based on the folin-chiocalteau reagent. The results obtained in the study shows that ethanol recovers more flavonoid and phenolic than the other solvents ($P < 0.05$). Besides, *T. procumbens* had more flavonoid and phenolic content compared to *M. micrantha* ($P < 0.05$). Nevertheless, the flavonoid and phenolic contents recovered from sun exposed plants was more than that recovered from shaded plants ($P < 0.05$). This leads to a conclusion that high light intensity can increase the concentration of flavonoid and phenolic of *M. micrantha* and *T. procumbens*.

Keyword: Flavonoid; light intensity; *Mikania micrantha*; phenolic, *Tridax procumbens.*

1. Introduction

Mikania micrantha and *Tridax procumbens* are plants belonging to the *Asteraceae* family. The former is also called Chinese creeper in English while the latter is commonly known as Coat buttons. The two species have a wide distribution. Moreover, they are regarded as invasive species because they occupy various regions in the world which were not their native environment. The plants are having recognition due to their antimicrobial, anticancer, antioxidant and other biological effects. *M. micrantha* for example is having applications in traditional medicine as an antidote for snake and insect bites as well as nausea and vomiting [1]. Ishak et al. [2] performed an extensive study on the vine and reported its antimicrobial, antioxidant, antiviral, and antidiabetic effects. Moreover, Jyothilakshmi et al. [3] reveals the antidermophytic effect of *M. micrantha*. *T. procumbens* is also use in traditional for treating diarrhoea. It is also having antifungal, antimicrobial and antioxidant effects. Both *M. micrantha* and *T. procumbens* were having bioactivity due to their flavonoid and phenolic contents.

Flavonoids (for example flavonol, flavones, and anthocyanin) are secondary metabolites produced by plants in response to cold, drought heat, salinity, UV radiation, pathogens. They function as detoxifying agents, allelopathic compounds, and signal molecules [4]. Flavonoids are not constantly produce by the plant, but rather, they are produced as response to a harsh condition [5]. Changes in the amount of light intensity a plant is receiving affects the secondary metabolites of the plant. For example, shading was reported to affect flavonoids concentration in leaves of *Litocarpus litseifolius* [6] because as the light intensity increases or decreases, the flavone accumulation was affected. Flavonoids are secondary metabolites that function in protecting the plants against harmful ultraviolet (UV) radiations [7]. Flavonoid accumulation in *Zingiber officinale* reduce when the light intensity increases [8] while *Centella asiatica* produces more flavonoids at high light intensity [9]. Other plants like *L. litseifolius* [6],

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e-ISSN: 2600-7924/penerbit.uthm.edu.my/ojs/index.php/jst

Piper aduncum [10] accumulates more flavonoid when the light intensity is moderate. Phenolics are plant secondary metabolites which are produced by plants in response to wound insects or pathogen attack, as well as UV radiation. Due to this, their concentration in plants varies depending on the light intensity received by the plant. For example, the phenolic of *Zingiber officinale* is higher when the light intensity is high [8]. Glycine max sprouts accumulates more phenolic under high light intensity [11]. Moreover, high level of phenolic was recorded for *Gracilaria chilensis* [12], *Labisa pumila* [13], and citrus plant [14]. Furthermore, *Zea mays* sprouts [15] that germinates under high light intensity contain more phenolic than those that germinate under dark condition. Besides, *Anoectochilus roxburghii* grown under red light had the highest phenolic than those grown under blue and yellow light [16].

The objective of the research is to determine the effect of varying light intensities and extraction solvent on the total flavonoid and total phenolic contents of *Mikania micrantha* and *Tridax procumbens* as well as how extraction solvent affects the flavonoid and phenolic contents recovered from the studied plants.

2. Materials and method

2.1 Plant material

The study area was at Gunung Ledang, Johor in Malaysia. Sun exposed and shaded *Mikania micrantha* and *Tridax procumbens* growing naturally in the forest were the studied plants. The plants were sampled and harvested. 100g was measured and dried in an oven at 45^{o}C for 42 hours. Sample was extracted based on Chai et al. [17] by grinding the dried leaves into powder. Furthermore, the dried powder was mixed with 2000ml of the solvents (ethanol, methanol and water) and incubated for 2 hours. The plant mixture was then filtered with a Whatman paper prior to centrifuging at 12000rpm, at 4^{o}C for 10 minutes. The supernatant was stored at low temperature and later used for determining total flavonoid and phenolic contents of the plants.

2.2 Determination of the total flavonoid contents (TFC)

Total flavonoid content of *Mikania micrantha* and *Tridax procumbens* was determined based on the aluminium chloride calorimetry method were 10% $AlCl_3$ and 1M potassium acetate were prepared based on standard protocol. The standard used for quantification of the flavonoids was quercetin and its stock solution was prepared by dissolving 1mg of quercetin in 1ml of distilled water. Moreover, serial dilution of the standard solution were prepared. Extract solution was then prepared by mixing 1ml of plant extract with 3ml of methanol, 200µl of 10%$AlCl_3$, 200µl of 1M potassium acetate and 5.6ml distilled water. Standard and blank solutions were prepared exactly as extract solution, except that for the former, 1ml of plant extract was replaced with several serial dilution of quercetin while the preparation of the latter was achieved by replacing 1ml of plant extract with ethanol [18]. Samples were then incubated at room temperature for 30 minutes prior to taking absorbance at 420nm. Quercetin calibration curve was generated from the data obtained and the total flavonoid content was calculated by dividing concentration of quercetin used and volume of extract by weight of the plant extract. Results were expressed as mg/g quercetin equivalents (QE).

2.3 Determination of the total phenolic contents (TPC)

Total phenolic content of *Mikania micrantha* and *Tridax procumbens* was determined using the folin-chiocalteau reagent. Quantification as done using UV-visible spectrophotometer. The procedure was based on Barku et al. [18]. Prior to quantification, 7.5% of $NaCO_3$ was prepared according to the standard protocol. Moreover, stock solution of standard (gallic acid) was prepared by dissolving 1mg of the acid in 1ml of distilled water and then making a serial dilution. In addition, the folin-chiocalteau reagent was prepared by mixing 2ml of the reagent with 20ml of distilled water. Besides, the extraction solution was then prepared. This was achieved by string 1ml of plant extract which was previously prepared with 5ml of folin-chiocalteau reagent. Alongside, 5ml of the formerly prepare $NaCO_3$ was added and stirred. The procedure followed for preparing extract

solution was followed exactly for preparing standard and blank solutions, except that for the former, 1ml of plant extract was replaced with several serial dilution of gallic acid while the preparation of the latter was achieved by replacing 1ml of plant extract with ethanol. Samples were then incubated for 20 minutes prior to taking absorbance at 760nm. Gallic acid calibration curve was generated from the data obtained and the total phenolic content was calculated by dividing concentration of gallic acid used and volume of extract by weight of the plant extract. Results were expressed as mg/g gallic acid equivalents (GAE).

2.4 Statistical analysis

Data were recorded in triplicates indicating the standard deviation of the means. Additionally, differences between the means of sun exposed *Mikania micrantha* and *Tridax procumbens* and shaded *M. micrantha* and *T. procumbens* were compared using paired sample T-test. Independent sample T-test was used to compare means of sun and shade *M. micrantha* as well as sun and shade *T. procumbens*. Likewise, means of different solvents employed were compared using one way ANOVA, were Tukey's multiple-range test was used to compare the differences in solvents that were significantly different form ANOVA test.

3. Results and Discussion

3.1 Total flavonoid content

The TFC of ethanol, methanol and ethanol extracts of *M. micrantha* and *T. procumbens* were represented in Fig. 1. Regarding the TFC of aqueous extract of the studied plants, it can be seen that the TFC of aqueous extract of *M. micrantha* is less than that of *T. procumbens* ($P < 0.05$). Moreover, the TFC of ethanol extract of *T. procumbens* was also higher than that of *M. micrantha*.

Out of all the three solvents, ethanol had the highest flavonoid extraction capacity in the studied plants. Nevertheless, solvent polarity plays a vital role in extraction of phytochemicals in plants. In an attempt to extract phytochemicals from *Pluchea indica* leaves, high amount of flavonoid was recovered using methanol, followed by ethanol while the least obtained was in aqueous extract [19]. In another study, TFC of *Amomum chinense* were obtained in highest concentration using 80% methanol followed by 80% ethanol while the least was obtained in aqueous extract [20]. Besides, Do et al. [21] reported that the highest flavonoid content of *Limnophila aromatica* was obtained in ethanol extract of the plant followed by methanol extract while the least was obtained in aqueous extract. In *Macademia tetraphyla*, extraction solvents have an influence in the TFC recovered from the plant [22]. In an increasing order, TFC of *M. tetraphyla* was lowest in aqueous extract compared to methanol and ethanol extract. Yet, methanol recovered the highest flavonoid compared to ethanol [22].

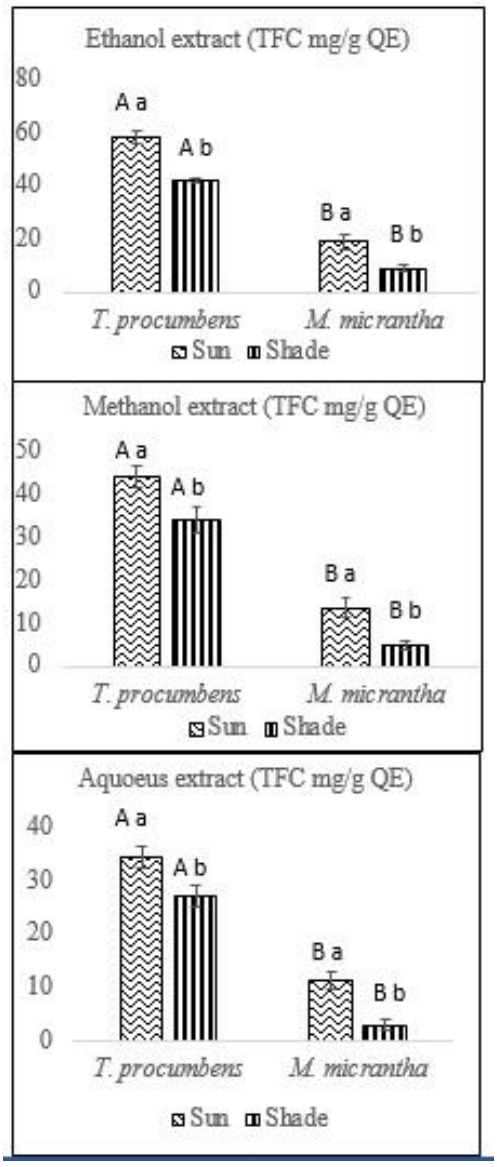

Fig. 1 Total flavonoid content of *M. micrantha* and *T. procumbens*

Different capital letters indicate significant differences among the plant species under the same light condition ($p < 0.05$); Different small letters indicate significant differences among two light conditions of the same plant species ($p < 0.05$).

Furthermore, the TFC of some medicinal plants as reported by Sultana et al. [23] were recovered in highest concentration when using 80% methanol, followed by aqueous ethanol. Though when comparing absolute methanol with aqueous methanol, the latter can extract more plant flavonoids than the former. This is also true when comparing absolute with aqueous ethanol [23]. In *Helicteres hirsute* leaves, TFC recovered in methanol and aqueous extracts was in decreasing order, with the highest recovered in methanol extract and the lowest in ethanol extract. In *Datura metel* [24], TFC recovered was highest in methanol compared to extracts from other solvents. Additionally, the TFC of *Phoradendron californicum* was highest in ethanol extract followed by aqueous, while the least was found in methanol extract [25].

To this point, it can be clearly seen that solvents extract flavonoids in a different manner depending on the plant. Concisely, plant inherent characteristics, type of flavonoid and polarity of the solvent plays an important role in the amount of flavonoid recovered from a plant extract. When comparing the means of TFC recovered from varying solvents (Table 1), it can be clearly seen that the difference in their means is statistically significant as the p value was less than 0.05.

Table 1 Total flavonoid content of *M. micrantha* and *T. procumbens* extracted with 3 solvents

Extract	LC	*T. procumbens*	*M. micrantha*
Ethanol	S	58.33±2.1[a]	19.00±0.9[a]
	SH	42.33±2.8[a]	9.00±1.1[a]
Methanol	S	44.34±2.6[b]	13.60±2.1[b]
	SH	34.00±3.1[b]	5.00±1.0[b]
Aqueous	S	34.67±2.1[b]	11.33±1.5[b]
	SH	27.33±2.2[b]	3.01±0.9[b]

LC, light condition; S, sun; SH, shade.
Different small letters indicate significant differences among different extract of the same plant species ($p < 0.05$).

Regardless of the extraction solvent, it is obvious that the TFC of the studied plants is higher in sun exposed plants compared to shaded plants. TFC of sun and shade plants varies according to plants. For example, *Zingiber officinale* [8] produce more flavonoid in shaded condition. *Centella asiatica* [9] accumulates more flavonoids in sun exposed condition while *Piper aduncum* [10] and *Lithocarpus litseifolius* [6] requires a moderate shading condition for their maximum accumulation of flavonoid. The TFC in methanol extract of *M. micrantha* was 2.07± 0.03 mg/g QE [26]. The TFC of *T. procumbens* was highest (61.28± 1.02 mg/g QE) in methanol extract, followed by methanol extract (55.43± 0.58 mg/g QE) and lowest in aqueous extract (42.17± 1.12 mg/g QE) [27]. In another study, TFC of *T. procumbens* leaves ranges from 25 to 50 mg/g QE [28].

3.2 Total phenolic content

The TPC of ethanol, methanol and ethanol extracts of *M. micrantha* and *T. procumbens* were represented in Fig. 2. As the TFC, the TPC of aqueous extract of *M. micrantha* is also less than that of *T. procumbens* ($P < 0.05$). It is clearly seen that the TPC of ethanol extract of *T. procumbens* was also higher than that of *M. micrantha*.

When comparing the TPC recovered from each of the three solvents, ethanol had the highest phenolic extraction capacity in the studied plants. Nevertheless, solvent polarity plays a vital role in extraction of phytochemicals in plants. The phenolic content of *Pluchea indica* leaves was high in methanol, followed by aqueous while the least obtained was in ethanol extracts [19]. In another study, TPC of *Amomum chinense* were obtained in highest concentration using methanol followed by ethanol while the least was obtained in aqueous extract [20]. Likewise, Do et al. [21] reported that the highest phenolic content of *Limnophila aromatica* was obtained in ethanol extract of the plant followed by methanol extract while the least was obtained in aqueous extract. In *Macademia tetraphyla*, the TPC was highest in aqueous extract compared to methanol and ethanol extract [22].

In some medicinal plants, the highest TPC was recovered when using 80% methanol, followed by aqueous ethanol [23]. In *Helicteres*

hirsute leaves, TPC recovered in aqueous extracts was higher than that recovered in methanol and ethanol extract. In *Datura metel* [24], TFC recovered was highest in methanol compared to extracts from other solvents. Additionally, the TFC of *Phoradendron californicum* was highest in ethanol extract followed by aqueous, while the least was found in methanol extract [25].

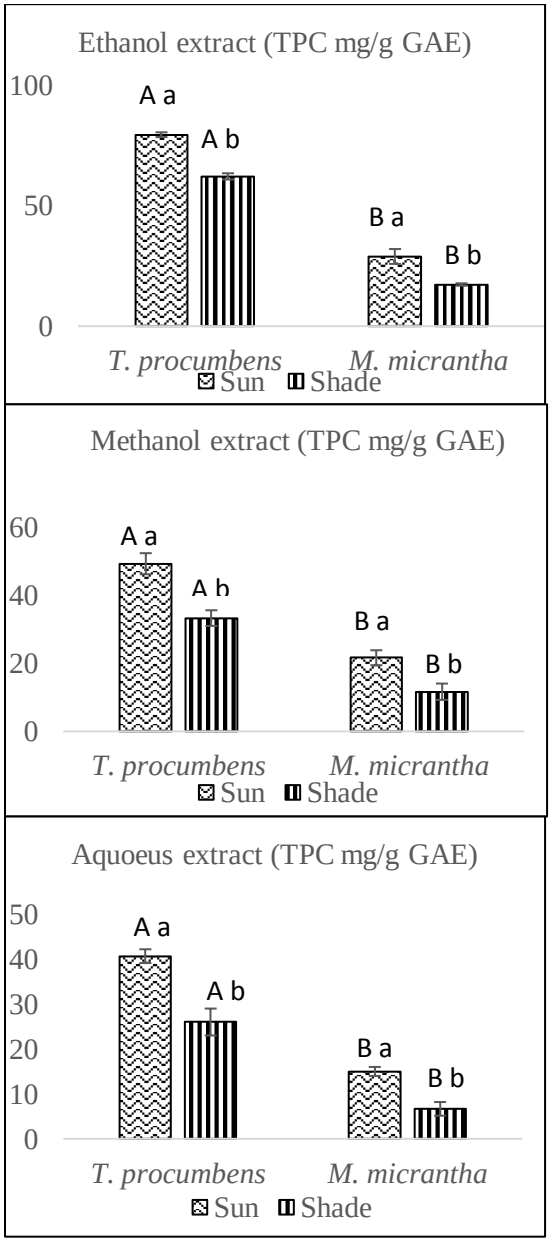

Fig. 2 Total phenolic content of *M. micrantha* and *T. procumbens.*

Different capital letters indicate significant differences among the plant species under the same light condition ($p < 0.05$); Different small letters indicate significant differences among two light conditions of the same plant species ($p < 0.05$).

Thus far, it is obvious that solvents polarity, plant inherent characteristics and the type of phenolic plays an important role when trying to recover the total phenolic present in a particular plant extract. Distinctly, analysis of variance shows that the differences in the TPC recovered from the three solvents is statistically significant ($P < 0.05$) as shown in Table 2.

Table 2 Total phenolic content of *M. micrantha* and *T. procumbens* extracted with 3 solvents

Extract	LC	*T. procumbens*	*M. micrantha*
Ethanol	S	79.67±1.00 [a]	29.00±1.3 [a]
	SH	62.33±3.1 [a]	17.33±0.5 [a]
Methanol	S	49.33±2.9 [b]	21.61±2.2 [b]
	SH	33.33±2.3 [b]	11.3±2.4 [b]
Aqueous	S	40.65±1.5 [b]	15.00±1.1 [b]
	SH	26.00±3.0 [b]	6.89±1.4 [b]

LC, light condition; S, sun; SH, shade.
Different small letters indicate significant differences among different extract of the same plant species ($p < 0.05$).

Light intensity indisputably affects the TPC of sun exposed and shaded plants studied. The sun exposed plants had the highest phenolic content compared to shaded plants. This is obvious because TPC of sun and shade plants varies according to the light intensity received by the plants. Most plants produced more phenolic content under high light intensity. This may be due to the photoprotective role of the phenolic contents. For example, glycine max sprouts [11], *Gracila pumila* [12], *Labisa pumila* [13], citrus plants [14] *Zingiber officinale* [8] and *Zea mays* sprouts [15] produce high TPC in sun exposed than in shaded conditions

4. Conclusion

The results lead to a conclusion that light intensity can change the concentration of flavonoid and phenolic of *M. micrantha* and *T. procumbens*. When considering the extraction solvent and the light condition, it is obvious that *T. procumbens* produces more flavonoids and phenolic than *M. micrantha*. It can also be concluded that ethanol is the best solvent to extract flavonoid and phenolic in the studied plants. Furthermore, sun exposed *M. micrantha*

and *T. procumbens* produces more flavonoid and phenolic than shaded plants. Nevertheless, phenolic content recovered from both plants exceeded the flavonoid contents recovered from the plants. A future study will be based on determining the antimicrobial activity of the flavonoid and phenolic contents.

Acknowledgements

The authors gratefully acknowledge the Universiti Tun Hussein Onn Malaysia (UTHM) for the facilities provided.

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