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Phytotoxic potential and nutritive values of *Bombax ceiba* L. leaveas

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This study was conducted to evaluate the phytotoxic potential and some nutritive values of *Bombax ceiba* L. leaves. For the first purpose, three different concentrations of the leaf aqueous extract were prepared: 0.5, 1 and 2 % dry weight v^{-1}, and consequently assayed on germination and seedling growth of three weed species: *Amaranthus viridis*, *Melilotus indicus* and *Trifolium resupinatum*. The profile of amino acids and mineral nutrients as well as some antioxidant vitamins; C and E, and the total antioxidant activity were measured to assess some nutritive values. In general, the aqueous extract reduced germination and seedling growth of the weed species. *A. viridis* was more sensitive to the extract than the others. The spectrophotometric analysis indicated the presence of appropriate amounts of phenolics and flavonoids. The results indicated the presence of many essential amino acids amongst which lysine and glutamine were abundant. The leaves were rich in potassium, ascorbate and α-tocopherols. These results suggest that the leaf extract was phytotoxic to some weeds, and therefore may be used as a bio herbicide. Presence of some nutrients, amino acids and vitamins in the *Bombax* leaves may give the potent to use the leaves of this species as a dietary supplement

Keywords: *Bombax ceiba*, phyto-toxicity, weeds, amino acids

INTRODUCTION

Bombax ceiba L. is a broad-leaved deciduous tree native to southern Asia and widely introduced in various localities all over the world (El-Hadidi and Boulos, 1988). It was also recorded in the dry rainforests of Australia, with a main trunk growing up to 20 m high and 100 cm thick (Brock, 2001). Recently, the *Bombax* trees were introduced to Egypt for ornamental purposes. Moreover, they are well established and able to survive and reproduce in some habitat conditions such as gardens, new urban areas and along the roadsides. The leaf litter of such tree is heavily found in different plant communities in winter (Murali and Sukumar 1993; Nanda et al. 2014; Hassan, 2018). It is also likely to persist somewhat on soil to summer. On the other hand, there is an evidence supporting that *B. ceiba* may be pollen-allelopathic (Khan and Jahan, 1988; Murphy, 1992). However, this is insufficient baseline work in the field of allelopathy, and, till now, the phytotoxic potential of *B. ceiba* is still lacking. It was therefore necessary to check this assumption.

On the other hand, the *Bombax* trees were widely used in folk medicine and pharmacological studies as they contain several bioactive compounds such as phenolics, flavonoids, terpenes and steroids (Chaudhary and Khadabadi, 2012; Hossain et al. 2013; Refaat et al.2013). These literatures have explored these ingredients in some parts of the tree such as roots stem, flowers and gums. However, they ignored, to some extent, some chemical ingredients of leaves which are involved in the antioxidant activity such as vitamin C and E. We therefore tested the existence of these vitamins as antioxidants in *Bombax* leaves. We also tested the hypothesis that the leaves of *B. ceiba* are more abundant in nutrients and amino acids if compared with some edible plants. They also have not mentioned the potential profile of amino acids and minerals. These ingredients may provide a baseline to use the *Bombax* leaves as a dietary supplement. Therefore, the main aims of this study are to (1) investigate the phytotoxic potential of the leaves of *B. ceiba* against some associate species, and the potent to use this plant as a bio-herbicide, and (2) give a further database for some bioactive constituents in the leaves of such species such as the amino acids, mineral nutrients and some vitamins (C and E) involved in antioxidant activity, and, thereby, the potent to use the leaves of such species in the dietary supplementations.

MATERIALS AND METHODS

Laboratory bioassay

Collection of leaf litter

From the areas cultivated by the trees, the leaf litter of *B. ceiba* was collected from the tree canopy in winter 2022, the period which is coinciding not only with the leaf abscission but also the phase of such tree affecting the plant cover of the understory species.

The target species

Three native target species namely: *Amaranthus viridis* (Linn.), *Melilotus indicus* (L.) All. and *Trifolium resupinatum* L. were used as test species because of their ecological importance. These species are common weeds in the agro-ecosystem (Gomaa et al. 2012) and found amongst the understory species (Hassan 2018). This investigation may indicate the level of *B. ceiba* interaction. The seeds of such species were collected when ripe from the natural plant communities.

Aqueous extraction

Twenty grams of the grinded leaf residue were extracted by soaking in 1 L distilled water at 20 ºC for 24 h with an effective shaking to give a concentration of 20 g dry tissue per litre (2 % w v^{-1}). The extract was filtered through two layers of filter papers (Whattman NO.1) to remove the plant debris. The supernatant was filtered again through Whatman NO.1 filter paper. Using the stock extract, different concentrations were prepared by subsequent dilutions with sterile distilled water to give the final concentrations of 0.5, 1 and 2 % w v^{-1}, while distilled water was used as control.

Germination and seedling growth

Germination test was conducted using the different concentrations of plant extract. The seeds of the target species were surface-sterilized with 5.25% w v^{-1} sodium hypochlorite solution for 15 minutes, rinsed twice with distilled water and placed on filter paper in sterilized 9 cm Petri dishes. Each Petri dish of 60 seeds receives 6 milliliters of the plant extract and distilled water for the control. All Petri dishes were placed in a dark chamber at 25 ± 2 ºC. All treatments were arranged in a completely randomized design with three replicates.

Total germination percentage was calculated using the germination data. This index represents the germination pattern in each treatment, The total germination percentage was calculated as G_T = (Nt × 100 / N), where Nt is the total number of germinating seeds after the incubation period and N is the number of seeds used in the bioassay. Measurement of both germination % and radicle and plumule lengths represents great significance in such experiment, as indicated by many authors (e. g., Hussain et al. 2011, Gomaa et al. 2014), and may reflect the effect of leaf litter on cover of the vegetation under field conditions. Radicle and plumule lengths were determined at the end of the experiment by measuring the most similar seedlings in each dish.

Some chemical constituents of *Bombax ceiba* leaves

Total soluble phenols and flavonoids

The total phenolics and flavonoids were measured according to Chun et al. (2003) and Zou et al. (2004), respectively. Three replicates were used for measurement. Standard calibration curves were constructed using different concentrations of gallic acid and rutin for determination of phenols and flavonoids, respectively. Total soluble phenols and flavonoids were expressed as µg gallic acid and rutin equivalents, respectively, per gram of dry leaf sample (µg g^{-1} dry weight).

DPPH Free Radical Scavenging Assay

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radicals scavenging activity of *B. ceiba* leaf extract was estimated according to the method explained by Cheung et al. (2003) with cerain modifications. Briefly, the crude extracts were suspended in methanol, and aliquots of 0.2 mM DPPH in methanol (160 µL) were mixed with each extract (40 µL, 0.01–1 mg mL^{-1}). The mixtures were left under subdued light for 10 min. The readings were monitored at 520 nm, using a UV-mini 1240 spectrophotometer (Shimadzu, Japan).The activity was expressed in µmol Trolox g^{-1} of sample dry weight. The calibration curve was constructed with the standard Trolox in the concentration range of 0 to 100 µM Trolox.

Amino acid profile

The free amino acids were measured according to Sinhaa et al. (2013).A 0.5 g dry *Bombax* leaves were homogenized by using a MagNALyser (Roche, Vilvoorde, Belgium) for 1 min, at 5000 rpm, in 5 mL 80% aqueous ethanol. The homogenate was centrifuged at 14,000 rpm for 20 min, the supernatant was evaporated under vacuum, and the pellet was re-suspended in 5 mL chloroform. Immediately, the residue was re-extracted with 1mL HPLC grade water using a MagNALyser, centrifugation was repeated (14,000 rpm for 20 min) and the supernatant was mixed with the pellet suspended in chloroform. Then, the mixture was centrifuged for 10 min at 14,000 rpm and the aqueous phase was filtered using Millipore micro filters (0.2 µM pore size) before assaying free amino acids levels. The amino acids were determined by using a Waters Acquity UPLC-tqd system (Milford, Worcester County MA, USA) equipped with a Sinhaa BEH amide 2.1 × 50 column.

Ascorbate and Tocopherols

Ascorbate content was determined by reversed phase HPLC separation, followed by UV detection according to the method described by Potters et al. (2004). Total antioxidant concentration (reduced +

oxidized) was determined after reduction with 0.04 M DTT for 10 min at room temperature, and the redox status was calculated as the ratio of the reduced form to the total concentration. Tocopherols were extracted with hexane, and measured according to the method proposed by Siebert 1999. The extract was dried under vacuum conditions (CentriVap concentrator, Labconco, KS, USA) and was re-suspended in hexane. Tocopherols were separated and quantified by HPLC (Shimadzu, Hertogenbosch, Netherlands) using normal phase conditions (Particil Pac 5 µm column material, length 250 mm, i.d. 4.6 mm). 5,7-dimethyltocol (DMT; 5 ppm) was used as an internal standard. Data were analyzed with Shimadzu Class VP 6.14 software provided by the HPLC system (Shimadzu, Tokyo, Japan).

Mineral profile

The dry leaf residueswere digested in a 5:1 ratio of HNO_3:H_2O in an oven. The macro-minerals and trace elements were determined by mass spectrometry (ICP-MS, Finnigan Element XR, Scientific, Bremen, Germany) according to Agusa et al.(2005). A mixture of standards was prepared in 1% nitric acid.

Statistical analyses

Data due to laboratory bioassay were analyzed using the SPSS Statistics software package, version 20 (IBM Corporation, USA) at $P \leq 0.05$ as a probability level. The Kolmogorov-Smirnov test was applied to check the data for normality and homoscedasticity, respectively. The normal data were analyzed by the parametric statistic, using one-way ANOVA. When the ANOVA revealed significant differences, we used the results of the Tukey's test for multiple comparisons of means of germination and the seedling growth. The data that were not normal were analyzed by nonparametric statistics, i.e., Kruskal-Wallis test.

RESULTS

Laboratory bioassay

The overall results indicated that the aqueous extract of the *Bombax* leaf litter reduced germination and seedling growth of the target species. Besides, the inhibitory effect was proportional to the extract concentration (Fig. 1). The lowest concentration of the extract significantly reduced germination of *A. viridis* and *M. indicus*. In addition, the highest inhibition was recorded for the former species at the extract concentrations 10 and 20 % (w/v) by 75.7 and 90.1 %, respectively. The lowest concentration also suppressed the radicle growth of only *A. viridis* if compared with the remaining species. Such concentration had no significant effect on shoot lengths of all test species. The inhibitory effect of the remaining concentrations was again more pronounced for *A. viridis* (Fig. 1). The general properties of the extracts were illustrated in Table 1. pH and osmotic potentials of the different concentrations varied significantly and gradually with respect to the extract concentration.

Table1: The properties of the different concentrations of the *Bombax ceiba* leaf extracts

Extract concentration (% w v^{-1})	pH	Osmotic Potential (Osm kg^{-1} H2O)
0	7.09a ± 0.04	0.015a ± 0.0010.
0.5	6.38b ± 0.072	0.058b ± 0.002
1	5.91c ± 0.035	0.122c ± 0.006
2	5.81c ± 0.04	0.180d ± 0.008

Phytochemical constituents of *Bombax* leaves

With respect to the amino acids detected in the leaves of *B. ceiba*, a total of fourteen free amino acids were found, amongst which lysine and glutamine were abundant (644.61 and 321.55 mg g^{-1} dry weight, respectively) (Table 2). Besides, the leaf residues contained moderate amounts of asparagine, phenylalanine and glutamate (42.05, 27.1 and 16.4 mg g^{-1} dry weight, respectively).The remaining amino acids were recorded as very few or traces (Table 2).

Table 2: Concentrations of the different amino acids (µg / g dry weight) detected in the leaves of *Bombax ceiba*

Amino acid	Content (mg g^{-1} dry weight)
Alanine	0.032±0.005
Arginine	0.014±0.003
Asparagine	42.05±13.35
Glutamate	16.40±1.43
Glutamine	321.55±16.94
Histidine	2.41±0.65
Isoleucine	0.2±0.03
Leucine	1.25±0.22
Lysine	644.61±174.15
Methionine	1.261±0.24
Ornithine	2.28±0.29
Phenylalanine	27.10±6.98
Threonine	0.27±0.05
Valine	1.13±0.36

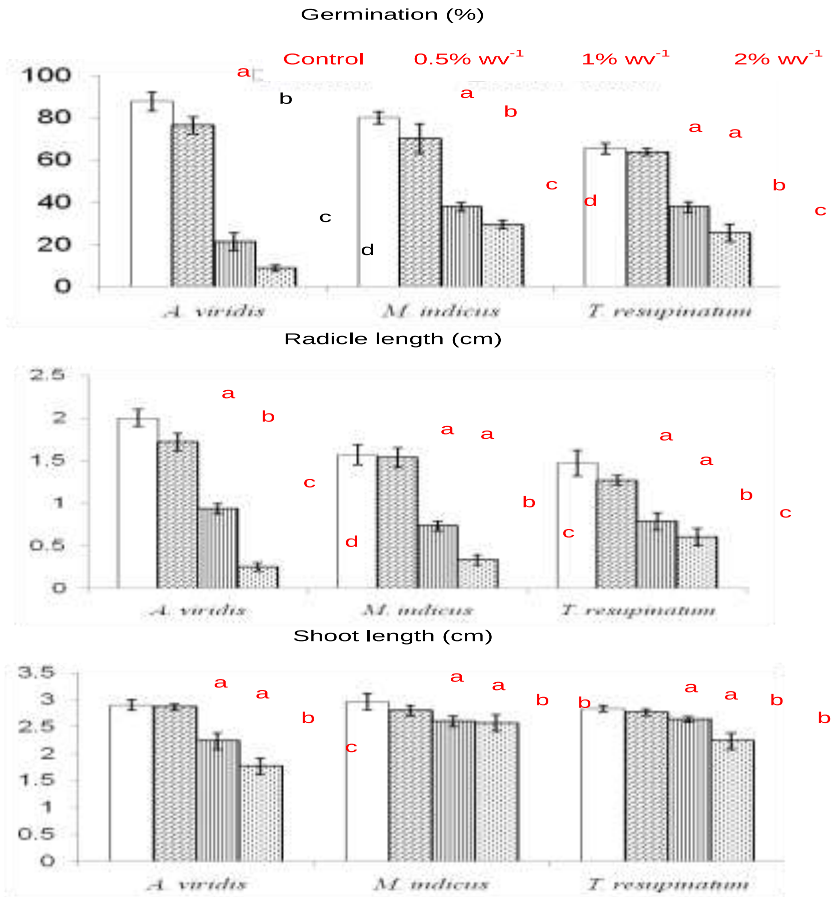

Figure 1: Effect of the aqueous extract of *B. ceiba* leaf litter on (a) germination (%), (b) radicle and (c) shoot lengths (cm) of the target species. Means of the same letter within the same species are not significantly different at p ≤ 0.05. Bars represent the standard deviations with three replicates.

The leaves of *B. ceiba* contained significant amounts of minerals (Table 3). In particular, potassium constituted the highest amount (387.43 mg 100g $^{-1}$ dry weight). In a descending order, phosphorus and magnesium were of moderate content (67.38 and 49.44 mg 100g $^{-1}$ dry weight, respectively). The contents of the remaining elements were less than unit.

Table 3: The mineral profile of the leaves of *B. ceiba*

Element	content (mg 100 g^{-1} dry weight)
K	387.43±5.84
Ca	0.47±0.041
Mg	49.44±3.01
P	67.38±4.57
Na	5.4±0.60
Cu	0.77±0.062
Fe	0.32±0.025
Mn	0.24±0.015
Zn	0.92±0.06

The total amount of the ascorbate content in the analysed leaves was 42.3 µg g^{-1} dry weight (Table 4). The total tocopherols detected were 1502.75 µg g^{-1} dry weights. α-tocopherols constituted the majority of them (1018.24 µg g^{-1} dry weight) (Table 4).

Table 4: The contents of total ascorbate, tocopherols, phenols, flavonoids and antioxidant activity of *B. ceiba* leaves

Compound	Content (µg g^{-1} dry weight)
Vitamins	
Vit C (total ascorbate)	2401.77±215.68
Vit E	
α-Tocopherols	1018.24±184.88
β-Tocopherols	215.76±39.74
ɣ-Tocopherols	246.04±42.61
δ-Tocopherols	22.71±5.67
Total Tocopherols	1502.75±272.90
Total phenols	3367.6± 534.77
Total flavonoids	4896.3± 37.82
Total antioxidant activity (µmol Trolox g^{-1} dry wight)	11.147±0.32

The spectrophotometric analyses also revealed the presence of appropriate amounts of phenolics and flavonoids (3367.6 and 4896.3 µg g^{-1} dry weight, respectively). The antioxidant activity of *B. ceiba* leaf extract was 11.147 µmol Trolox g^{-1} dry wight.

DISCUSSION

The present study showed that the aqueous extract of *Bombax ceiba* leaf litter significantly reduced germination and seedling growth of the tested weeds. This effect could be attributed to some phytotoxins in the leaf litter. Some flavonoids such as apigenin, isovitexin, kaempferol and quercetin, and other glycosides were detected in *B. ceiba* (Refaat et al. 2012). Moreover, the spectrophotometric analysis confirmed that the leaf litter contained considerable amounts of phenolics and flavonoids. These compounds were recorded as allelochemicals that may inhibit germination and growth of the target species (Hiag, 2008). Increasing the inhibitory effect by increasing the extract concentration is in accordance with previous studies (Gomaa and AbdElgawad, 2012; Gomaa et al. 2014). The response of an organism to chemical compounds depends on the dose (Belz and Hurle, 2005). The results showed also that rootsof the target species were more sensitive to the extract that shoots. This result was consistent with other studies (e.g., Qasem, 1995; Gomaa et al. 2014; Hassan et al. 2014).The strong inhibitory effects of extract on roots might be related tothe direct contact of the extract with roots and subsequently with inhibitory effect of the allelochemicals as described in earlier works with various crops and weeds (Qasem, 1995). The results confirmed that *Amaranthus viridis* was more affected by the extract if compared with the other species. This result could be attributed to seed size. Considerably, it was demonstrated that small-seeded plants are more affectedby the phytotoxins than the larger-seeded ones (Liebman and Sundberg, 2006). On the other hand, the extract may also exert negative osmotic effects on the test species (Souza et al. 2010; Gomaa et al. 2014). Generally, reduction in the seedling growth of the target weeds may be attributed to reduced cell division of the seedlings, because the phenolic compounds could inhibit cell division and alter the ultrastructure of the cells (Li et al. 2010). On the other hand, the osmotic potentials and pH of the different concentrations of the extract varied. Gomaa et al. (2014) showed that 0.1 Osm kg^{-1} H_2O inhibited germination and growth of *Melilotus indicus* and other weeds. Therefore, it is worth mentioning that the osmotic potential of *B. ceiba* leaves had a negative effect on germination and growth of the target species. Higher osmotic potentials of the extract could be attributable to high potassium contents of the leaves. Although pH of the solutions varied, their values lies on normal ranges for germination of seed plants.

Amino acid composition revealed that the leaf litter of *B. ceiba* contained the majority of essential amino acids: lysine, isoleucine, leucine, methionine, threonine, valine, histidine and phenylalanine. The litter was rich in lysine and glutamin. Their contents in the litter were relatively higher than that for other African staple cereals (rice, maize, millet and sorghum) (World Health Organization 1985). They are also exceeding the amino acid content in hungry rice (*Digitaria exilis*) as a cereal crop cultivated in West Africa (Glew et al. 2013). Furthermore, these amino acids constituted higher contents relative to some amino acids in some varieties of dates in KSA (Hamad et al. 2015).

The nutrient profile of this study showed that the litter was rich in minerals. The contents of K, Fe, and Cu in the litter were relatively higher than that recorded in the brown rice (Marles, 2017). Besides, the K content of the litter was higher than that found in some varieties and cultivars of dates (Mohamed et al. 2014; Assirey 2015; Hamad et al. 2015). Also, such potassium content was higher than that found in some prepared Ghanaian foods (Annor et al. 2016). When compared with the hungry rice in West Africa, the sampled *Bombax* leaves were more rich in K, Na and Cu (Glew et al. 2013).

The analyses confirmed the presence of considerable amounts of vitamin C and E (tocopherols). In comparison with some cruciferous vegetables, vitamin C content in *Bombax* leaves was higher (Kapusta-Duch and Leszczyńska, 2013). In this study, α-tocopherol was the main contributor in in total tocopherol content. This result was consistent with Lobo et al. (2010) and Hamad et al. (2015). As far as we could ascertain, there are no previous reports measuring ascorbate and tocopherol contents in the leaf litter of *B. ceiba*. The content of tocopherols in the litter may exceed that was found in many genotypes of wheat (Lampi et al. 2008).In addition to phenolics and flavonoids, ascorbic acid and tocopherols are considered as strong antioxidant molecules that play an important role against oxidative stress and also have nutritional value (Gill and Tuteja, 2010).

Based on the result using the DPPH method, the leaves of *B. ceiba* recorded a considerable antioxidant activity. Such activity could be attributed to the leaf content of phenolics and flavonoids. Tiveron et al. (2012) confirmed the positive correlation between the phenolics and the antioxidant activity. Moreover, Enujiugha et al. (2012) attributed the free radical scavenging and antioxidant properties of the African yam bean seeds to their contents of phenolics and flavonoids. When compared to some Brazilian vegetables, the phenolic content and the antioxidant activity were relatively more abundant in the leaves of *B. ceiba* (Tiveron et al. 2012).

CONCLUSIONS

The present study may be the first to assess the phytotoxic potential of *B. ceiba* leaf litter, and it certainly provides a database for some nutritional values of *Bombax* leaves. Phytotoxicity could be attributed to some phenolic and flavonoid compounds that may reduce germination and seedling growth of the target weeds. A further study may be necessary to evaluate the allelopathic potential of *B. ceiba* under field conditions.

This study therefore represents a potent to use the leaf litter of this species as a bioherbicide. As they rich in amino acids, potassium, and antioxidant molecules (phenols, flavonoids, vitamin C and E), the leaves of such tree may be used as a dietary supplement.

Supplementary materials

The supplementary material / supporting for this article can be found online and downloaded at: https://www.isisn.org/article/

Author contributions

Mahmoud Omar Hassan[1]and Sanad M. contribute equally

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Conflict of interest

The authors declared that present study was performed in absence of any conflict of interest.

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