Herbicidal Activity of Mimosine and Its Derivatives

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Additional information is available at the end of the chapter

1. Introduction

Mimosine [β-[N-(3-hydroxy-4-oxypyridyl)]-α-aminopropionic acid] is a non-protein amino acid, and is a major compound present in all plant parts of *Mimosaceae*, which includes Leucaena (*Leucaena leucocephala*), *Leucaena glauca*, and other legumes belonging to *Mimosa* spp.. Structurally, mimosine is an analog of dihydroxyphenylalanine with a 3-hydroxy-4-pyridone ring instead of a 3,4-dihydroxy-phenyl ring (Fig. 1). Although Leucaena has a rich protein content and high annual yield, the presence of mimosine has limited the wide use of this plant as animal feed. This compound causes alopecia, growth retardation, cataracts and infertility in animals [1]. Mimosine can be degraded to DHP [3-hydroxy-4(4H)-pyridone] (Fig. 2) by microorganisms in the rumen and bacteria in rhizome nodules of Leucaena, by endogenous enzymes in the Leucaena plants, or by HCl hydrolysis. Although DHP is also toxic, it exerts lower toxicity than mimosine [2]. Mimosine possesses antimitotic activity that blocks the cell cycle in the large G1 phase [3] and inhibits DNA synthesis, which prevents the formation of the replication fork by altering deoxyribonucleotide metabolism [4]. The amino acid may also act as a tyrosine analogue which incorporates biologically vital proteins and, in turn, causes hair loss [1].
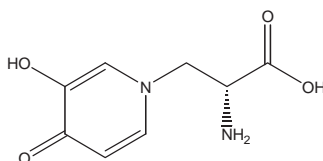

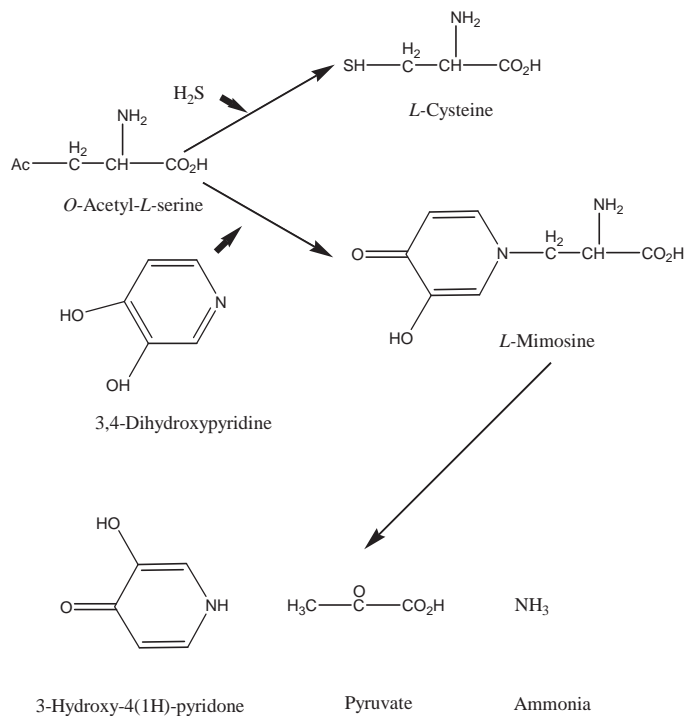**Figure 1.** Chemical structure of mimosine

Figure 2. Metabolic relations between cysteine, mimosine and DHP [3-hydroxy-4(1H)-pyridone] [41]

Mimosine has been known as an allelochemical, which is responsible for the strong allelopathic activity of Leucaena and *Mimosa* spp., by suppressing growth of plants and plant fungi [2,5]. Our previous work examined the possibility of exploiting higher plants selected from the plant ecosystems in Southeast Asia for paddy weed control, of which *Leucaena glauca* was among those which reduced paddy weeds up to 70% and increased rice yield by 20% [6]. We concluded that mimosine was responsible for significant weed reduction of *L. glauca* in paddy fields, and rich nutrients contained in plant materials of the legume also contributed to the increase of the rice yield.

Because mimosine is a compound that is responsible for many interesting biological activities as mentioned above, many works have been conducted to purify mimosine, typically from Leucaena because it contains higher mimosine content than other species of Mimosaceae. However, most methods published so far have been too complicated and costly to successfully

yield mimosine in highly pure grade. Our group has already developed a simple method to purify mimosine at an industrial scale by the use of ion exchange resin. Although mimosine shows herbicidal and antifungal activity, the synthesis of mimosine-derived compounds is indispensable because of the need to find mimosine derivatives with stronger suppression against pests and fungi. Synthesized compounds exerting strong activity and require a simple process for synthesis will be selected for development of novel bioactive pesticides. However, very little success with synthesis of mimosine derivatives has been reported.

This paper describes the allelopathic interaction of mimosine as well as its efficacy in application for biological control of weeds and pests. The analytical and purified methods of this compound developed in our laboratory and the synthesis of its derivatives and their suppressive activities against plants and fungi are also demonstrated.

2. Discovery of mimosine

Mimosine was first isolated from the sap of *Mimosa pudica* by Renz [7] and was given the name "mimosine". Later, minosine was biologically characterized from *M. pudica* by Nienburg and Taubõck [8]. From the extraction of ground *Leucaena glauca* seeds, Mascré [9] successfully isolated an optically inactive crystal line solid, m.p.287°C named "leucenol". Its empirical formula was elucidated to be $(C_4H_5O_2N)\chi$, and further experimental research has shown that it contains an α-amino acid including a phenolic hydroxyl. Bickel and Wibaut [10] named leucaenol and concluded the formula of mimosine was $C_8H_{10}N_2O_4$; it was chemically synthesized by Adams and Johnson [11]. Wilbaut and Klipool [12] isolated mimosine from *Leucaena leucocepphala* which they named "leucaenine" and verified that the three different substances are analogs. The chemical structure of mimosine was determined by Bickel [13] as β-N-(3-hydroxy-4pyridone)-α-amino propionic acid. The structure of mimosine is similar to dihydroxyphenylalanine with a 3-hydroxy-4-pyridone ring instead of a 3,4-dihydroxy-phenyl ring (Fig.1).

3. Content of mimosine

Mimosine exists in large quantities in leaves, pods and seeds of tropical legumes of the genus *Leucaena.* This compound is present in a much greater quantity in Leucaena than in *Mimosa* [14]. The Leucaena hybrid has lower mimosine content than the original *Leucaena leucocephala* [15]. In the non-hybridized Leucaena legume plant, mimosine accounted for 2-5% of fresh weight, and the level of concentration could increase to 10% in young leaves[16]. Small amounts of mimosine were in the nodules and the root exudates of Leucaena as well. The seed, stem, pod, and leaf tissue of different Leucaena species contained 1-12% mimosine, whereas the highest amount of mimosine was found in growing tips of Leucaena [14,16,17]. More recently, Xuan et al. [5] noted that all plant parts of Leucaena contained mimosine; however, the amount of mimosine in the young leaves and mature seeds was the highest, varying from

2.4 to 2.7% of the fresh weight, whereas the lowest mimosine content was in the root xylems and xylems (0.11 to 0.18%, respectively). Our research team did not find mimosine content greater than 5% in any plant parts of Leucaena observed in previous reports [5].

The quantity of mimosine in Leucaena plants is species dependent. *Leucaena leucocephala* has a medium level of mimosine, whereas, *L. collinsii, L. diversifolia, L. escuienta, L. greggii,* and *L. pallida* have low mimosine content [18]. In addition, leaf size also showed different mimosine concentrations, with smaller-leaved species having lower mimosine content [18]. The afore-mentioned evidences suggest that mimosine concentration may be related to the genetic variation in Leucaena species. Among ecotypes, content of mimosine and DHP in the dry season were higher than in the rainy season [19,20]. The main difference may involve the anti-nutritional factors. The concentration of mimosine contained in Leucaena plants also fluctuates with the time of year and is proportionally related to growth rate. For example, better growth of Leucaena leads to higher mimosine content [19]. Moreover, some abiotic factors of envi-ronmental stress such as drought and moisture stress can dramatically increase mimosine levels in both new and old leaves [21].

4. Purification of mimosine

Although mimosine levels are high in Leucaena, it is not easy to isolate pure mimosine. The determination of mimosine via extracting solvents and analytical instruments and spectro-photometrics was conducted [22,23]. However, it is complicated to separate mimosine from other amino acids in Leucaena, the cost of mimosine purchased from chemical companies is rather high. In our laboratory, mimosine can be easily purified at an industrial scale by the use of ion exchange resin. By this process, 6 kg of freshly harvested Leucaena leaves were immersed in 30 L of boiling water for 10 min. The water extract was cooled to room temperature and filtered with 300 mesh sieve. Ultrafiltration was carried out at 4 atm, 30°C and 700 rpm using a Filtron Miniset Omega equipped with the cassette system membrane. The filtrate was passed through a column packed with acid form Amberlite IRA (technical grade). The resin was washed with 1 L of 2N NH_4OH. About 30 g of relatively pure mimosine was obtained after adjusting the pH to 4.5~5.0. We have examined various conditions for mimosine purification and observed that the type of ion exchange resin and adjustment of pH are crucial conditions to obtaining the maximum quantity and high purity of mimosine (5 g per 1 kg fresh Leucaena leaves, purity>95%).

5. Mimosine acts as an allelochemical

Mimosine is considered as an allelochemical and is responsible for the allelopathic activity of the *Leucaena* genus and other species belonging to *Mimosa* spp. Leucaena is popular in intercropping with annual crops, using as a hedgerow, and alley cropping for yield promotion and weed control [24]. In bioassays, this compound exerted inhibition against seedlings of

mung bean (*Phaseolus aureus*) [25,26], lettuce [27,28]; hemp sesbania (*Sesbania exaltata*), ryegrass (*Lolium perenne* L), sicklepod (*Senna obtusifolia*), wheat (*Triticum aestivum*)[29], and rice (*Oryza sativa*)[28,30]. Similar to other phytotoxins, effects of mimosine against plant germination and growth are proportional to applied doses. Chou and Kuo [28] indicated that at 20 ppm, mimosine significantly suppressed growth of lettuce, rice and ryegrass; however, *Miscanthus floridulus* and *Pinus taiwanensis* were not inhibited by the mimosine at 200 ppm. Mimosine exhibited selective influence against the germination and growth of certain indicator plants including hair beggarticks (*Bidens pilosa* L), creeping grass (*Mimosa pudica* L), cabbage (*Brassica rapa*), Italian ryegrass (*Lolium multiflorum* L), and kidney bean (*Phaseoulus vulgaris* L) at 50-100 ppm. However, the effect of mimosine was the lowest against plants which are mimosine producers (*M. pudica* and *L. leucocepphala*) [5].

Mimosine also shows selective influence against certain bacteria and fungal growth. Some bacteria were inhibited, whereas growth of several bacteria was promoted by mimosine. Soedarjo and Borthakur [31] reported that growth of some root nodule bacteria was inhibited by mimosine. In contrast, some Leucaena-nodulating *Rhizobium* strains could utilize mimosine as a source of carbon and nitrogen. *Rhizobium* sp. strain TAL 1145 is such a strain that can catabolyze mimosine, which provides it a competitive advantage for nodulation of Leucaena [17]. Tawata et al. [32] revealed that *Escherichia coli* Crooks (1222) growth was inhibited by mimosine, but increased by DHP. *Aerobacter aerogenes* (1232) growth was increased by both mimosine and DHP. *Coryne bacterium psudodiphterium* (1471) growth was inhibited by DHP, but increased by mimosine.

There were 38 unknown microorganisms collected from the Leucaena population growing around Campus of University of the Ryukyus, Okinawa, Japan, including 12 from roots, 13 from top soil, and 8 from deep soil, and the remaining was from Leucaena stems; they were examined against mimosine and DHP. Among the unknown microorganisms, fungus D6-31 growth was inhibited by DHP, but increased by mimosine, whereas that of fungus D6-30 was inhibited by mimosine, but increased by DHP. The population of fungus D6-27 was dramatically increased by both mimosine and DHP, however, that of fungus D3-6 was inhibited by both mimosine and DHP. These four unknown fungi were selected for future research [32]. Other reports such as Murugesan and Radha [33] demonstrated that mimosine inhibited growth of bacteria and fungi, including *Alternaria* sp., *Cercospora canescens*, *Colletotrichum indemuthianum*, *Diplodia natalensis*, *Sclerotium rolfsii*, *Dreschlera oryzae*, and *Rhizoctonia solani*. Anitha et al. [34] noted that mimosine was toxic against fungi rather than bacteria.

On the other hand, mimosine released from rhizomes and foliated leaves to soil caused inhibition of plants in the vicinity of Leucaena [5,20]. Soils amended with mimosine retarded growth of *Brassica rapa* [5]. Hong et al. [6] evaluated the potential of weed suppression of various plants collected from plant ecosystems in Southeast Asia. Several species showed the potential for weed suppression up to 70% and increased rice yield to 20%, including *Leucaena glauca*. Because of its weed suppression and rich nutrients as well as the wide adaptation of Leucaena in the tropics, the biomass of this plant is useful for weed control and serves as a source of natural fertilizer.

6. Synthesis of mimosine and its derivatives

Mimosine toxicity is ascribed to the presence of –OH and –O in the pyridine ring and known to suppress iron-containing enzymes and compete with tyrosine [35]. The characteristic activity of growth inhibitory properties of mimosine is a hydroxyl group α to the oxo function of the pyridone ring (Fig.1). The location of the amino acid side chain seems to be less critical and an isomer (Fig.1). The synthesis of two mimosine isomers with the position of the α-hydroxy-oxo function in the pyridine ring of mimosine was at least as active *in vitro* and *in vivo* as the natural amino acid [36]. The constituent properties of the α-hydroxy-oxo group are involved in the biological activity of mimosine and other systems and may play a key factor in growth suppression [25,37-39]. The structure of the heterocyclic ring in mimosine is possible to modify the chelate properties of the molecule and their biological activity which could lead to the design of a mimosine analogue [36].

Even though mimosine shows a great potential as an allelochemical, it is difficult to apply this amino acid as a natural herbicide because it may be unstable in natural conditions. Mimosine can be easily degraded in soil by soil factors such as nutrients, minerals, pH and microorganisms. Therefore, synthesis of mimosine derivatives with stronger activity and greater stability is needed. Although many interesting experiments on mimosine have been conducted, very sporadic work on the synthesis of mimosine-derived compounds has been carried out and reported. This is the first synthesis of propionates as mimosine derivatives and was carried out in our laboratory [32].

6.1. Synthesis of propionates as mimosine derivatives

Each of 2-hydroxypyridine (material A) and 4-hydroxypyridine (material B) were well blended with each 12 different acrylates (Fig. 3), at 90-110°C for 4-6 h to receive oily substances with deep yellow and brown color (Fig. 4). The reactive products were applied to TLC for purification. Solvents of TLC were benzene: methanol (1:1 or 1: 2, v/v). Yielded compounds were recovered by methanol and subsequently subjected to ^{1}H-NMR, ^{13}C-NMR and IR to determine their chemical structures. The synthesized propionates are shown in Fig. 5. Herbicidal and antifungal activities of the propionates were examined against growth of *Brassica rapa* and two noxious fungi *Schlerotium dellfinii* and *Rhizoctonia solani* at 100 ppm, respectively.

6.2. Herbicidal activity

Among synthesized propionates, two compounds including A2 and B2 [chloro-3-(2-oxohydoropyridyl) and chloro-3-(4-oxohydoropyridyl) propionates] exhibited the strongest herbicidal activity against growth of *B. rapa* (50-70% of inhibition) (Fig. 6). On the other hand, lengths of radicle and hypocotyl were either promoted or inhibited by the propionates A3, A4, A11, B4, B5, B6, B11, and B12. The other compounds reduced growth of *B. rapa* by lower magnitudes (20-40%). The chloric group in the two propionates A2 and B2 may be responsible for the greater herbicidal activities than other compounds. However, none of these synthesized propionates could exert stronger herbicidal activities than mimosine, which showed a 80-90% inhibition.
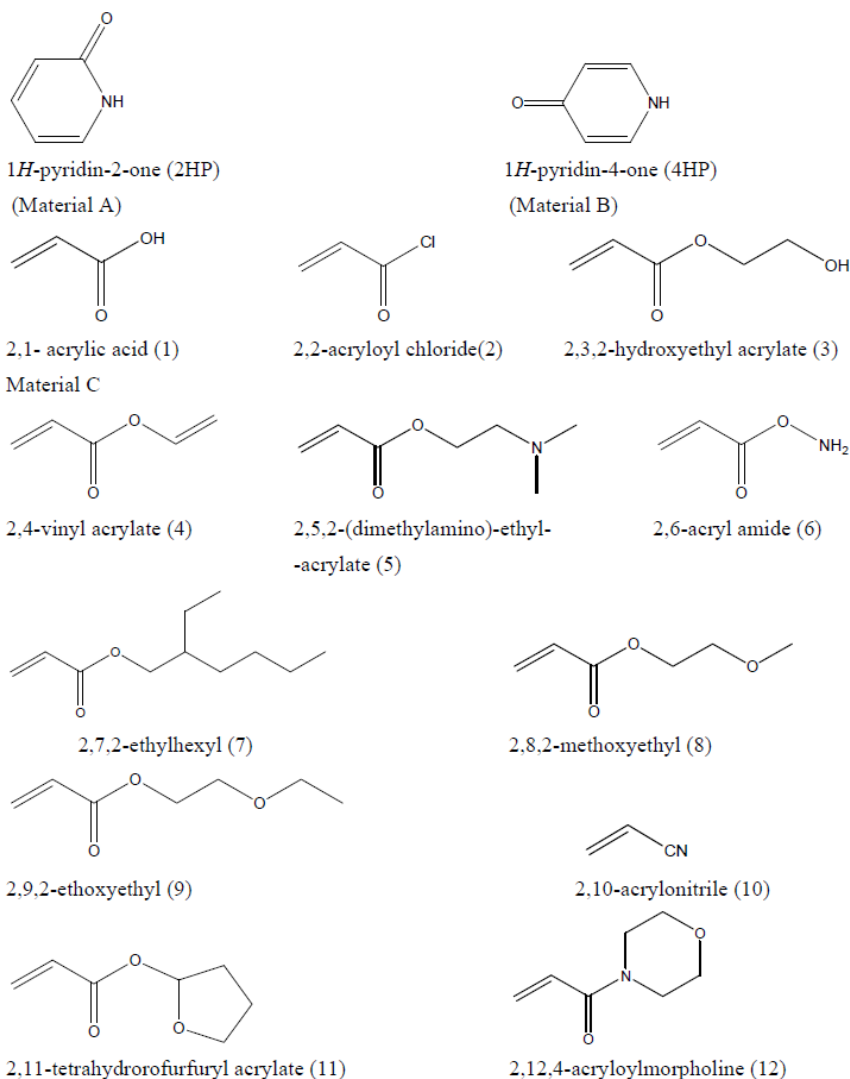
1H-pyridin-2-one (2HP)
(Material A)

1H-pyridin-4-one (4HP)
(Material B)

2,1- acrylic acid (1)
Material C

2,2-acryloyl chloride(2)

2,3,2-hydroxyethyl acrylate (3)

2,4-vinyl acrylate (4)

2,5,2-(dimethylamino)-ethyl-
-acrylate (5)

2,6-acryl amide (6)

2,7,2-ethylhexyl (7)

2,8,2-methoxyethyl (8)

2,9,2-ethoxyethyl (9)

2,10-acrylonitrile (10)

2,11-tetrahydrorofurfuryl acrylate (11)

2,12,4-acryloylmorpholine (12)

Figure 3. Chemical structures of materials for propionate synthesis

6.3. Antifungal activity

The fungal activity varied among the mimosine derivatives. The compounds A1, A2, A11, B6, and B8 were the most inhibitive against both *R. solani* and *S. dellfinii* (50-70% inhibition) (Fig. 7), whereas there were 5 propionates B3, B4, B5, B11, and B12 that stimulated growth of the two fungi up to 20%. Growth of *R. solani* and *S. dellfinii* were either stimulated or suppressed
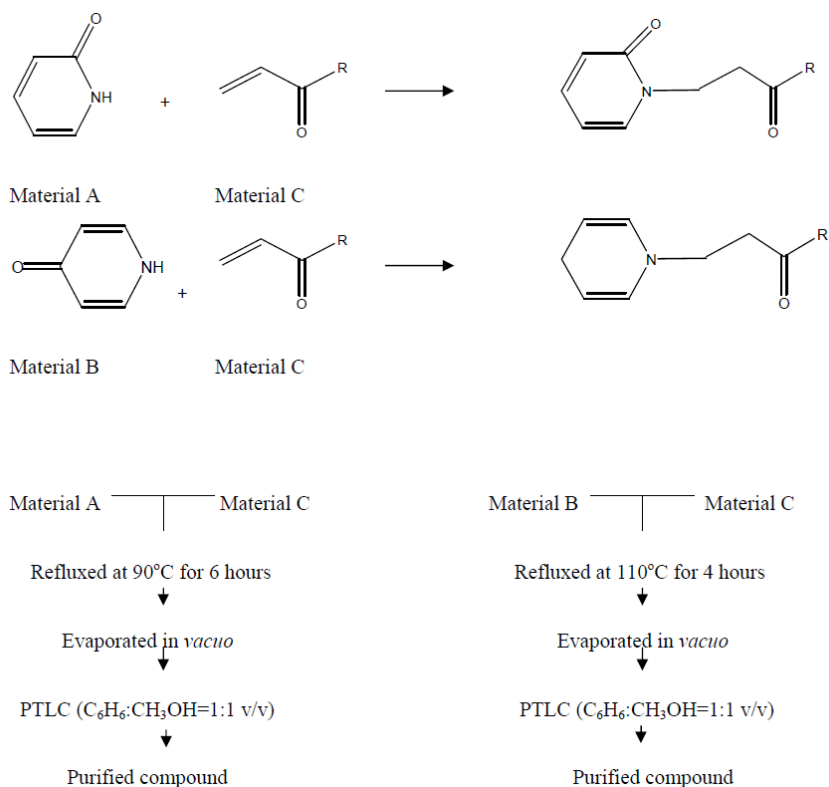
Figure 4. Synthesis route of propionates

by A3 and A4. The other propionates exerted fungal activity by 10-40%. The compounds chloro-3-(2-oxohydoropyridyl) and chloro-3-(4-oxohydoropyridyl) propionates (A2 and B2) showed good antifungal activity, whereas the chloro-3-(4-oxohydoropyridyl) propionate exhibited weak suppression of *R. solani* (about 10% inhibition). The two compounds, A2 and B2, were the most potential among synthesized propionates for obtaining herbicidal and antifungal activities. Mimosine did not show any effects against *S. dellfinii*, but inhibited growth of *R. solani* by about 30%. The antifungal strength of these synthesized propionates was greater than that of mimosine, with the exception of compounds A4, A8, B4, B5, B6, B11, and B12 (Fig. 7).
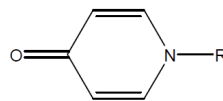
Several compounds among the synthesized propionates from this research showed good herbicidal and antifungal activities. In general, antifungal activity of these propionates was greater than their herbicidal activity. The most promising compounds were chloro-3-(2-oxohydoropyridyl) and chloro-3-(4-oxohydoropyridyl) propionates.

A   B
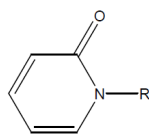
Synthesize propionates

A1, B1: R= 2,1- acrylic acid	A2, B2: R= 2,2-acryloyl chloride
A3, B3: R= 2,3,2-hydroxyethyl acrylate	A4, B4: R= 2,4-vinyl acrylate
A5, B5: R= 2,5,2-(dimethylamino)-ethyl-acrylate	A6, B6: R= 2,6-acryl amide
A7, B7: R= 2,7,2-ethylhexyl	A8, B8: R = 2,8,2-methoxyethyl
A9, B9: R= 2,9,2-ethoxyethyl	A10, B10: R = 2,10-acrylonitrile
A11, B11: R= 2,11-tetrahydrorofurfuryl acrylate	A12, B12: R= 2,12,4-acryloylmorpholine

Figure 5. Chemical structures of synthesized propionates

7. Analytical determination of mimosine

Paper and thin layer chromatography were used to identify mimosine [28]; however, mimosine content could not be quantified. Gas-liquid chromatography, liquid chromatography, and reversed-phase ion-pair high-performance liquid-chromatography were also applied for mimosine determination. However, these methods require elaborate preparation of samples, but with no appreciable improvement in the range of sensitivity [23]. Other methods were the coupling of mimosine with *p*-nitroaniline [22] or mimosine with N-1(naphthyl)ethylenedia-mine (NEDA) forming a pink-colored azodye with an absorbance of 540 nm [23], and the use of indirect spectrophotometricity which is based on its reaction with diazotized sulfanilamide (DZSAM). These methods were reported to increase the sensitive estimation of mimosine. A useful HPLC system to determine mimosine and DHP contents that influenced *Rhizobium* isolates was reported by Soedarjo et al. [40]. They applied a C18 HPLC column, UV detection at 280 nm, a solvent system of 0.2% orthophosphoric acid to detect mimosine and DHP at 2.7 and 4.8 min, respectively.

Our laboratory also developed a simple method using HPLC to determine mimosine and DHP. This method is nottime consuming, uses simple reagents and procedures, and has a high level of accuracy. Of which, the HPLC system includes an 880-PU pump and column (Fine pak Sil C18, Nihonbunko company). The mobile phase employed was a mixture solution of 10 mM potassium-dihydrogen phosphate, 10 mM phosphoric acid, acetonitrile (45:45:10), and finally, 0.1% sodium 1-octanesulfonate was added to the mixture as the surface active agent. The flow rate was 1.5 mL per min. Mimosine and DHP were detected at a wave length of 280 nm. The fresh samples from Leucaena (leaves, stems, or roots) were boiled for 10 min, cooled at room temperature, centrifuged, filtered and injected into HPLC at 2-5 μL. The peaks of mimosine
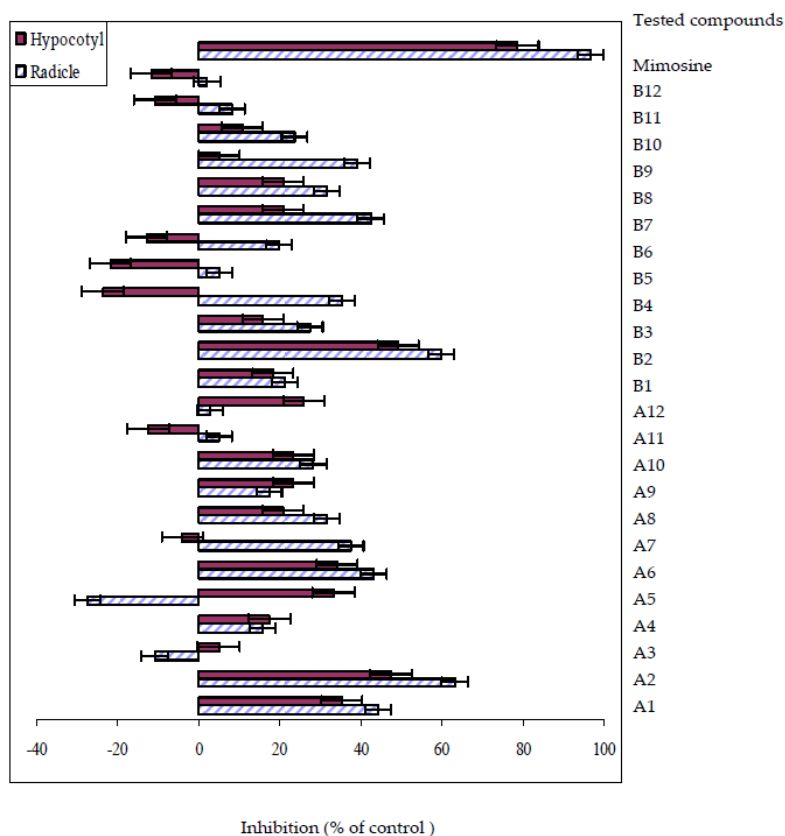
Figure 6. Herbicidal activity of mimosine and its propionate derivatives against *Brassica rapa* (100 ppm)

and DHP appear at 2.5 min and 7.5 min retention time, respectively. However, these retention times varied among columns and HPLC conditions.

8. Conclusion

Mimosine is a major secondary metabolite in Leucaena and Mimosaceae plants and is responsible for the biological activities of these plants. Its allelopathic interaction includes both inhibition of plants, fungi, and bacteria, and stimulation of several strains of bacteria. The biomass of Leucaena in the tropics is a potential source for reduction of weed emergence in paddy fields and simultaneous utilization as green manure. Although we have synthesized several propionates which exerted potent antifungal activity, further synthesis should be
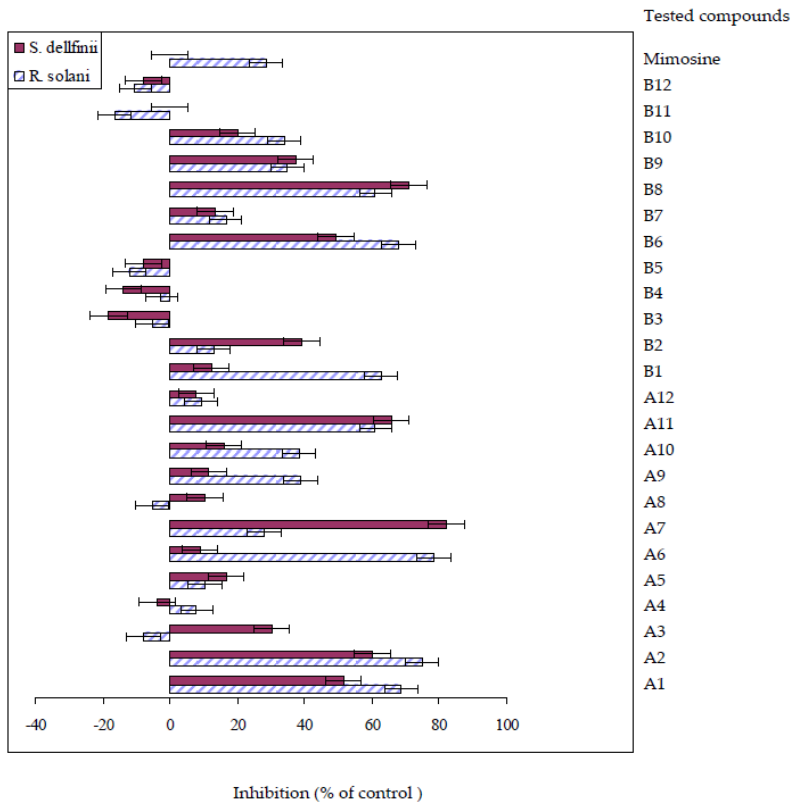
Figure 7. Antifungal activity of mimosine and its propionate derivatives (100 ppm)

continued to yield novel derivatives of mimosine which can obtain stronger inhibition on plant growth than their parent, mimosine.

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