
The molecular and chemical profiling of *Ageratum conyzoides* as organic fertilizer: A comprehensive nuclear study

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Abstract *Ageratum conyzoides* (Babandotan) is an invasive tropical weed that, despite its allelopathic and noxious characteristics, holds potential agricultural value as a feedstock for compost-based organic fertilizer. However, the detailed molecular-level information on the humic acid derived from *A. conyzoides* compost remains limited, as previous studies have primarily focused on bulk functional group characterization. The molecular composition of humic acid extracted from composted *A. conyzoides* using ¹³C nuclear magnetic resonance (NMR) spectroscopy is elucidated. The resulting compost exhibited a near-neutral pH (6.09 ± 0.08), high total organic carbon content ($40.2 \pm 1.05\%$), and a carbon-to-nitrogen ratio of 24.9 ± 1.2 . ¹³C NMR spectra revealed five major carbon regions, for instance aliphatic, carbohydrate, aromatic, carboxyl, and carbonyl with aromatic, carbohydrate, and carbonyl carbon representing the dominant fractions, indicating an advanced degree of humification. The combined presence of recalcitrant aromatic structures and carboxyl/carbonyl functional groups suggested that *A. conyzoides*-derived humic acid possesses both long-term carbon stabilization potential and nutrient-chelating capacity relevant to organic fertilizer application. These findings are supported the feasibility of converting this invasive weed into a value-added organic soil amendment, contributing to sustainable weed management in tropical agricultural systems.

Keyword: ¹³C NMR, Carbon stabilization, Compost, Humic acid, Soil amendment

Introduction

Ageratum conyzoides (locally known as Babandotan) is an herbaceous weed native to Central and South America that has become widely naturalized throughout the tropical and subtropical regions of Southeast Asia (Babu Shrestha *et al.*, 2016; Baral *et al.*, 2022). Characterized by its fast growth, hairy stems and leaves, and small purple-to-white flowers, this species is generally regarded as an aggressive and noxious weed that readily colonize backyards, roadsides, and various agricultural plantations, often resulting in considerable crop losses (Gusain and Suthar, 2020; Wang *et al.*, 2011). *Ageratum conyzoides* is also known to produce several toxic

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allelochemicals, including stigmasterol, dimethoxy-ageratochromene, and caryophyllene, including stigmasterol, dimethoxy-ageratochromene and caryophyllene, which contribute to its allelopathic and invasive behavior. Despite its negative agronomic impact, *A. conyzoides* also contains valuable secondary metabolites such as benzofurans, coumarines, and flavonoids, and has been explored for several beneficial application, including essential oil extraction for plant protection and its uses as organic fertilizer through composting (Chahal *et al.*, 2021; Maturi *et al.*, 2024).

During composting, organic residues undergo microbially mediated transformation into more chemically stable compounds, particularly humic substances (HS). HS are formed through the chemical and biological transformation of plant and animal residues together with microbial metabolic byproducts and constitute the major reservoir of organic carbon at the Earth's surface, playing a critical role in regulating ecological and environmental processes (Canellas *et al.*, 2015). Based on their solubility behavior, HS are conventionally classified into three operationally defined fractions; humin, which is insoluble in both acid and base; humic acid, which is soluble at pH 2; and fulvic acid, which remains soluble across the entire pH range (Tipton *et al.*, 1992; von Wandruszka, 2000). The functional group composition of humic acid varies considerably depending on feedstock origin, age, climatic conditions, and the extraction method employed (De Melo *et al.*, 2016).

A range of spectroscopic techniques, including fluorescence excitation-emission matrix (EEM) spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, ultraviolet-visible (UV-Vis) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy, have been employed to investigate the structural composition of compost-derived humic substances (Liu *et al.*, 2021). Among these, NMR spectroscopy offers a particularly powerful and complementary approach for elucidating the molecular structure of humic materials at a resolution beyond what conventional functional-group-based methods can provide (Helal *et al.*, 2011). However, despite growing interest in the agricultural utilization of *A. conyzoides* compost, and the detailed molecular-level characterization of its derived humic acid remains scarce were investigated.

Materials and methods

Study site and weed biomass collection

Ageratum conyzoides biomass was collected from the Agricultural Experimental Zone, University of Bengkulu, Bengkulu City, Bengkulu Province, Indonesia. A total of 500 kg of fresh weed was collected, chopped into 3–5 cm fragments.

Compost preparation

The chopped biomass was mixed with 5 kg of cattle manure, 100 cc of effective microbe's inoculant per liter of water, with additional water added until the mixture reached a moist consistency. The composting materials were stacked in layers 15–20 cm thick, thoroughly mixed, and covered with blue plastic sheeting for 6 weeks. The pile was turned and watered every three days throughout the composting period. Compost maturity was assessed visually, based on a dark brown color, non-clumping texture, and absence of unpleasant odor.

Nutrient analysis of compost and weed biomass

Organic carbon content was determined using the Walkley-Black method, total nitrogen was determined using the *Kjedahl* method, phosphorus content was determined using Bray-I, potassium was determined using extraction by NH_4OAc pH 7, calcium, and magnesium concentrations were determined using wet extraction method. Compost pH was measured using a pH meter in a 1:1,25 (w/v) compost-to-distilled water suspension. Additional compost properties analyzed included sulfur, iron, copper, zinc, manganese, carbon-to-nitrogen (C/N) ratio, cellulose, lignin, and hemicellulose content. The same nutrient parameters were analyzed for the raw (uncomposted) weed biomass, excluding pH and C/N ratio. All analyses were performed in triplicate, and results are expressed as mean $\pm$ standard deviation.

Extraction and fractionation of humic substances

Total Humic substances. Compost was extracted with 0,1 N *Sodium pyrophosphate* + *Sodium hydroxide* for 24 hours by shaker and allowed to stand overnight. The day after that, extract filtered by filter paper to separate humin and humic substances, such as humic acid and fulvic acid. Filtrate was pipetted as 5 ml (5 gr) into 100 ml volumetric flask. Adding 5 ml 1 mol L^{-1} $\text{K}_2\text{Cr}_2\text{O}_7$ and mixed them. Adding 7,5 ml 96-97% H_2SO_4 and diluted with 50 ml distilled water. After standing overnight, the humic and fulvic acid absorbance were measured by spectrophotometry with 561 nm wavelength (used glucose standard 0-250 g kg^{-1}).

Humic acid. It was pipetted 5 ml filtrate into 50 ml volumetric flask and added 1 ml 5 mol L^{-1} H_2SO_4 . After overnight, filtered with filter paper and rinsed extract that not passed by filter paper with 20 ml 0.01 mol L^{-1} H_2SO_4 . Rinsed again with 0.1 ml L^{-1} NaOH (70-80°C) slowly. Pipetted 5 ml extract into 100 ml volumetric flask. Adding 5 ml 1 mol L^{-1} $\text{K}_2\text{Cr}_2\text{O}_7$ and mixed them. Adding, 7.5 ml 96-97% H_2SO_4 and diluted with 50 ml distilled water. After standing overnight, the humic acid and fulvic acid absorbance

were measured by spectrophotometry with 561 nm wavelength (used glucose standard 0-250 g kg⁻¹).

The humic and fulvic acid content were calculated as

$$\text{Humic \& fulvic acid (\%)} = (\text{C-humic \& fulvic acid}) \times 1.79 \quad (\text{a})$$

$$\text{Humic acid content (\%)} = \text{C-humic content} \times 1.79 \quad (\text{b})$$

$$\text{Fulvic acid content (\%)} = (\text{a}) - (\text{b})$$

NMR analysis

Solid humic acid (1 g) was dissolved in 1 mL of deuterium oxide (D₂O) and vortexed for one minute, followed by sonication for 5 minutes. The mixture was centrifuged at 10,000 rpm for 5 minutes, and the resulting supernatant was transferred into an NMR tube for analysis. ¹³C NMR spectra were acquired using a JNM-ECZ500R, 500 MHz super conductive magnet, with 64 scans. Chemical shifts were referenced to TMS and reported in parts per million (ppm). Spectral data were processed using *MestReNova* ver. 17, and peak assignments followed established chemical shift regions: aliphatic carbon (0–50 ppm), carbohydrate carbon (51–110 ppm), aromatic carbon (111–160 ppm), carboxyl carbon (161–190 ppm), and carbonyl carbon (191–220 ppm), according to Hatcher *et al.* (1983) and Chefetz *et al.* (1998).

Results

General compost properties

Babandotan (*A. conyzoides*) was a local weed that easily to find and growth massively in around, like backyards, garden, and roadsides. This weed has wide advantages for human, as the medicines, organic fertilizers like compost or soil ameliorant, and hyperaccumulator plant for tailing in post-mining waste area (Wang *et al.*, 2011; Babu Shresta *et al.*, 2016; Maturi *et al.*, 2024; Althaaf *et al.*, 2025). As a compost, this weed has potential nutrients for maintaining soil fertility and supplying nutrition for plant growth and yield.

This Babandotan compost contains pH with neutral level (6.09) with moisture level 73%. This compost is found to be high salinity (4.89 dS/m), Total Organic Carbon (TOC) (40.2%), Total Kjeldahl Nitrogen (TKN) 1.61%, cation of Na⁺ was high (2.43 g kg⁻¹), cations of K⁺ were high (26.02 g kg⁻¹) and high C/N ratio (24.9). On the other side, this compost has lignin of acid-insoluble lignin 21.22% and acid-soluble lignin 0.94%, cellulose 31.34% and hemicellulose 36.23% (Table 1). If compared with vermicompost as result from Devi and Khwairakpam (2020), raw matter vermicompost from Babandotan resulting lower characteristics than compost. In vermicompost, TOC is 38.37% while TKN higher (2.28%) than compost.

Table 1. The chemical properties of *Ageratum conyzoides* compost

Variables	<i>A. Conyzoides</i> compost
pH	6.09 ± 0.08
Moisture content (%)	73.2 ± 1.78
EC (dS/m)	4.89 ± 0.12
TOC (%)	40.2 ± 1.05
TKN (%)	1.61 ± 0.03
Na ⁺ (g kg ⁻¹)	2.43 ± 0.8
K ⁺ (g kg ⁻¹)	26.03 ± 2.43
Ca ²⁺ (g kg ⁻¹)	4.13 ± 1.03
C/N ratio	24.9 ± 1.2
Lignin	
a. Acid-insoluble lignin (%)	21.22
b. Acid-soluble lignin (%)	0.94
Cellulose (%)	31.34
Hemicellulose (%)	36.23

Source: *Maturi et al.*, (2024)***¹³C-NMR spectroscopic characteristics of humic acids***

The ¹³C NMR spectra indicated the presence of several types of carbons, including aliphatic, carboxyl and carbonyl carbons, with a wide variation in the abundance of aliphatic. The relative concentration of various groups was calculated by integrating areas of corresponding peaks in the solid NMR spectrum. Generally, ¹³C NMR exhibited common major peaks on five regions. The region was the aliphatic region (0-50 ppm), aromatic carbon (111-160 ppm), the carboxyl group (161-190 ppm), carbohydrate (51-110 ppm), and carbonyl group (191-220 ppm). Generally, humic acid from this compost showed common peaks at carbohydrate, aromatic carbon and carbonyl group.

Table 2. Major bands of ¹³C NMR of humic acids

Humic acids	Carbonyl shift range (191 – 220 ppm)	Carboxyl shift range (161 – 190 ppm)	Aromatic shift range (111 – 160 ppm)	Carbohydrate shift range (51 – 110 ppm)	Aliphatic shift range (0 – 50 ppm)
<i>Ageratum conyzoides</i>	221.53, 217.41, 210.66, 206.34, 199.55, 193.11, 190.82	190.82, 183.31, 1176.20, 166.74, 160.85, 158.02,	160.85, 158.02, 153.42, 143.73, 139.56, 132.54, 126.85, 116.51	107.24, 101.95, 91.73, 88.29, 76.51, 65.00, 67.24, 57.94, 53.74	45.27, 43.11, 32.40, 27.91, 18.93, 13.31

The aliphatic region was intensified in three spectra (Table 1, Figure 1), such as amina bromide (45.47 ppm and 43.11 ppm), alkil bromida (32.40), alkana (27.92 ppm; 18.93 ppm; 13.31 ppm). Humic acid having the strongest

peak near 32.40 ppm that corresponded to methylene groups near points of carbon branching. Meanwhile, the region of carbohydrate range (51-110 ppm) shows aliphatic alcohol or ether region. It was resonance into peak between 60 – 110 ppm characterized of carbon atoms singly bonded to oxygen or nitrogen in alcoholic, etheric or amine carbons.

Humic acid from this compost exhibited some peaks in between aromatic range (111-160 ppm), one peak showed a peak near 111 ppm (Table 1, Figure 1). The bands between 112-160 ppm are aromatic and phenolic carbons and the peaks in this region mainly correspond to aromatic and olefinic carbons. The intensity of peaks varied, but generally weaker peaks were centered near 130 ppm in the spectra, indicating ring carbons in which the ring contains a strong electron donor such as oxygen or nitrogen. The peak between 116 – 126 ppm indicated an alkyl aromatic carbon was substituted by a nitrogen or oxygen. Furthermore, there were also peaks near at 143 ppm and 153 ppm that indicated aromatic carbons substituted by functional groups containing oxygen and nitrogen such as -OH, -OR, or -NH₂. These findings are clearly indicated that aromatic carbons are more common in this humic acid.

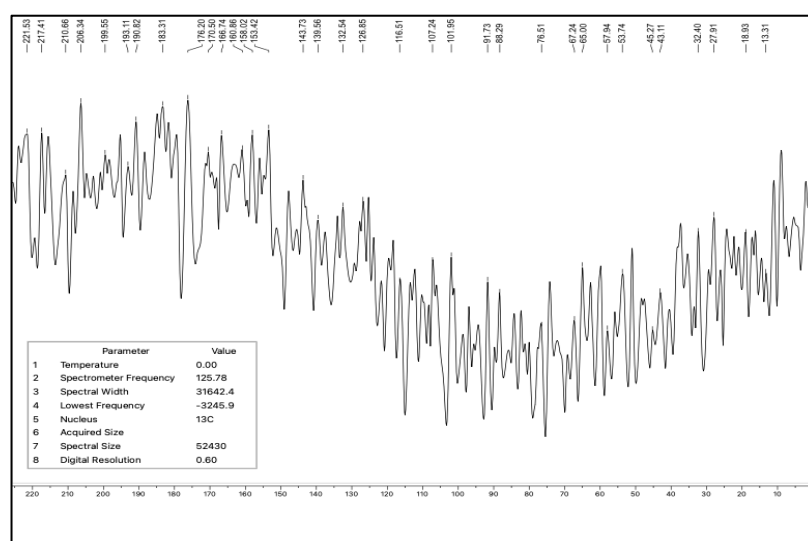


Figure 1. ¹³C NMR spectra of humic acid extracted from composted *Ageratum conyzoides*

The carboxyl and carbonyl region (160 – 220 ppm) showed the strong peaks at 166.74, 176.20, and 206.34 ppm, respectively (Figure 1). The peaks in this region correspond to carbonyls of carboxylic acids, but the region between 170 – 176 ppm most likely corresponds to ester linkage. Meanwhile, the peak range of 191 – 220 ppm was chemical shift of carbonyl carbon of aldehydes, ketones, and quinones.

Discussion

The ^{13}C NMR spectra obtained in this study revealed that the humic acid extracted from composted *A. conyzoides* was dominated by aromatic, carbohydrate, and carbonyl/carboxyl carbon signals, with a comparatively lower contribution from aliphatic carbon. This distribution pattern is generally indicative of a relatively advanced degree of humification, as the depletion of simple aliphatic structures relative to aromatic and oxidized carbon forms is commonly associated with progressive microbial decomposition and stabilization of organic matter during composting (Mao *et al.*, 2000; Chefetz *et al.*, 1998).

The aliphatic carbon fraction observed in this study, although less dominant than other regions, remains agronomically relevant. Aliphatic carbon is the simplest organic carbon structure, composed solely of carbon and hydrogen, and has previously been shown to correlate strongly with organic carbon inputs to soil (Simon, 2005) as well as with soil microbial biomass and aggregate stability ($r = 0.91$) (Capriel *et al.*, 1990). The hydrophobic nature of this fraction contributes to the water stability of soil aggregates, while its relatively higher biodegradability compared to aromatic carbon suggests that this portion of the humic acid may serve as a more readily available carbon source for soil microorganisms following application. Vranova *et al.* (2013) further noted that aliphatic compounds can contribute to the phytoremediation of contaminated soils by influencing the availability of heavy metals and organic pollutants, suggesting an additional environmental benefit beyond nutrient supply.

The prominent carbohydrate-region signals (51–110 ppm) detected in this study likely originate from residual polysaccharide structures, such as cellulose and hemicellulose, which were also identified in substantial proportions in the raw compost material. The persistence of carbohydrate carbon at this stage of composting indicates that a fraction of the original plant structural carbohydrates had not yet been fully mineralized, which may represent a continued, slow-release carbon and energy source for soil microbial communities after the compost is applied to soil.

The aromatic carbon fraction was one of the most prominent regions identified in this study, consistent with the relatively high lignin content of *A. conyzoides* biomass which is given that lignin-derived phenolic structures are a primary precursor of aromatic carbon in humic substances. Aromatic hydrocarbons, including benzene derivatives such as toluene and xylene isomers, tend to bind tightly to soil organic matter and mineral colloids and are generally resistant to microbial decomposition, allowing them to persist in soil over extended periods (Kariyawasam *et al.*, 2022; Rusanescu *et al.*, 2025). The relative abundance of aromatic carbon in this humic acid therefore suggests that *A. conyzoides*-derived compost may contribute to long-term soil

organic carbon stabilization rather than serving solely as a short-term nutrient source.

Beyond aromatic and aliphatic carbon, the strong carboxyl and carbonyl signals identified in this study are of particular agronomic significance. Carboxyl and carbonyl functional groups are the primary contributors to the cation exchange capacity of humic acids and play a central role in the chelation and complexation of polyvalent metal cations, including micronutrients such as Fe, Cu, Zn, and Mn analyzed in this study's compost characterization. This chelating capacity can enhance micronutrient availability to plants while buffering against nutrient leaching, supporting the broader role of *A. conyzoides*-derived humic acid as a functional component of organic fertilizer rather than merely a source of bulk organic carbon.

Taken together, these molecular-level findings are provided mechanistic support for previous agronomic observations on the benefits of *A. conyzoides*-based organic amendments, including increases in soil organic carbon, organic matter content, and nutrient availability, alongside reductions in soil cation exchange capacity following compost application (Anhar *et al.*, 2018; Baral *et al.*, 2022; Kumar *et al.*, 2018). The combination of recalcitrant aromatic carbon for long-term carbon stabilization, labile aliphatic and carbohydrate carbon for short-term microbial activity, and carboxyl/carbonyl groups for nutrient chelation are collectively supported the potential of *A. conyzoides* compost as a multifunctional organic fertilizer, while simultaneously offering an environmentally sound strategy for managing this invasive weed species.

Conflicts of interest

The authors declare no conflict of interest.

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