Original Research Article

Phytochemical Screening of *Etlingera elatior* Cultivated on Different Dosage of Biochar

Abstract

This study was to investigate the impact of biochar on phytochemical composition in plant. The phytochemical composition was extract from different dosage application of biochar (0%,5% and 20% by weight). These treatments found significantly increased in the phytochemical composition. It is recommended that application of biochar not only improve the growth rate but also enhance the phytochemical of the plants. **The Fourier Transform Infrared spectra confirmed that the biochar rich with humus like-compounds which increase the nutrient content of the soil.

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Keywords: phytochemical, biochar, ethanolic extract, Cultivated

Introduction

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Recently, food safety is becoming a major issue. Eventhough the advances in technology have been applied to agricultural industry, the global food supply is yet insufficient to meet the markets demands. A prominent environmental issues such as climate change, desertification, and soil pollution are still remain to be resolved for the agriculture sector [1]. Soil quality and agricultural productivity become deteriorated due to the impact of industrialization. The pollutans like heavy metals, engineered nanomaterials, polycyclic aromatic hydrocarbons (PAHs), and persistent organic and inorganic chemicals as derivatize from the industrial process acceleratedd the soil contamination [2]. These anthropogenic sources have constantly affected plant environments, thereby causing harm to the terrestrial food. Many studies have witnessed the negative impacts of contaminants on soil composition and biodiversity.

Pyrolisis and composting technologies had introduced biochar into global agriculture attention due to its benefits [3,4]. Biochar produced by biomass pyrolysis [5]. Its elemental composition consists of carbon, nitrogen, hydrogen, potassium, and magnesium all of which can serve as major nutrients in plant growth. The addition of biochar increases the amount of organic matter in the soil (e.g, organic carbon), thereby improving soil physicochemical and biological properties. Biochar can positively or negatively affect the soil microbial growth to alter the agricultural environment [7].

Biochar is a very stable, carbon-based material obtained from the pyrolysis of biomass under anaerobic conditions, and is highly recalcitrant in soils. The parent material or biomass can be obtained from agricultural, municipal, animal, or industrial sources. The pyrolysis temperatures generally employed range from 300 to 1000 °C. Biomass is largely composed of organic compounds such as cellulose, hemicellulose, and lignin. Lignin is the most stable of these compounds, and is resistant to degradation at even higher temperatures. In contrast, temperatures above 300 °C can decrease the cellulose and hemicellulose contents. The temperature and duration of pyrolysis are determined based on the target purpose. In some cases, catalytic additives such as K3PO4 and clinoptilolite are used to reduce the pyrolysis temperature. During pyrolysis, water and volatile organics from the biomass may evaporate, thereby increasing the aromatic content. The parent biomass source and pyrolysis temperature affect the physiochemical properties of the biochar obtained. For instance, pyrolysis temperature is the main control on atomic ratio and structural composition. Although raw biomass is slightly acidic, pyrolysis at high temperatures increases its alkalinity. This is due to the partial detachment of the functional groups leading to the formation of unpaired negative charges such as carboxyl (COO^{-}) and hydroxyl groups (OH^{-}) that have the ability to attract positive charges [9]. High-temperature pyrolysis also causes release of hydrogen- and oxygen-containing groups, contributing to increased carbon content. The tendency of the surface functional groups to attract positive charges enhances the cation exchange capacity, which is an important property of biochars for remediation of metal-contaminated soils. Furthermore, the porosity, pore size, and surface area of biochars depend on pyrolysis temperature as the high temperature of pyrolysis leads to formation of pores via the release of volatile organics. Thus biochar's properties can be targeted to a range of different purposes by adjusting the pyrolysis temperature.

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Author: the introduction was updated

Application of biochar has been shown to yield a wide range of benefits to plant growth and stress management. Studies have documented the role of biochar in improving agronomical parameters and environments for various plant species. Addition of less than 5% biochar enhanced the germination, yield, and root development of the halophytes such as sesbania and seashore mallow. Rice hull-based biochar applied at a rate of 2% to a sandy soil increased the biomass yield and sucrose content of sugarcane plants.

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Application of biochar also can improve nutrient cycles. In wheat culture, biochar addition at 10% in combination with urea improved agronomic efficiency of N by 63%; biochar mixed with 10% KOH significantly increased Si uptake by plants. It is noted that Si plays a vital role in both biotic and abiotic stress management in plants. P-loaded biochar at 30 g kg^{-1} also improved the bioavailability of P. Sewage sludge biochar at 50% acts as a soil conditioner and was able to stimulate turf grass growth, even in urban soils. Other studies have found that biochar can expand plant diversity by benefitting reforestation activities in low-carbon soils. The reforestation capacity is due to increased total soil carbon in the reforested sites.

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Author: Etlingera elatior is a medicinal plant. Its importance was included in the text.

In this study, it was hypothesized that apllication of biochar to *E. elatior* with different dosages of biochar might affect the type of secondary metabolite. Therefore, this present study aimed to evaluate the effect of biochar at two dosages (10 and 20% of fresh mixture weight) on the type of secondary metabolite extracted from the *E. elatior*.

Materials and Method

Cultivation of *Etlingera elatior*

Etlingera elatior was cultivate on the pot with 20 cm diameter and 35 cm height. 3 replicates for pots of *Etlingera elatior* was cultivated and label as 0%, 5% and 20%. The cultivation soil used was non-fertilized soil(0%) fertilized soil with biochar; 5% and 20%. The pots was placed in the netting house and the growth of *Etlingera elatior* was monitored. Watering process was done twice a day. The biochar used in this study were purchased from Black Owl Biochar Products. The dosage of biochar used was calculated over the weight of soil. The soil treatments are as follows:

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Better write soil without biochar, with 5% ...
2. What is the nature of the basic soil used in the experiment?
Author; the report was corrected, the soil was obtained from a farm .

a) Non-fertilized soil (0%), b) non-fertilized + biochar (5% and 20%)

Sample extraction

Phytochemical was extracted according to [12,13,14] with some adjustments. 100 gm of the dry leaf powder of *E. elatior* were weighted, transferred to a flask, soaked with ethanol until the powder was fully immersed and incubated overnight. The extracts were then filtered through Whatman filter paper No.1 along with 2 gm sodium sulfate anhydrous to remove the traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with 95% ethanol. The filtrates were then air dried and subjected to gas chromatography-mass spectrometry analysis.

Fourier Transform Infrared (FTIR)

The biochar samples were analysed using an ATR-FTIR equipped with diamond crystal, controlled by OMNIC software (Thermo Nicolet Analytical Instruments, Madison, WI). A flat tip powder press was used to achieve even distribution and contact. All spectra were collected at 12 scans with a resolution of 4 cm-1 in the range 4000–670 cm-1. The spectrum of each sample was ratioed against a fresh background spectrum recorded from the bare ATR crystal. The ATR crystal was cleaned with ethanol.

Gas Chromatography Mass Spectrometry (GCMS) analysis

The gas chromatography mass spectrometry analysis was performed by using a non-polar BPX-5 capillary column with an initial temperature of 50^{o}C hold for five minutes and then increased to 300^{o}C at a rate of 5.0^{o}C per minutes and hold 10 minutes. The temperature of the injector and detector were set at 320°C respectively. 1µl of the fractions was diluted in 100µl hexane was introduced into the gas chromatography. The gas used as the carrier was Helium. Interpretation of mass-spectrum was conducted using the database of National Institute Standard and Technology (NIST17). The spectrum of the secondary metabolites components was compared with the spectrum of known components stored in the NIST library. The name, molecular mass and structure of the components of the test materials were ascertained.

Results and discussions

The Fourier Transform Infrared spectra shown in Figure 1. The spectra with pronounced bands at 1591 cm^{-1} corresponding to carboxyl group of protein compounds. Broad bands at 1700 cm^{-1} due to stretching of C-O bond in polysaccharide. However, polysaccharide would have degraded during the process of biochar, whilst the phosphate content in biochar increase. The phosphate band can be observed at 1029 cm^{-1}. These findings confirm that the biochar was rich in humus compounds which is probably from mixture of poultry materials.

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The gas chromatography's chromatograms of *Etlingera elatior* obtained from ethanolic extract are shown in Figure 2. The chromatograms demonstrate different peaks for different dosage of biochar (Figure 2(a)-(c)). Different pattern of chromatogram was observed on different dossage of biochar applied. The major compounds with their percentage area (PA%)

are summarize in Figure 3. The results revealed that phytol (13%), Hexadecanoic acid (9.76%), Neophytadiene (6.51%), coumarin (5.65%), precocene (5.27%) and caryophyllene (4.59%) were among the major compounds identified from *Etlingera elatior* etahnolic extract on 0% biochar (Figure 3(a)). Increasing biochar dosage to 5%, the identified compounds shows that, the 6 major compounds are Dihydrocucurbitacin (13.69%), Niacinamide (11.02%), α-Limonene (10.01%), Phyrahen (9.23%), Phytol (7.24%) and Neophytadiene (5.75%) shown in Figure 3(b). In 20% biochar the major compounds of *Etlingera elatior* are Linoleic acid (39.98%), 2-pinen-4-ol (12.32%), Hexatriacontyl pentafluoropropionate (6.89%), Benzofuran (5.12%), Acethophenon (4.41%) and furfural (4.03%) shown in Figure 3(c). Most of the compounds known to be secondary metabolite which are rich in medicinal values.

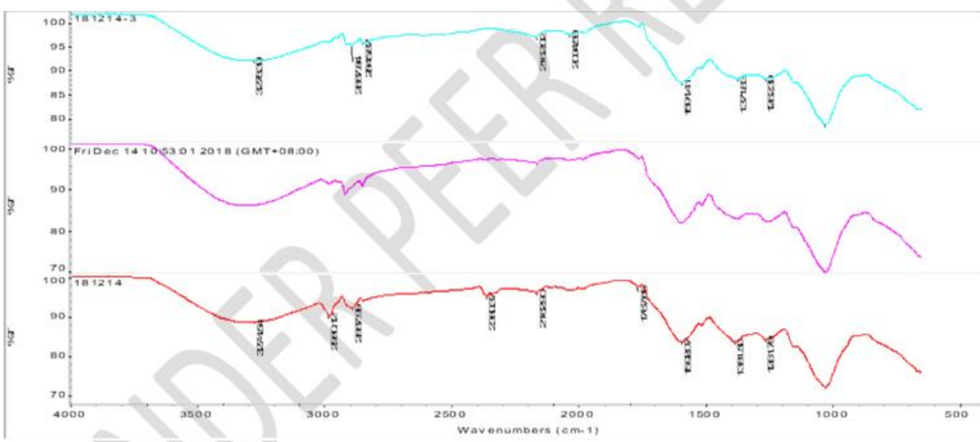

Figure 1. Stack spectra of biochar.

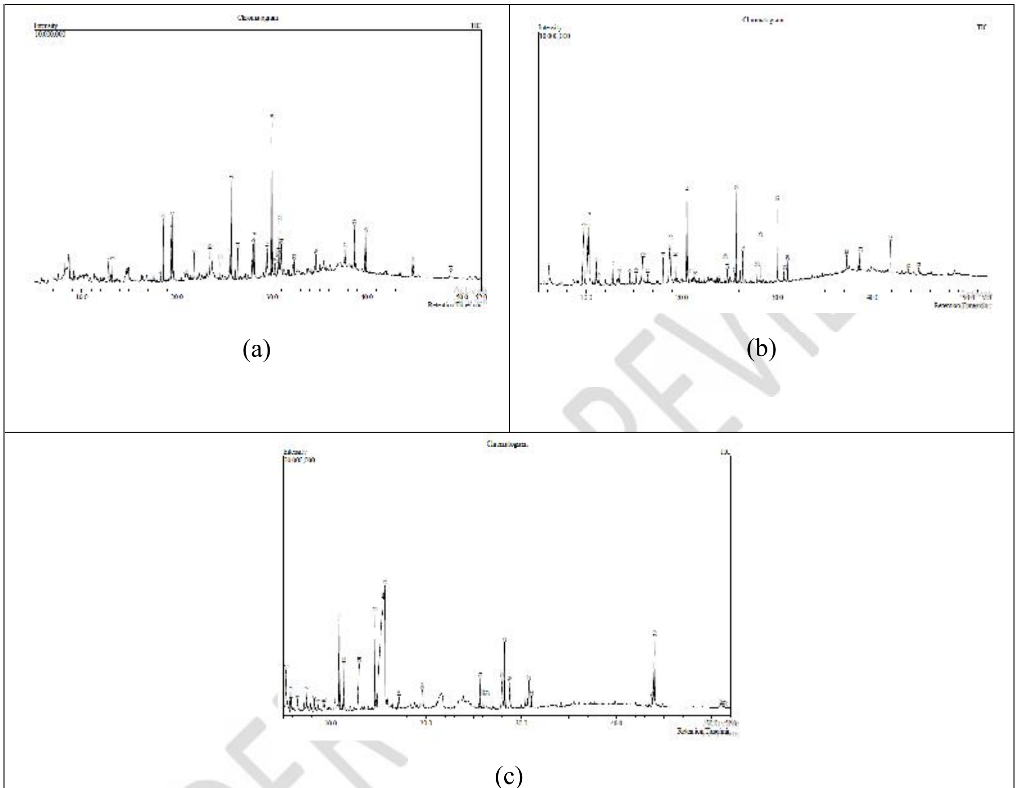

Figure 2. Chromatogram of phytochemical from *Etlingera elatior* at different dosage of biochar; (a) 0% biochar, (b) 5% biochar and (c) 20% biochar.

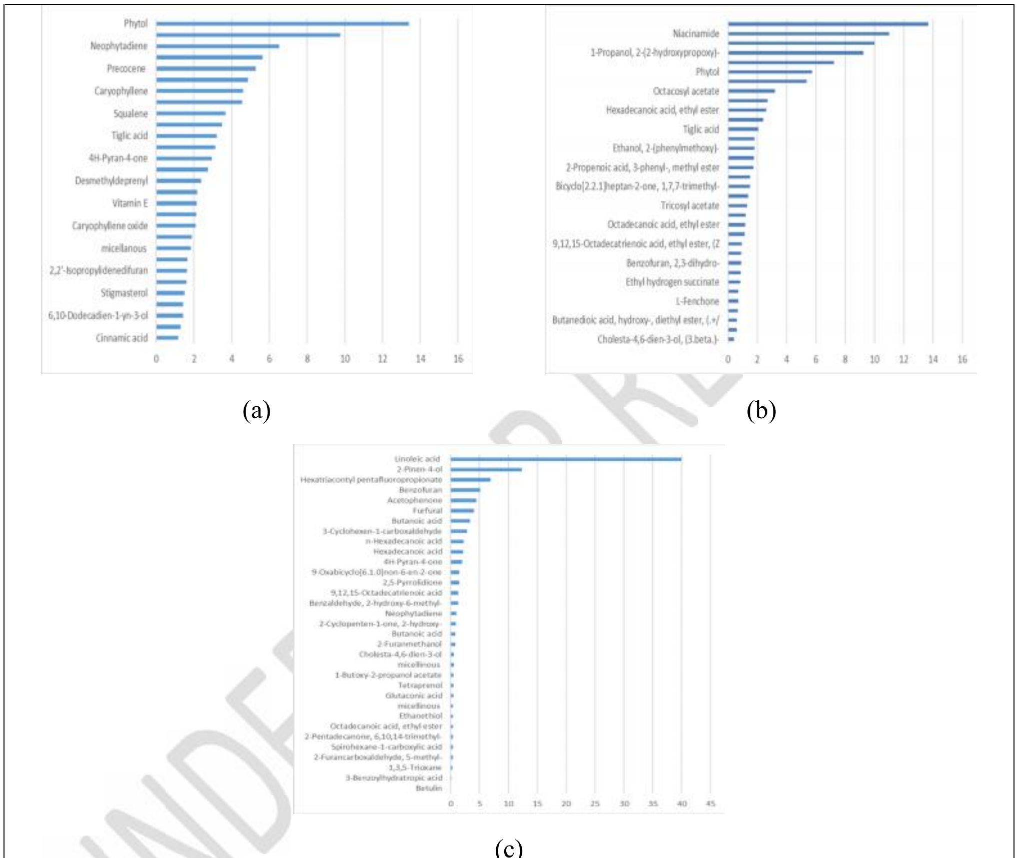

Figure 3. Peak area percentage of compounds extracted from *Etlingera elatior* at different dosage of biochar; (a) 0% biochar, (b) 5% biochar and (c) 20% biochar.

Conclusion

Application of biochar on soil can increase nutrient availability and enhance the development of phytochemical composition in plants. Without biochar, the chemical composition *Etlingera* *elatior* extract was slighlyt low. At 5% and 20% biochar, some compounds are increasing and new compounds are develop compared to 0% biochar. This suggest that the biochar not only able to increase the growth rate of plants but also the nutrients of the plants.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

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