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Metabolites, elemental profile and chemical activities of *Pinus strobus* high temperature-derived pyroligneous acid

Raphael Ofoe*, Lokanadha R. Gunupuru and Lord Abbey*

Abstract

Background: Pyroligneous acid (PA) is an aqueous smoky fraction produced during pyrolysis of biomass. The chemical composition of PA from different plant biomass has been studied, but reports on PA metabolites and elemental profiles are rare. In this study, we examined the metabolites, elemental profiles and the associated chemical activities of PA derived from white pine (*Pinus strobus*) at 1100 °C compared to similar work done elsewhere using different biomass at lower temperatures.

Results: PA from *P. strobus* biomass exhibited a lower electrical conductivity (2.05 mS/cm), salinity (1.03 g/L) and total dissolved solids (1.42 g/L) but higher °Brix content (9.35 ± 0.06) compared to PA from other feedstock. The *P. strobus* PA showed a higher antioxidant activity characterized by enhanced radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl free-radical (78.52%) and accumulation of higher total phenolic (95.81 ± 1.45 gallic acid equivalents (GAE)/mL) and flavonoid content (49.46 µg quercetin/mL). Metabolite profiling by direct injection mass spectrometry with a reverse-phase liquid chromatography–mass spectrometry (DI/LC–MS/MS) identified a total of 156 metabolites. Four (4) main groups including organic acids (90.87%), hexose (8.60%), carnitine (0.3%) and phospholipids (0.24%) were found in the PA. Mineral element analysis revealed that the *P. strobus* PA contained high concentrations of nitrogen (N), potassium (K), calcium (Ca) and zinc (Zn), while the content of sodium (Na) and trace/heavy metals were present at levels below the reported limit.

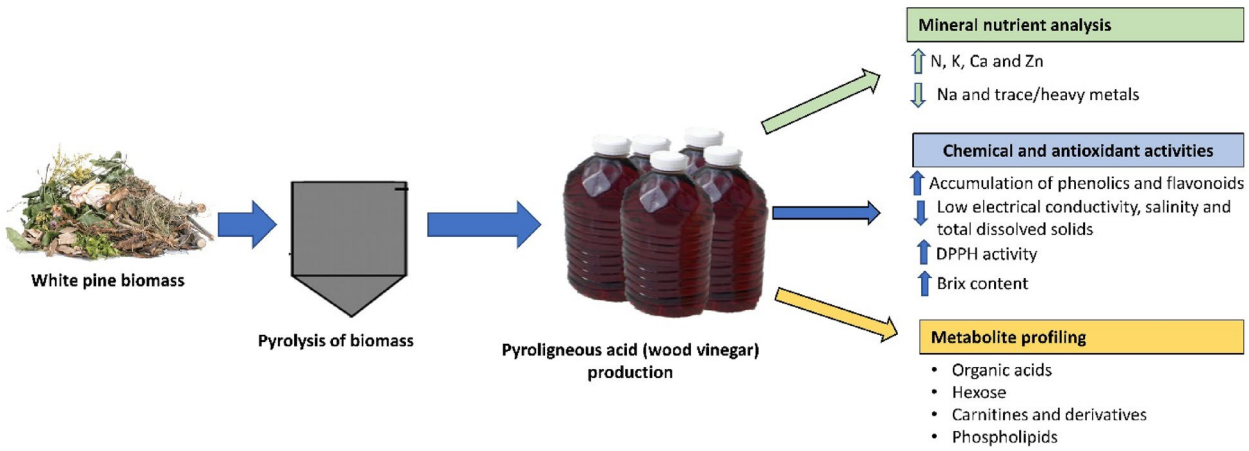
Conclusion: This study indicates that *P. strobus* PA is a valuable product that can be used in agriculture to improve plant growth and productivity under normal and environmentally stressful conditions.

Keywords: Wood vinegar, White pine, Pyrolysis, Biostimulant, Plant growth promoter

*Correspondence: rapheal.ofoe@dal.ca; loab07@gmail.com

Department of Plant, Food, and Environmental Sciences, Faculty of Agriculture, Dalhousie University, 50 Pictou Road, P.O. Box 550, Bible Hill, NS B2N 5E3, Canada

Graphical Abstract



Introduction

Pyroligneous acid (PA) is a yellow or reddish-brown translucent aqueous fraction produced by the pyrolysis of organic biomass in the presence of limited oxygen [1]. It is also known as wood vinegar, smoke water or wood distillate; and comprises a complex mixture of over 200 different aqueous organic compounds. Water, phenolic compounds, and organic acids are typically, the major constituents of PA but also contain nitrogen, sugar derivates, alcohols, and esters [1–3]. However, the chemical composition of PA depends on the feedstock, temperature, heating rate, and residence time [1].

Pyroligneous acid has been recognized as a safe natural product and used extensively in diverse areas including agriculture, medicine and food [1, 4]. In agriculture, it is used as a biostimulant to enhance seed germination, crop yield and productivity [5–8]. It has been shown to have no adverse effect on biodiversity and other bioindicators [9]. Additionally, it is used as a herbicide [10], insect repellent [11], soil conditioner [12] and antimicrobial agent for controlling several plant diseases [13, 14]. In medicine, PA has been reported to exhibit anti-inflammatory effects against 2,4-dinitrochlorobenzene (DNCB) induced dermatitis by inhibiting the proliferation of epithelial cells in mice [15]. Other biological activities such as antifungal, termiticidal, antitermitic and strong antioxidant effects have also been reported [2, 16–18].

These beneficial effects of PA have been attributed to its numerous bioactive compounds. For instance, the antioxidant and anti-lipid peroxidation capacity of PA is due to its phenolic compounds, which induce high ROS-scavenging and reducing power activities [2, 19]. The antimicrobial and anti-inflammatory activities of PA were positively correlated to its high organic acid and phenolic compounds [2, 16, 17, 20]. All these results indicate that PA possesses a great potential to be used as a sustainable product. However, these biological activities can be influenced by the pyrolytic temperature as high pyrolytic temperatures between 311 and 550 °C was demonstrated to have the strongest antioxidant activity [2].

Moreover, various PA have been produced from different plant materials including pineapple (*Ananas comosus*) waste [21], bamboo (*Dendrocalamus asper*) [5, 22], oak (*Quercus* sp.) [23], *Eucalyptus grandis* [22, 23], *Rhizophora apiculate* [19, 24], *Litchi chinensis* [18], walnut (*Juglans regia*) shell [2, 25], coconut (*Cocos nucifera*) shell [26], *Leucaena leucocephala*, *Azadirachta indica*, *Eucalyptus camaldulensis*, and *Hevea brasilien*sis [22]. Generally, most PAs are produced at a pyrolysis temperature range of 200–550 °C [2, 27]. However, little is known about the chemical composition of PA produced from temperatures above 1000 °C. Many studies have reported that PA produced from different plant species might present varying quantities of bioactive constituents and levels of bioactivity. However, no studies have investigated the metabolic and mineral element profile of PA produced at high pyrolytic temperature (> 1000 °C). In this paper, we examined the metabolic and mineral element profile, and the antioxidant activity of PA produced from *P. strobus* at 1100 °C.

Materials and methods

Study location and materials

This study was performed in the Department of Plant, Food, and Environmental Sciences, Faculty of

Agriculture, Dalhousie University, Truro. PA was prepared from white pine (*P. strobus*) biomass using fast pyrolysis. The front portion of the 22 ft long tube through which the biomass traverses receive a maximum heat flux of 22.6 W/sq inches. The average heat flux imposed on the tube was 15 W/sq. inches. The PA was produced at a pyrolysis temperature of 1100 °C and prepared by Proton Power Inc. (Lenoir City, USA). The mass yield of PA produced from 40,000 MT of pine wood biomass was 10% with density of 1.0 g/cc and a moisture content of 85%. The PA was stored in the dark at room temperature until further analysis. Chemicals used for this study were of analytical grade. The chemical reagents were 1,1-diphenyl-2-picrylhydrazyl (DPPH), trichloroacetic acid (TCA), ferric chloride ($FeCl_3$), gallic acid, quercetin, Folin–Ciocalteu reagent and aluminum chloride ($AlCl_3$) from Thermo Fisher Scientific Co. (Canada); and potassium ferricyanide [$K_3Fe(CN)_6$], butylated hydroxyanisole (BHA), dithiothreitol (DTT), ethylene diamine tetra acetic acid (EDTA), L-Ascorbic acid and sodium carbonate from BioShop Canada Inc. (Canada).

Mineral nutrient and chemical analysis

Complete elemental composition of PA was determined at the RPC Science and Engineering facility in Fredericton, New Brunswick using inductively coupled plasma-mass spectrometry (ICP-MS) [28]. For the chemical analysis, PA samples were poured into a 100-mL beaker and 500 µL was used for °Brix content determination using a handheld refractometer (Atago, Japan). The pH, salinity, total dissolved solids (TDS) and electric conductivity (EC) were measured using a 3-in-1 multimeter (EC 500 ExStik II S/N 252957, EXTECH Instrument, Taiwan).

Total phenolics

Total phenolics content (TPC) of PA was determined using the Folin–Ciocalteu method described by Dudonne et al. [29] with little modification. Briefly, 200 µL of PA was added to 400 µL of 10% (v/v) Folin–Ciocalteu reagent and mixed thoroughly for 5 min. A solution of 1.6 mL of 700 mM Na_2CO_3 was then added to the mixture. After incubation in the dark at room temperature for 30 min, the absorbance was measured at 765 nm against a blank. TPC was calculated using a gallic acid standard curve and expressed as mg gallic acid equivalent.

Total flavonoid

Total flavonoid of PA was estimated following the colorimetric method described by [18] with little modification. One mL of PA was added to equal volume of 95% methanol and 1 mL of the resultant mixture was mixed with 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M potassium acetate, and 2.8 mL distilled water. The reaction was incubated at room temperature for 40 min and absorbance was read at 415 nm against a blank without $AlCl_3$. Total flavonoid content was estimated using the quercetin standard curve.

DPPH free-radical scavenging capacity

The DPPH free-radical scavenging capacity of PA was determined using the method described by Dudonne et al. [29] with slight modification. 100 µL of PA in methanol (1:1, v/v) was added to 2.9 mL of 60 µM fresh DPPH methanolic solution. The mixture was vortexed and incubated in the dark at room temperature for 30 min. The absorbance of the reaction mixture was measured at 515 nm against a methanol blank and the radical scavenging activity was calculated using the formula:

$$\text{Inhibition}\,(\%) = [(A_B - A_S)/A_B] \times 100\%,$$

where A_B is the absorbance of the blank sample and A_S is the absorbance of the PA sample.

DI/LC–MS/MS method

Targeted metabolic analysis was performed at the metabolic core facility, The Metabolomics Innovation Centre (TMIC), Alberta, Canada. A targeted quantitative metabolomics approach was applied to analyze the PA samples using a combination of the direct injection mass spectrometry with a reverse-phase LC–MS/MS (DI/LC–MS/MS) custom method [30]. This method involved using multiple reaction monitoring (MRM) pairs to merge analytes extraction and derivation, and selective mass spectrometric detection. This identifies and quantifies up to 207 endogenous metabolites including amino acids, biogenic amines and derivatives, acylcarnitines, glycerophospholipids, sphingolipids, uremic toxins and sugars. The assay includes a 96 deep-well plate with an attached filter plate with sealing tape and solvents and reagents for developing the plate assay. To prepare all metabolites except organic acids, the PA sample was vortexed followed by centrifugation at 13,000 × *g*. After, 10 µL of the sample was loaded onto the middle of the filter on the upper 96-well plate and dried through a stream of nitrogen. Phenyl-isothiocyanate was subsequently added for derivatization and incubated at room temperature for 20 min. The filter spots were further dried using an evaporator. The targeted metabolites were extracted by adding 300 µL of extraction solvent (5 mM ammonium acetate in methanol) to each well. The extracts were obtained by centrifugation from the 96-well filter plate to a Nunc® 96 DeepWell™ plate. Finally, the extracts were further diluted with MS running solvent and metabolites quantification was based on isotope-tagged internal standards and other endogenous standards. The first 14

wells were used for one blank, three zero samples, seven standards and three quality control samples. For absolute qualification of organic acid, 150 µL of ice-cold methanol and 10 µL of the isotope-labelled internal standard mixture was added to 50 µL of a serum sample for overnight protein precipitation followed by 20 min centrifugation at 13,000 × *g*. 50 µL of supernatant was pipetted directly into the centre of each well of a Nunc® 96 DeepWell™ plate. 3-nitrophenylhydrazine (NPH) reagent was added to each well and the plate was incubated at room temperature for 2 h. The reaction mixture was further diluted with water and stabilized with butylated hydroxytoluene (BHT) for LC–MS/MS analysis. The mass spectrometric analysis was carried out on an ABSciex 4000 Qtrap® tandem mass spectrometry instrument (Applied Biosystems/MDS Analytical Technologies, Foster City, CA) equipped with an Agilent 1260 series UHPLC system (Agilent Technologies, Palo Alto, CA). The samples were delivered to the mass spectrometer by an LC method followed by a direct injection (DI) method.

Statistical analysis

The experimental results were expressed as mean and standard deviation (SD) of quadruplicate measurements. Metabolic profile data analysis was done using Analyst 1.6.2 and MultiQuant 3.0.3 software.

Results and discussion

Chemical qualities and antioxidant activities

Table 1 shows the chemical qualities of PA, which represent important summation of its chemical constituents. The moisture content of the PA obtained from pine was 85% which was similar to those reported by other authors [22, 27, 31, 32]. This indicates that organic content fraction could account for 15%. The PA from pine had an acidic pH of 2.3, which was lower than those of other PAs reported in the literature (Table 1). For instance, Wei et al. [2] reported a pH range from 2.98 to 3.32 for walnut shell PA produced at varying pyrolytic temperatures between 90 and 550 °C; while a pH of 4.4 was reported for pineapple waste at varying pyrolytic temperature between 200 and 500 °C [21]. Similarly, the pH of eight PAs produced from five different wood species ranged between 2.9 and 3.50 [22]. Zhai et al. [25] reported that the pH of walnut shell PA increased from 2.51 to 3.92 as pyrolysis temperature was increased. The differences in pH (2.3) of the white pine PA in the present study compared to those of previous studies can be attributed to differences in feedstock and the pyrolytic temperature used [1, 2]. Moreover, wood biomass is composed of cellulose, hemicellulose and lignin which forms a major constituent of plant cell wall [27]. During pyrolysis, these structures are disintegrated to produce a wide range of organic compounds with organic acid being the major component of the derived PA [1, 2]. Also, increased pyrolysis temperature could result in a significant reduction in moisture content, thereby concentrating the amount of organic constituents [31]. Thus, the low pH of the PAs can be explained by the increase in the production of organic acids from structural compounds subjected to high temperature during pyrolysis.

Table 1 Chemical characteristics of wood vinegar obtained from *Pinus strobus*

Chemical indices	Content
Moisture content (%)	85
pH	2.30 ± 0.03
Salinity (g/L)	1.03 ± 0.01
TDS (g/L)	1.42 ± 0.01
EC (mS/cm)	2.05 ± 0.01
°Brix	9.35 ± 0.06

Results are means of four individual replicates

TDS total dissolved solids, *EC* electrical conductivity

Pyroligneous acid contains water-soluble salts consisting of sodium and potassium salts [27]. We found that the salt content of pine PA was 1.03 g/L (Table 1). Generally, the salt content of PA is strongly influenced by the chemical composition of the plant biomass [27]. Salt content typically constitutes 15% of herbaceous plant biomass whereas woods contains lower salt content (i.e. 0.3–0.4% per dry weight) [33]. Hence, the low salt content of the pine wood PA. A recent study revealed that the salinity of a solution exhibits a strong association with its total dissolve solids (TDS) and electrical conductivity (EC) [34]. TDS content represents the amount of inorganic salt and little organic substances dissolved in a solution. In this study, the TDS content of PA was 1.42 g/L, which was considerably lower than that reported by Shen et al. [35]. They showed that carbonation of corn (*Zea mays*) straw at 600 °C yielded a PA with TDS of 35.0 g/L with a EC value of 2.05 mS cm^{-1}, which was slightly lower than the ECs of other PA (e.g., 3.54 mS cm^{-1} [36]). The lower TDS and EC values could be attributed to the low inorganic salt content in woody plants compared to herbaceous plants [33]. Furthermore, the analysis of °Brix content of the white pine PA (Table 1) showed a higher value than PA produced from other wood species. For examples *D. asper*, 5.60; *H. brasiliensis*, 6.00; *L. leucocephala*, 3.80–4.60; *A. indica*, 3.00–3.40; and *E. camaldulensis*, 3.40–4.60 [22], bamboo, 6.6; Oak, 1.70; and pine, 2.00 [37].

Generally, total phenolic and flavonoid are common compounds found in plant extracts. These compounds have been reported to induce multiple biological effects such as antioxidant activity and are used to categorize products considered as a source for natural antioxidants

[18, 21]. The total phenolic and flavonoid contents in the PA were estimated to be 95.81 mg GAE/mL and 49.46 µg quercetin/mL, respectively (Fig. 1). The total phenolic and flavonoid content of the PA is relatively higher than those reported from other plant sources [18, 21]. DPPH radical scavenging assay has been widely used to assess the antioxidant capacity of PA from diverse plant sources [2, 18, 19, 24, 25]. It involves reducing organic nitrogen free-radicals with hydrogen-donating antioxidants leading to the generation of a non-radical form. In the present study, the PA exhibited a stronger DPPH radical scavenging activity of 78.5% (Fig. 1). The results of the DPPH scavenging activity is comparable to earlier reports [2, 21]. Previous studies have demonstrated that the significant ROS-scavenging activity of PA is due to its rich phenolic compounds that cause a strong antioxidant activity [2, 19]. Moreover, the search for natural antioxidants has increased significantly due to the adverse effect of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) [38]. The present results suggest that PA produced from pine at high temperature (1100 °C) could be a significant source of natural antioxidant that can be used in food, medicine, and agriculture.

Metabolites profile of PA

The metabolites profile analyses of PA produced from white pine at higher pyrolysis temperature revealed a total of 156 metabolites. These metabolites can be categorized into organic acids, hexose, carnitine and derivatives of phospholipids (Fig. 2).

Organic acids and hexose

Organic acids are the major class of metabolites in the white pine PA and comprised approximately, 90.9% (i.e. 8446.12 µM) of the total metabolites identified (Fig. 2; Table 2). The total organic acids are made up of 25 individual organic acids with varying levels. Notably, propanoic acid recorded the highest organic acid accounting for approximately, 48.8% of the total organic acids whereas methylmalonic acid, indole acetic acid, oxaloacetic acid, abscisic acid, aconitic acid and jasmonic acid were comparatively low (Table 2). Apart from propanoic acid, other organic acids influenced the high total organic acid content of the PA and include malic acid, butyric acid, succinic acid, glutaric acid, glyceric acid, β-hydroxybutyric acid, lactic acid, benzoic acid, oxalic acid, salicylic acid, citric acid, pyruvic acid and α-ketoglutaric acid. The high composition of organic acid has been reported as the major component of PA by several authors [22, 25, 30]. Total organic acids constituted 60.6% in walnut shell PA [25], 30.8% in walnut tree branches PA [2] and 2.7% in pineapple waste PA. Specifically, the high propanoic acid content in the white pine PA in the present study is consistent with the results of previous studies [2, 25, 39]. During pyrolysis, the integral component of wood (i.e. cellulose, hemicellulose and lignin) undergoes pyrolytic chemical reactions resulting in the formation of different compounds [1]. Degradation of hemicellulose presents the main origin to produce organic acids including propanoic acid [39–41]. Although the chemical properties of the feedstock were not investigated, it can be surmised that the high organic acid composition of PA could be due to a high hemicellulose content of the feedstock. Furthermore, the presence of phytohormones (i.e. salicylic acid, indole acetic acid, abscisic acid and jasmonic acid) in PA could explain its biostimulatory properties in regulating physiological and cellular responses of plants when applied [7, 8].

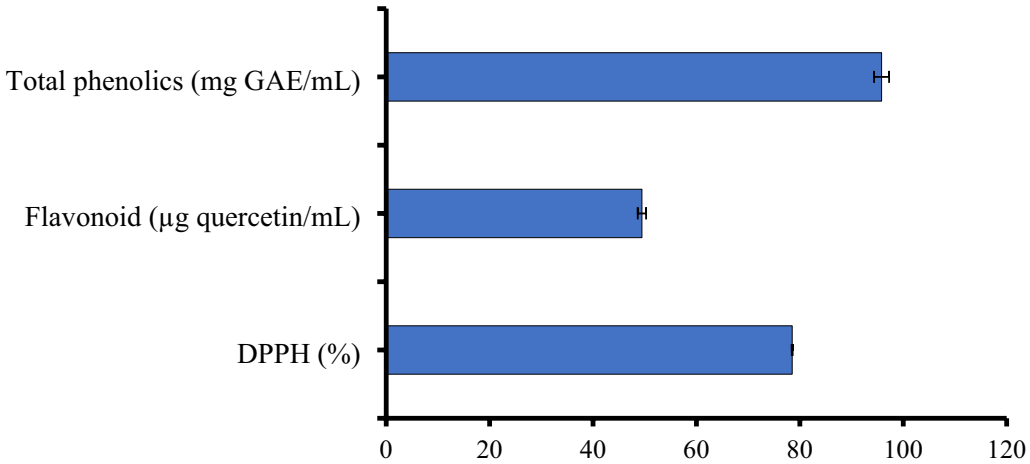


Fig. 1 Antioxidant activity of wood vinegar obtained from *Pinus strobus*. *DPPH* DPPH free-radical scavenging ability. Results are mean ± SD of four individual measurements

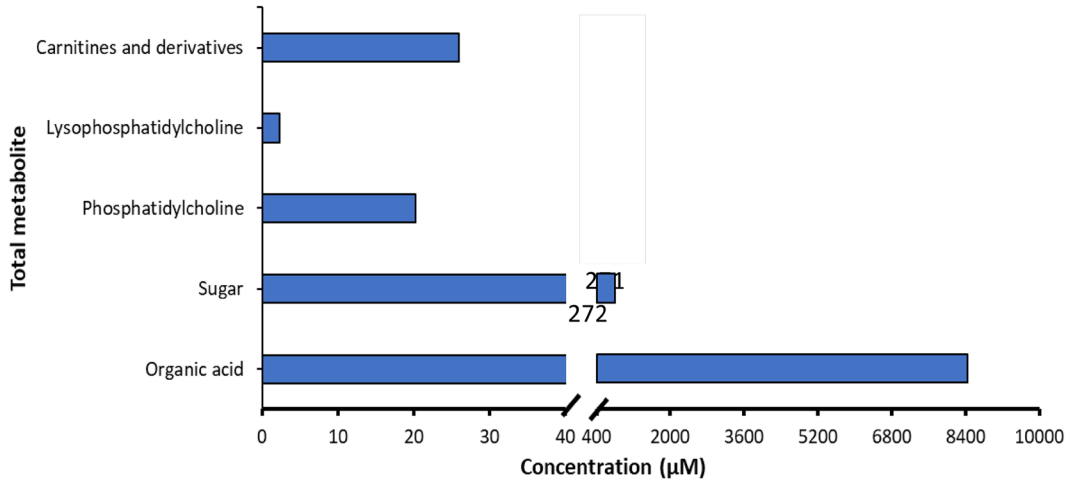


Fig. 2 Total metabolite composition of PA obtained from *Pinus strobus*

Table 2 Metabolic profile of organic acids in PA obtained from *Pinus strobus*

Metabolite name	LOD (µM)	Content (µM)
Propionic acid	0.03	4120.00
Succinic acid	0.02	1022.00
Lactic acid	0.87	809.00
Butyric acid	0.08	568.00
Benzoic acid	0.01	494.00
Glutaric acid	0.03	436.00
Pyruvic acid	0.06	434.00
Glyceric acid	0.04	238.00
β-Hydroxybutyric acid	0.04	97.60
Malic acid	0.06	70.30
Valeric acid	0.05	64.80
Oxalic acid	0.10	47.80
para-Hydroxyphenylacetic acid	0.07	11.30
α-Ketoglutaric acid	0.02	10.30
Citric acid	0.18	9.83
Salicylic acid	0.06	8.33
Fumaric acid	0.03	1.99
Shikimic acid	0.32	1.87
HPHPA	0.01	0.99
Methylmalonic acid	0.01	0.01
Oxaloacetic acid	0.08	< 0.09
Jasmonic acid	0.09	< 0.09
Abscisic acid	0.04	< 0.04
Aconitic acid	0.03	< 0.03
Indole acetic acid	0.02	< 0.02
Total		8446.12
Hexose	18.50	800.00

HPHPA,3-(3-hydroxyphenyl)-3-hydroxypropionic acids

LOD limit of detection

Intriguingly, the rich organic acids in PA were demonstrated to possess numerous adsorption sites, which can chelate heavy metals and render them less toxic in soils and unavailable for plant uptake [42–44]. Thus, this study suggests that PA can be used appropriately to remediate heavy metal pollutants in degraded soils [45, 46].

Thermal degradation of cellulose has been reported to yield the production of sugars [39]. In this study, hexose, a simple sugar with six carbon atoms accounted for 8.6% (800 µM) of the total metabolites (Table 2). Similar reports have indicated that PA contains sugar and its derivatives, and their concentration is dependent on the feedstock and pyrolysis temperature [1, 2, 21, 25]. The white pine PA sugar content as confirmed by the °Brix (Table 1) were considerably higher than those reported in the literature because of the higher pyrolytic temperature. Cellulose is composed of a linear homopolysaccharide of glucose moieties that are connected to a beta-glycosidic bond. It has been demonstrated that at a higher pyrolytic temperature above 300 °C, cellulose depolymerizes to glucose monomers and subsequent removal of water. This results in levoglucosan formation [47] and thereby, reducing the simple sugar levels in PA. Besides, Hu et al. [48] reported that pyrolysis of pine biomass at 500 °C yields the highest levoglucosan content. Nevertheless, the level of levoglucosan was not examined and will require further investigation.

Carnitine and derivatives

Carnitine and its derivatives are ubiquitous compounds identified in all living organisms including plants, which are known to play numerous critical biological, physiological and metabolic functions [49]. In this study, our result revealed the presence of a significant amount of

carnitine and its derivates in PA obtained from white pine (Fig. 2; Table 3). Carnitine and its derivatives accounted for 0.3% of the total identified metabolites and consisted of 39 individual carnitines (Table 3). The presence of carnitines in PA could be due to the feedstock used. Moreover, previous studies have reported the presence of carnitines and its derivatives in several plant species including avocado (*Persea americana*) and cauliflower (*Brassica oleracea var. botrytis*) [50], barley (*Hordeum vulgare*) [51], pea (*Pisum sativum*) [52–54], tobacco (*Nicotiana tabacum*), *Arabidopsis thaliana*, flax (*Linum usitatissimum*) and rape (*Brassica napus*) seeds [55]. Although no studies have reported the presence of carnitine in woody plants, it is plausible that our finding of carnitine in PA can be attributed to its presence in the pine feedstock. Interestingly, carnitines are heat stable when exposed to a temperature range of 80–130 °C [56]. Furthermore, its presence in PA produced at 1100 °C could suggest that carnitines are stable even at higher temperatures and could implicate a critical role in plants.

Functionally, carnitines are known to be involved in fatty acid metabolism by shuttling activated fatty acids into the mitochondrion as acylcarnitine for β-oxidation reaction [49]. Additionally, environmental stress studies revealed that carnitine and its derivatives can accumulate at high concentrations and act as osmolytes to protect cells against salt and chilling-induced osmotic stress [57–59]. It has been suggested that carnitines could facilitate fatty acid intercellular transport as an important membrane lipids adjustment process during chilling stress [57]. In maize (*Zea mays*), exogenous carnitine effectively enhanced seedling growth under both normal and cold stress conditions via modulation of photosynthesis, nitrogen assimilation and antioxidant enzyme activities [60]. Interestingly, carnitine modified saturated and unsaturated fatty acids ratio in maize cell membranes in support of more unsaturated fatty acid production to safeguard cellular membranes from cold stress damage [60]. Evidence revealed that exogenous application of 5 µM carnitine stimulated significant internal accumulation in 7-day-old *A. thaliana* seedling without causing developmental defects leading to induced antioxidant properties and increased seedling growth and salt stress tolerance [59]. Therefore, the utilization of PA in crop production could provide a sustainable avenue for environmental stress mitigation.

Table 3 Metabolic profile of total carnitine and derivatives in PA obtained from *Pinus strobus*

Metabolite name	Formula	LOD (µM)	Content (µM)
Carnitine	C0	0.22	6.73
Acetylcarnitine	C2	0.13	1.37
Propionylcarnitine	C3	0.08	0.68
Propenoylcarnitine	C3:1	0.03	0.28
Hydroxypropionylcarnitine	C3OH	0.03	0.84
Butyrylcarnitine	C4	0.05	0.50
Hydroxybutyrylcarnitine	C4OH	0.02	0.44
Butenylcarnitine	C4:1	0.02	0.23
Valerylcarnitine	C5	0.03	0.25
Glutarylcarnitine	C5DC	0.02	0.62
Methylglutarylcarnitine	C5MDC	0.02	0.39
Hydroxyvalerylcarnitine	C5OH	0.03	0.47
Tiglylcarnitine	C5:1	0.03	0.14
Glutaconylcarnitine	C5:1DC	0.03	0.37
Hexenoylcarnitine	C6:1	0.02	0.15
Pimelylcarnitine	C7DC	0.02	0.30
Octanoylcarnitine	C8	0.03	0.32
Nonaylcarnitine	C9	0.01	0.25
Decanoylcarnitine	C10	0.06	0.31
Decenoylcarnitine	C10:1	0.12	0.12
Decadienylcarnitine	C10:2	0.05	0.45
Dodecanoylcarnitine	C12	0.04	0.52
Dodecenoylcarnitine	C12:1	0.08	5.91
Dodecanedioylcarnitine	C12DC	0.01	0.11
Tetradecanoylcarnitine	C14	0.03	0.22
Tetradecenoylcarnitine	C14:1	0.07	0.25
Hydroxytetradecenoylcarnitine	C14:1OH	0.06	0.25
Tetradecadienylcarnitine	C14:2	0.05	0.37
Hydroxytetradecadienylcarnitine	C14:2OH	0.05	0.60
Hexadecanoylcarnitine	C16	0.03	0.30
Hydroxyhexadecanoylcarnitine	C16OH	0.04	0.12
Hexadecenoylcarnitine	C16:1	0.02	0.31
Hydroxyhexadecenoylcarnitine	C16:1OH	0.05	0.23
Hexadecadienylcarnitine	C16:2	0.05	0.27
Hydroxyhexadecadienylcarnitine	C16:2OH	0.04	0.27
Octadecanoylcarnitine	C18	0.02	0.21
Octadecenoylcarnitine	C18:1	0.04	0.29
Hydroxyoctadecenoylcarnitine	C18:1OH	0.06	0.20
Octadecadienylcarnitine	C18:2	0.06	0.26
Total			25.92

LOD limit of detection

Phospholipids

Total phospholipids contents represented 0.24% of the total identified metabolites and comprised 76 phosphatidylcholines (PC, Table 4A) and 14 lysophosphatidylcholines (LysoPC, Table 4B). Both PC and LysoPC accounted for about 0.22% and 0.03%, respectively (Fig. 2). Moreover, lignocellulosic-derived feedstocks are composed of phospholipids found in the plasma membrane of cells [61]. A previous study revealed that PCs are the main phospholipids in the outermost sapwood of pine, and vital for plasma membrane formation [62]. Similarly, characterization of pyrolysis tar obtained from

Table 4 Metabolic profile of phospholipids in PA obtained from *Pinus strobus*, (A) phosphatidylcholine (B) lysophosphatidylcholine

Metabolite name	Formula	LOD (μM)	Content (μM)
(A) Phosphatidylcholine			
Phosphatidylcholine diacyl	PC aa C24:0	0.02	0.10
Phosphatidylcholine diacyl	PC aa C26:0	0.27	0.51
Phosphatidylcholine diacyl	PC aa C28:1	0.05	0.06
Phosphatidylcholine diacyl	PC aa C30:2	0.02	0.08
Phosphatidylcholine diacyl	PC aa C30:0	0.02	0.14
Phosphatidylcholine diacyl	PC aa C32:3	0.03	0.34
Phosphatidylcholine diacyl	PC aa C32:2	0.04	6.68
Phosphatidylcholine diacyl	PC aa C32:1	0.06	1.10
Phosphatidylcholine diacyl	PC aa C32:0	0.01	0.13
Phosphatidylcholine diacyl	PC aa C34:4	0.02	0.03
Phosphatidylcholine diacyl	PC aa C34:3	0.01	0.07
Phosphatidylcholine diacyl	PC aa C34:2	0.07	0.23
Phosphatidylcholine diacyl	PC aa C34:1	0.11	0.23
Phosphatidylcholine diacyl	PC aa C36:6	0.13	0.08
Phosphatidylcholine diacyl	PC aa C36:5	0.05	0.13
Phosphatidylcholine diacyl	PC aa C36:4	0.05	0.13
Phosphatidylcholine diacyl	PC aa C36:3	0.08	0.11
Phosphatidylcholine diacyl	PC aa C36:2	0.02	0.27
Phosphatidylcholine diacyl	PC aa C36:1	0.01	0.19
Phosphatidylcholine diacyl	PC aa C36:0	0.08	0.15
Phosphatidylcholine diacyl	PC aa C38:6	0.81	0.13
Phosphatidylcholine diacyl	PC aa C38:5	0.55	0.08
Phosphatidylcholine diacyl	PC aa C38:4	0.08	0.08
Phosphatidylcholine diacyl	PC aa C38:3	0.08	0.06
Phosphatidylcholine diacyl	PC aa C38:1	0.05	0.07
Phosphatidylcholine diacyl	PC aa C38:0	0.11	0.09
Phosphatidylcholine diacyl	PC aa C40:6	0.11	0.12
Phosphatidylcholine diacyl	PC aa C40:5	0.02	0.12
Phosphatidylcholine diacyl	PC aa C40:4	0.16	0.12
Phosphatidylcholine diacyl	PC aa C40:3	0.09	0.07
Phosphatidylcholine diacyl	PC aa C40:2	0.81	0.06
Phosphatidylcholine diacyl	PC aa C40:1	0.58	0.05
Phosphatidylcholine diacyl	PC aa C42:6	1.18	0.07
Phosphatidylcholine diacyl	PC aa C42:5	0.47	0.14
Phosphatidylcholine diacyl	PC aa C42:4	0.09	0.35
Phosphatidylcholine diacyl	PC aa C42:2	0.06	0.09
Phosphatidylcholine diacyl	PC aa C42:1	0.18	0.08
Phosphatidylcholine diacyl	PC aa C42:0	0.11	0.05
Phosphatidylcholine acyl-alkyl	PC ae C30:2	0.07	0.05
Phosphatidylcholine acyl-alkyl	PC ae C30:1	0.07	0.05
Phosphatidylcholine acyl-alkyl	PC ae C30:0	0.08	0.11
Phosphatidylcholine acyl -alkyl	PC ae C32:2	0.02	3.42
Phosphatidylcholine acyl-alkyl	PC ae C32:1	0.04	0.18
Phosphatidylcholine acyl-alkyl	PC ae C34:3	0.29	0.07
Phosphatidylcholine acyl-alkyl	PC ae C34:2	0.36	0.32
Phosphatidylcholine acyl-alkyl	PC ae C34:1	0.35	0.46
Phosphatidylcholine acyl-alkyl	PC ae C34:0	0.07	0.29
Phosphatidylcholine acyl-alkyl	PC ae C36:5	0.05	0.11

Table 4 (continued)

Metabolite name	Formula	LOD (μM)	Content (μM)
Phosphatidylcholine acyl-alkyl	PC ae C36:4	0.07	0.07
Phosphatidylcholine acyl-alkyl	PC ae C36:3	0.04	0.03
Phosphatidylcholine acyl-alkyl	PC ae C36:2	0.01	0.11
Phosphatidylcholine acyl-alkyl	PC ae C36:1	0.02	0.13
Phosphatidylcholine acyl-alkyl	PC ae C36:0	0.02	0.12
Phosphatidylcholine acyl-alkyl	PC ae C38:6	0.04	0.10
Phosphatidylcholine acyl-alkyl	PC ae C38:5	0.06	0.07
Phosphatidylcholine acyl-alkyl	PC ae C38:4	0.06	0.04
Phosphatidylcholine acyl-alkyl	PC ae C38:3	0.03	0.05
Phosphatidylcholine acyl-alkyl	PC ae C38:2	0.02	0.07
Phosphatidylcholine acyl-alkyl	PC ae C38:1	0.01	0.10
Phosphatidylcholine acyl-alkyl	PC ae C38:0	0.12	0.12
Phosphatidylcholine acyl-alkyl	PC ae C40:6	0.08	0.11
Phosphatidylcholine acyl-alkyl	PC ae C40:5	0.01	0.07
Phosphatidylcholine acyl-alkyl	PC ae C40:4	0.01	0.06
Phosphatidylcholine acyl-alkyl	PC ae C40:3	0.01	0.06
Phosphatidylcholine acyl-alkyl	PC ae C40:2	0.01	0.06
Phosphatidylcholine acyl-alkyl	PC ae C40:1	0.07	0.07
Phosphatidylcholine acyl-alkyl	PC ae C42:5	0.03	0.23
Phosphatidylcholine acyl-alkyl	PC ae C42:4	0.01	0.18
Phosphatidylcholine acyl-alkyl	PC ae C42:3	0.01	0.09
Phosphatidylcholine acyl-alkyl	PC ae C42:2	0.01	0.06
Phosphatidylcholine acyl-alkyl	PC ae C42:1	0.01	0.06
Phosphatidylcholine acyl-alkyl	PC ae C42:0	0.02	0.06
Phosphatidylcholine acyl-alkyl	PC ae C44:6	0.02	0.07
Phosphatidylcholine acyl-alkyl	PC ae C44:5	0.02	0.12
Phosphatidylcholine acyl-alkyl	PC ae C44:4	0.01	0.11
Phosphatidylcholine acyl-alkyl	PC ae C44:3	0.00	0.16
Total			20.21
(B) Lysophosphatidylcholine			
Lysophosphatidylcholine acyl C14:0	LysoPC a C14:0	0.19	0.35
Lysophosphatidylcholine acyl C16:0	LysoPC a C16:0	0.02	0.30
Lysophosphatidylcholine acyl C16:1	LysoPC a C16:1	0.05	0.22
Lysophosphatidylcholine acyl C17:0	LysoPC a C17:0	0.15	0.26
Lysophosphatidylcholine acyl C18:0	LysoPC a C18:0	0.03	0.17
Lysophosphatidylcholine acyl C18:1	LysoPC a C18:1	0.05	0.17
Lysophosphatidylcholine acyl C18:2	LysoPC a C18:2	0.27	0.17
Lysophosphatidylcholine acyl C20:3	LysoPC a C20:3	0.02	0.02
Lysophosphatidylcholine acyl C20:4	LysoPC a C20:4	0.03	0.20
Lysophosphatidylcholine acyl C24:0	LysoPC a C24:0	0.13	0.12
Lysophosphatidylcholine acyl C26:0	LysoPC a C26:0	0.03	0.10
Lysophosphatidylcholine acyl C26:1	LysoPC a C26:1	0.03	0.09
Lysophosphatidylcholine acyl C28:0	LysoPC a C28:0	0.02	0.09
Lysophosphatidylcholine acyl C28:1	LysoPC a C28:1	0.08	0.06
Total			2.34

LOD limit of detection

different wood species showed that pine tar contains significantly higher hydrophobic extractives including phospholipids compared with that of oak and bamboo [63]. Additionally, high phospholipid accumulation was noted to increase in twig bark and wood of poplar plants and functions to harden plants during frost stress [64]. The transformation of these phospholipids during pyrolysis is unknown and requires further investigation. However, the presence of phospholipids in PA from the white pine biomass can further confirm their ubiquitous nature in plant extracts.

Intriguingly, PCs are the most abundant phospholipids and function in maintaining cell membrane integrity even under stressful environmental conditions [65]. PCs are known to play critical roles in mediating physiological and cellular metabolisms including cell elongation and signalling and defence via association with other membrane proteins [66]. Also, PCs act as precursors for the biosynthesis of diverse signalling molecules including LysoPC, diacylglycerol (DAG), phosphatidic acids and arachidonic acid [65]. LysoPC serves as a secondary messenger that mobilises vacuolar proton pool and stress-activating enzymes for defence response [67]. A recent study demonstrated that exogenous PC treatment of peach (*Prunus persica*) seedlings expanded guard cells to maintain sufficient gas exchange, promote proline accumulation, lowered lipid peroxidation and increase cell membrane integrity, which enhanced tolerance to drought stress [68]. Similarly, LysoPC has been reported to stimulate the immune response of solanaceous plants against potato (*Solanum tuberosum*) virus Y and *Phytophthora infestans* by restricting pathogen proliferation [69]. All these results imply that the white pine PA can be highly efficacious for use to enhance plant growth by protecting membrane integrity and activating biological processes and components against both biotic and abiotic stresses.

Table 5 Mineral nutrient composition of PA obtained from *Pinus strobus*

Mineral element (mg/L)	RL	Content
Kjeldahl nitrogen	50.00	460.00
Nitrate and nitrite	50.00	100.00
Aluminum	1.00	2.00
Antimony	0.10	< 0.10
Arsenic	1.00	< 1.00
Barium	1.00	< 1.00
Beryllium	0.10	< 0.10
Bismuth	1.00	< 1.00
Boron	1.00	< 1.00
Cadmium	0.01	0.20
Calcium	50.00	100.00
Chromium	1.00	< 1.00
Cobalt	0.10	< 0.10
Copper	1.00	< 1.00
Iron	20.00	< 20.00
Lead	0.10	0.60
Lithium	0.10	< 0.10
Magnesium	10.00	< 10.00
Manganese	1.00	1.00
Molybdenum	0.10	< 0.10
Nickel	1.00	< 1.00
Potassium	20.00	180.00
Rubidium	0.10	0.10
Selenium	1.00	< 1.00
Silver	0.10	< 0.10
Sodium	50.00	< 50.00
Strontium	1.00	< 1.00
Tellurium	0.10	< 0.1.00
Thallium	0.10	< 0.10
Tin	1.00	< 1.00
Uranium	0.10	< 0.10
Vanadium	1.00	< 1.00
Zinc	1.00	10.00

RL reporting limit

Mineral element content

The mineral nutrient analysis showed 33 mineral elements in PA. Several elements were detected, which includes macroelements [nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg)], microelements [Iron (Fe), zinc (Zn), copper (Cu), boron (B), manganese (Mn), molybdenum (Mo)], trace and/or heavy metals (aluminum, antimony, arsenic, barium, beryllium, bismuth, cadmium, chromium, cobalt, lead, lithium, nickel, rubidium, selenium, silver, strontium, tellurium, thallium, tin, uranium, vanadium) and sodium (Na) (Table 5). The highest elemental content was noted for Kjeldahl N followed by K, nitrate and nitrite, Ca, and Zn (Table 5). In this study, the total Kjeldahl N which includes organic N, ammonia and ammonium was 460 mg/L, whereas other inorganic N such as nitrate and nitrite recorded 100 mg/L. Potassium content recorded 180 mg/L which was higher than previous studies. For instance, PA derived from oak contained 13.23 mg/L of K [70], mangrove wood PA contained moderate levels of K [71], fruitwood PA contained 0.01 mg/L [36] while 3.63–11.79 mg/L of K was recorded in Durian (*Durio zibethinus*) PA produced between 350 and 550 °C [72]. Also, Ca content in the white pine PA was 100 mg/L which was higher than Durian PA (14.72 mg/L) produced at 550 °C [72] and mangrove wood (8.82 mg/L) [71]. Conversely, the Ca content was lower when compared to other PAs produced from corn straw (276 mg/L) [35]

and oak (375.88 mg/L) [70]. Zn content in this study was 10 mg/L which was higher than PA derived from fruitwood (5.5 mg/L) [36] and mangrove wood PA [71], but lower than PA obtained from oak PA (16.71 mg/L) [70] and Durian PA (36.52–42.71 mg/L) produced at pyrolysis temperature between 350 and 550 °C [72]. Additionally, Mg, Fe, Cu, B, Mn, Mo, Na and all the trace and heavy metals were present at levels below the reported limits (Table 5). In contrast, Setiawati et al. [72] and Rui et al. [70] reported high levels of Mg (5.92–10.42 mg/L and 133.43 mg/L), Fe (233.02–282.02 mg/L and 578.62 mg/L) and Na (14.03–14.72 mg/L and 146.15 mg/L) in durian and oak PA, respectively compared to the PA values for the white pine in the present study. Furthermore, these differences in PA elemental compositions can be attributed to the different feedstock, plant growth conditions and the pyrolysis temperature.

Generally, plant growth and development are influenced extensively by mineral nutrient availability. While N is a key factor that modulate vegetative growth through its involvement in proteins, enzymes and chlorophyll synthesis, K function in enzyme activation, photosynthesis and water relation improvement, and assimilate transportation [73–75]. Also, Ca is critical for cell elongation and division, facilitate soil nutrient uptake and ameliorate plant tissue resistance against biotic and abiotic stresses by acting as a signalling messenger for downstream activation of defensive genes and pathways [76–79]. Thus, the presence of high N, K and Ca contents suggest that PA can be used as an alternative nutrient source in crop production for promoting plant growth and yield. Additionally, the presence of very low contents of Na and heavy metals in PA derived from the white pine could imply that using PA for crop production may not increase soil salinity and heavy metal.

Conclusion

Pyroligneous acid obtained from *Pinus* (white pine) was analysed for its chemical qualities, antioxidant activities and complete metabolite and mineral nutrient profile. The results revealed that white pine PA had a lower electrical conductivity, salinity, and total dissolved solids but higher in °Brix. Moreover, the antioxidant activity assay showed a higher radical scavenging activity against DPPH which could be ascribed to the high total phenolic and flavonoid contents. Metabolic profiling indicates that white pine PA contains 156 metabolites of which organic acids represent 90.9% followed by hexose, carnitines, and phospholipids. Mineral elements analysis showed higher contents of N, K, Ca and Zn with lower content of the other trace elements or heavy metals. Therefore, the presence of these bioactive compounds and elements in white pine PA offers a potential use of PA as an alternative source of plant growth promoter for enhancing plant productivity and resilience to environmental stresses.

Abbreviations

PA: Pyroligneous acid; DNCB: 2,4-Dinitrochlorobenzene; DPPH: 1,1-Diphenyl-2-picrylhydrazyl; TCA: Trichloroacetic acid; BHA: Butylated hydroxyanisole; BHT: Butylated hydroxytoluene; DTT: Dithiothreitol; EDTA: Ethylene diamine tetra acetic acid; TDS: Total dissolved solids; EC: Electric conductivity; TPC: Total phenolics content; PC: Phosphatidylcholine; LOD: Limit of detection.

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Author contributions

Conceptualization; LA, RO. Data curation and formal analysis; RO, LRG. Funding acquisition; LA. Investigation; RO and LRG. Methodology; RO and LRG. Project administration; LA. Resources; LA. Supervision; LA. Validation; RO, LA and LRG. Writing—original draft; RO. Writing—review and editing; RO, LRG, LA. All authors read and approved the final manuscript.

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Availability of data and materials

The data sets used in this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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