RESEARCH

Banana-pseudostem sap growing media as a novel source of phytochemicals and mineral nutrients: Influence on seedling growth of sweet corn

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ABSTRACT

Phytochemicals in plant extracts help plants to grow, develop and survive. This study aimed to identify the phytochemicals and quantify soluble nutrients present in banana (*Musa acuminata* Colla)-pseudostem sap, and to observe the subsequent effect on seedling growth of sweet corn (*Zea mays* L. var. *saccharata* (Sturtev.) L.H. Bailey). The collected banana pseudostem sap was extracted with methanol followed by a liquid-liquid extraction procedure. The extracted fractions were analyzed by liquid chromatography mass spectrometry (LC-MS). Seven concentrations of fresh banana pseudostem sap, i.e., 5%, 10%, 15%, 25%, 50% aqueous, and 100% raw sap with control (distilled water) were replicated three times following a completely randomized design. The LC-MS analysis identified 86 various phytocompounds, while inductively coupled plasma (ICP) quantified different essential plant nutrients in the fresh sap, confirming the benefits of phytocompounds and mineral nutrients on sweet corn. The sap showed 44 beneficial plant compounds such as 1-amino-1-carboxycyclopropane, 4-aminobenzoic acid, glutamic acid, 1,4-benzoquinone, proline betaine, butanoic acid, hydroxysouric acid, 2,4-dihydroxybenzoic acid and aluminium acetate. These are vital for plant growth and development. The results confirmed that a high number of beneficial secondary metabolites and mineral nutrients in aqueous banana pseudostem sap, especially in lower concentrations (5%-15%), significantly influenced seedling growth over higher concentration and control. However, compared to control, 10% aqueous banana pseudostem sap resulted in 21.2% larger root length, 19.6% shoot length, 32.0% dry biomass, and 17.4% chlorophyll content, respectively. It is concluded that a 10% aqueous solution of fresh banana pseudostem sap will be more effective for crop production.

Key words: Banana pseudostem sap, mineral nutrients, phytochemicals, seedling growth, sweet corn.

INTRODUCTION

Banana (*Musa acuminata* Colla) belong to Musaceae family is an exoteric tropical fruit and an important source of food throughout the world. Generally, after harvesting banana, producers simply leave the pseudostem (overlapping leaf sheaths of the trunk) in the field (Sharma et al., 2017). However, sap is the dominant fluid in banana plant and especially in the pseudostem (85%), and it should be saved for use as an effective organic fertilizer (Cao et al., 2018). This substances contains a high concentration of nutrients (Bahtiar et al., 2017). Banana and plantains are rich in nutrient, especially K and Ca (Pradhan and Deo, 2019). Mohapatra et al. (2010) studied the large amounts of P, K, Ca, and Mg in banana pseudostem compared to other parts of the banana plant. It contains a lot of phytocompounds depending on the genotypic variation, age, and plant parts it is situated in (Deng et al., 2020). The extraction technique and type of tools used may influence the

identification of phytocompounds existing in the plant. Currently, liquid chromatography mass spectrometry (LC-MS) methodology-based metabolic profiling has been improved and it can investigate the diversity of non-target phytochemicals via a special library. Structural information can be obtained without extra MS/MS analysis (Matsuda et al., 2009).

Plants contain diverse secondary metabolites which are produced in the plant metabolic process and make a great contribution to growth, development and defense mechanism (Erb and Kliebenstein, 2020). Some compounds occurring in extracts from plant materials such as flavonoids, phenolic compound, alkaloid organic acids (Mohapatra et al., 2010), protein, and non-protein amino acid molecules (Qiu et al., 2020), may play a key role in plant growth and development. The chlorophyll content, shoot length, and biomass of sweet corn increased when amino acid was applied (Tadros et al., 2019). Moreover, amino acids *viz*., tryptophan, glycine, and glutamic acid elevated the hydroponic production of lettuce (Khan et al., 2019), and these were present in banana pseudostem (Deng et al., 2020). The phenolics and flavonoids in Calotropis plant (milkweed, Sodom-apple) extract led to a higher seed germination in wheat and barley (Radwan et al., 2019). Apart from these, oligosaccharides in banana help to grow beneficial bacteria and act as cofactors in the metabolic process (Mohapatra et al., 2010). The 3-amino-2-naphthoic acid and cyclopentene-1-acetic acid extracted from banana pseudostem (Deng et al., 2020) individually influenced plant growth, metabolism; they were also evaluated for their anti-pathogenic activity in maize and okra plants (Zhang et al., 2017). Using phytocompounds to supplement traditional fertilizers will lead to better plant protection, growth, development, yield, and quality of the final product (Sharma et al., 2014).

To date, researchers have reported the presence of phenolics, flavonoids, alkaloids, protein, saponins, tannins, and glycosides in banana pseudostem sap and banana flower extract (Onyema et al., 2016). Borges et al. (2014) characterized secondary metabolites in banana plants. Similarly, Ulfa et al. (2013) observed auxin, gibberellin, and cytokinin in banana-hump and leaf. The phenolics, flavonoids, and antioxidant capacity of banana flesh, peel, and boiled peels were evaluated by Khawas and Deka (2016). Results from various experiments confirmed that the sap from banana pseudostem extraction contained large amounts of phenolics, flavonoid and alkaloids, while saponin in the same samples was only slightly detected (Pradhan and Deo, 2019). In another study, Pothavorn et al. (2010) reported the existence of dopamine, *N*-acetyl serotonin, hydroxy cinnamic acids, glycosides, glucose, and rhamnose in the sap of banana. Recently, Deng et al. (2020) identified a large number of bio-active compounds in banana pseudostem such as alkaloids, amino acids and their derivatives (L-lysine and L-tryptophan, L-glutamic acid, phenylalanine), flavonoids, lipids (palmitoleic acid, and α-linolenic acid), nucleotides (adenine, guanosine), and organic acids. These are important nutritional components of the human diet. The inclusive metabolome characterization of banana fruit revealed that it is a source of several secondary metabolites including organic acids, amino acids, peptides, alkaloids, benzenoids, and phenylpropanoids and derivates.

Most of the research done on phytochemicals in banana plants has been to assess their use in the food manufacturing industry. Roy et al. (2006) examined the seedling growth of vegetables treated with different concentrations of banana plant extract. Syawal et al. (2018) studied banana pseudostem sap for corn production. Higher nutrient uptakes were recorded in fenugreek through the application of enriched banana pseudostem sap (Misal et al., 2019). Apart from banana extract, Sharma et al. (2014) identified bio-stimulants in seaweed sap for plant use. Talukder et al. (2015) examined herbal plants extract and its ability to germinate different vegetable seeds. However, very little research has focused on chemical constituents of banana pseudostem sap for crop production. There is little published literature on the influence of secondary metabolites in banana pseudostem sap and how they help seedlings' growth and development. To the best of our knowledge, this study is the first evaluation of banana pseudostem sap on seedling growth, the roles of both phytocompounds and mineral nutrients are considered. It will further explore the potential of banana pseudostem sap for use as an organic liquid fertilizer in sustainable agriculture and aspects of pharmacological research. Hence, the study was executed to identify the beneficial phytochemicals and nutrient composition in banana pseudostem sap and their subsequent impact on the seedling growth of sweet corn.

MATERIALS AND METHODS

Collection and preparation of banana pseudostem sap

Mature and non-productive banana pseudostems were collected from a banana field located in Banting (2°48'16.1" N, 101°30'10.99" E), Selangor, Malaysia, on 1st May 2020 during the dry season. The banana pseudostems were immediately washed with fresh tap water after removing the rotted or infected sheath to clean the dirty surface. The pseudostems were unfolded and then washed twice with distilled water, then chopped into small pieces and the sap was collected via mortar

and pestle operation. About 1.5 L banana pseudostem sap was collected and retained in a clean airtight glass jar. Half of the collected sap was immediately preserved in a refrigerator at -16 °C for phytocompounds analysis, while the other half was preserved at 4 °C to determine the chemical properties at the facilities of the Department of Land Management, Faculty of Agriculture, University Putra Malaysia.

Extraction of banana pseudostem sap with methanol solvent

About 200 mL fresh banana pseudostem sap was put into a 1000 mL volumetric flask separately and the required volume was made up with methyl alcohol 99.95% (HPLC grade, Sigma-Aldrich, St. Louis, Missouri, USA). Thereafter, 48 h of continuous shaking was done via an orbital shaker at 250 rpm. The sample solution was filtered with filter paper Whatman 125 mm (Grade 1; Whatman/GE Healthcare Companies, England). The filtered solution was then preserved in a refrigerator at 4-5 °C after being wrapped in aluminum foil to protect the sample from light. Alcohol and excess water were evaporated using a rotary evaporator (CCA-111, EYELA, Tokyo, Japan) where the heater and cooler temperature were maintained at 41 °C with 6 rpm and 10 °C, respectively. Finally, 5 mL of concentrated sample solution were collected in a 10 mL glass vial and analyzed at the laboratory utilizing liquid chromatography-mass spectrometry (LC-MS).

Liquid chromatography-mass spectrometry (LC-MS) studies

The phytochemical compounds of the methanol sap were identified using LC-MS equipped with a binary pump (Agilent, Santa Clara, California, USA). The LC-MS was an interfaced Agilent 1290 Infinity LC system coupled with Agilent 6520 accurate-mass Q-TOF mass spectrometer with a dual electrospray ionization (ESI) source. Full-scan mode from m/z 50 to 1000 was executed with a source temperature of 125 °C. The column of Agilent Zorbax eclipse XDB-C18, narrow-bore 2.1×150 mm, 3.5 microns (P/N: 930990-902) served to maintain the column temperature of 30 °C for analysis where 0.1% formic acid in water and 0.1% formic acid in methanol were used as solvent A and B, respectively. Solvents were delivered at a total flow rate of 0.1 mL min^{-1} and run by isocratic elution. The MS spectra were acquired in both the positive and negative ion modes. The temperature of the drying gas was 300 °C, at a gas flow rate of 10 L min^{-1}, with 45 psi nebulizing pressure. About 1 mL concentrated sample sap was diluted with methanol and filtered with a 0.22 µm nylon filter before analysis. A volume of 1 µL sap was injected into the analytical column for analysis. The mass fragmentations were identified using a spectrum database for organic compounds in Agilent mass hunter qualitative analysis B.07.00 (Metabolomics-2019 m) application.

Determination of chemical properties in the banana pseudostem sap

The pH was measured from banana pseudostem sap samples utilizing a digital pH meter (HI 2211 pH meter, Hanna Instruments, Woonsocket, Rhode Island, USA), whereas the electrical conductivity (EC) in the same samples was determined by digital EC meter (Hanna 2300). The total N was determined by TruMac CNS analyzer (Leco, St. Joseph, Missouri, USA). The collected banana pseudostem sap was diluted 50 times with distilled water in order to determine the properties of total nutrients, namely P, K, Ca, Mg, Na, Zn, Cu, and B through inductively coupled plasma (ICP)-optical emission spectroscopy (Optima 8300, PerkinElmer Corporation, Norwalk, Connecticut, USA) with the standard solutions. The chemical properties of banana pseudostem sap samples are described more detail in Table 1.

Table 1. Chemical properties of banana pseudostem sap.

Chemical characters	Banana pseudostem sap
pH	5.29 ± 0.20
Electrical conductivity, µS cm^{-1}	6.47 ± 0.26
Total N, g L^{-1}	4.25 ± 0.13
Total P, g L^{-1}	0.92 ± 0.03
Total K, g L^{-1}	2.03 ± 0.06
Total Ca, mg L^{-1}	6.00 ± 0.19
Total Mg, mg L^{-1}	83.39 ± 1.91
Na, mg L^{-1}	2.30 ± 0.08
Cu, mg L^{-1}	2.50 ± 0.09
Zn, mg L^{-1}	1.00 ± 0.02
B, g L^{-1}	0.25 ± 0.01

Means ± standard deviations are the average of three replicates.

Preparation of sap for seedling growth

The collected fresh sap (without methanol) was filtered and diluted with distilled water to prepare 5%, 10%, 15%, 25%, and 50% aqueous solutions. Meanwhile raw sap was considered as 100% banana pseudostem sap solution to evaluate the germination and growth of seedlings. Three pieces of filter paper were placed in a sterilized Petri dish. According to treatments, 8 mL of above mentioned aqueous fresh sap were poured into a Petri dish where distilled water was used as the control. Sweet corn seeds (*Zea mays* L. var. *saccharata* (Sturtev.) L.H. Bailey; Hybrid F1 592, Leckat, Kepong, Malaysia) were soaked for 12 h in water before 10 sprouting seeds were placed in the Petri dish with an equal distance between them. The study was carried out in the Laboratory of Land Management Department, University Putra Malaysia, where the ambient temperature was 26 °C, humidity 70%, and normal light (daytime) conditions. An equal volume (5 mL) of a similar solution in each Petri dish was previously added in order to dry the Petri dish, which was continued for all 10 d of the experimental period. The data on germination (%), root length (cm), shoot length (cm), fresh weight (digital balance), biomass weight (oven dry method), and chlorophyll content (SPAD-502Plus, Konica Minolta, Tokyo, Japan) were recorded. The root and shoot lengths of corn seedlings were measured from a photography produced with Image J software (Rasband, W.S., U.S. National Institutes of Health, Bethesda, Maryland, USA).

Statistical analysis

Data were subjected to statistical analysis with the use of PROC software following completely randomized design (SAS 9.4; SAS Institute, Cary, North Carolina, USA). Treatment means were compared by least significant difference (LSD) test at the 5% probability level.

RESULTS

Characterization of phytocompounds in fresh banana pseudostem sap

Secondary metabolites identified with methanolic extraction of fresh banana pseudostem sap using LC-MS are shown in Table 2. A total of 86 compounds of positive ($M\text{-}H^+$) and negative ($M\text{-}H^-$) charge were detected by given chromatographic pick under the retention time 0.63 to 21.59 min (Figures 1 and 2), including chemical formula. All 86 molecules were grouped into various classes such as amino acid, phenolic compounds, fatty acid, flavonoid, alkaloid, carbohydrates, organic acids, sulphur compound, glycosides, and others (Table 2). Among the identified molecules, 18 amino acids, namely 2-methanidylpropane, prolamine, 1-amino-1-carboxy cyclopropane, *N*,*N*-diethylglycine, glutamic acid, cysteinyl-alanine, isoleucylglycine, tetramethylguanidinyl azide, *S*-amino methyl dihydro lipoamide, benzyloxy carbonyl arginine, alanyl tryptophan, asparaginyl-hydroxyproline, and *N*-methacryloyl glycine were obtained. Besides, 25 molecules *viz*; *N*-methylacetoacetamide, pyridate, 2,4-dihydroxybenzoic acid, cinnamic acid, gingerol, 3,5-diazidobenzoic acid, 4-aminobenzoic acid, trimethylgallic acid, 2-oxo-4-methylthiobutanoic acid, 3-amino-2-naphthoic acid, hydroxybutyric acid, piscidic acid and acetic acid-aluminum salt (3:1) were under the phenolic group. Similarly, the butanoic acid, trimethyluric acid, sorbitolamine, abscisic acid, benzenesulfonic acid, itaconic acid, and fumaric acid were in the organic acid class. The compounds sucrose, 3-*O*-methylglucose, deoxyribose, 1,4-*d*-xylobiose, d-glucose, ketolactose were discovered as carbohydrates while 5-hydroxysouric acid and usambarensine were deemed to be alkaloidal compounds. In addition, vitamins (ethyl 6-hydrazinonicotinate), sulphur compounds (sinalexin), and many others were identified from the banana pseudostem sap sample. The overall study found 44 molecules that will benefit plants and 50 molecules that can assist human health (Table 2). The LC-MS chromatogram confirmed the existence of different metabolites with a particular retention time illustrated in Figures 1 and 2.

Effects of fresh pseudostem sap on seedling growth of sweet corn

The concentration of banana pseudostem sap significantly influenced ($p \leq 0.05$) seed germination of sweet corn (Table 3, Figure 3). The largest percentage concerning the germination of sweet corn seeds was 92.3% in 5% aqueous sap, which was significantly identical to 91.7%, 90.0%, and 90.0% in 10%, 15% aqueous sap, and control, respectively. The smallest germination (57.7%) was detected in 100% raw sap treated seeds followed by other treatments. Results also showed that 5% aqueous fresh sap provided (2.57%) higher seed germination while 100% raw sap had 35.9% lower germination over the control. On the other hand, 10% concentration of fresh sap resulted in a higher root length (15.5 cm) which was identical to root length recorded in 5% (14.66 cm) and 15% aqueous sap (15 cm), respectively.

Table 2. Identification of beneficial phytochemicals in banana pseudostem sap by liquid chromatography mass spectrometry.

Serial Nr	Compounds name	Chemical group	Chemical formula	Retention time (min)	M/Z ratio	Mass	Plant beneficial Yes/Not	Health beneficial Yes/Not	Others
1	Prolamine	Amino acid	C_4H_9N	1.34	72.08	71.07	Y	N	-
2	Pipecolic acid		$C_6H_{11}NO_2$	1.36	130.08	129.08	Y	N	-
3	1-Amino-1-carboxycyclopropane		$C_4H_7NO_2$	1.53	102.05	101.05	Y	N	-
4	*N*,*N*-Diethylglycine		$C_6H_{13}NO_2$	2.18	132.10	131.09	Y	N	-
5	Glutamic acid		$C_5H_{10}N_2O_3$	1.22	147.07	146.07	Y	N	-
6	Cysteinyl-alanine		$C_6H_{12}N_2O_3S$	0.694	193.06	192.06	N	Y	-
7	Isoleucylglycine		$C_8H_{16}N_2O_3$	14.16	187.10	188.11	Y	Y	-
8	Tetramethylguanidinyl azide		$C_5H_{12}N_6$	14.42	157.12	156.11	Y	N	-
9	*S*-aminomethyldihydrolipoamide		$C_9H_{20}N_2OS_2$	1.81	259.09	236.10	Y	N	-
10	Benzyloxycarbonylarginine		$C_{14}H_{20}N_4O_4$	13.80	307.14	308.14	N	Y	-
11	Alanyl tryptophan		$C_{14}H_{17}N_3O_3$	9.00	276.13	275.13	N	Y	-
12	Asparaginyl-hydroxyproline		$C_9H_{15}N_3O_5$	3.20	268.09	245.10	Y	N	-
13	*N*-methacryloyl glycine		$C_6H_9NO_3$	4.55	144.06	143.06	Y	N	-
14	Sulfonamide		$C_{10}H_{13}NS$	1.09	180.08	179.07	Y	N	-
15	Benzphetamine		$C_{17}H_{21}N$	12.97	274.13	239.16	N	Y	-
16	2-[(*Z*)-9-Octadecenylamino] pyridine		$C_{23}H_{40}N_2$	21.14	343.31	344.31	N	Y	-
17	Glutamyl-glutamic acid		$C_{15}H_{23}N_3O_{10}$	1.15	404.13	405.13	Y	Y	-
18	Proline betaine		$C_7H_{13}NO_2$	4.69	166.08	143.09	Y	N	-
19	*N*-Methylacetoacetamide	Phenolic	$C_5H_9NO_2$	1.14	116.06	115.06	Y	N	-
20	Pyridate		$C_{19}H_{23}ClN_2O_2S$	1.16	377.10	378.12	Y	N	-
21	2,4-Dihydroxybenzoic acid		$C_7H_6O_4$	2.07	153.02	154.02	N	N	-
22	Cinnamic acid		$C_9H_{10}O_3$	8.40	165.05	166.06	N	Y	-
23	Gingerol		$C_{17}H_{26}O_4$	14.00	293.17	294.18	N	Y	-
24	3,5-Diazidobenzoic acid		$C_7H_4N_6O_2$	1.79	203.03	204.04	N	Y	-
25	4-Aminobenzoic acid		$C_7H_7NO_2$	1.15	138.05	137.05	Y	N	-
26	Trimethylgallic acid		$C_{10}H_{12}O_5$	9.71	213.07	212.06	N	Y	-
27	2-Oxo-4-methylthiobutanoic acid		$C_5H_8O_3S$	19.32	149.02	148.02	Y	Y	-
28	Diethyl (2*R*, 3*R*)-2-methyl-3-hydroxysuccinate		$C_9H_{16}O_5$	8.75	203.09	204.10	N	Y	-
29	2-(2-Bicyclo[2.2.1]heptanyl)acetic acid		$C_9H_{14}O_2$	14.23	155.10	154.10	Y	N	-
30	3-Amino-2-naphthoic acid		$C_{11}H_9NO_2$	8.73	205.09	187.06	Y	N	-
31	Hexadecanamide		$C_{16}H_{33}NO$	21.04	256.26	255.25	Y	N	-
32	Acetic acid, aluminum salt (3:1)		$C_6H_9AlO_6$	1.56	222.05	204.02	Y	N	-
33	*S*-Heptyl carbamothioate		$C_8H_{17}NOS$	1.25	198.09	175.10	Y	N	-
34	Hydroxy glutaric acid		$C_6H_8O_5$	2.53	131.03	132.03	N	N	-
35	Hydroxybutyric acid		$C_4H_8O_3$	1.53	105.05	104.04	Y	N	-
36	*P*-*O*-Methayl piscidic acid		$C_{12}H_{14}O_7$	0.89	274.09	256.06	N	Y	-
37	1-Benzoyl-3, 3-bis(propan-2-yl) thiourea		$C_{14}H_{20}N_2OS$	10.24	282.16	264.13	N	Y	-
38	Phenyl acetaldehyde		C_8H_8O	2.141	138.09	120.06	Y	Y	-
39	Methylheptane		$C_8H_{18}N_4S$	15.69	203.13	202.12	Y	N	-
40	Henicosane-9-thiol		$C_{21}H_{44}S$	21.36	327.30	328.31	N	Y	-
41	3-Nonyl-3-sulfanyloxetane-2,4-dione		$C_{12}H_{20}O_3S$	13.77	245.12	244.11	Y	N	-
42	2,3-Butanedione oxime thiosemicarbazone		$C_5H_{10}N_4OS$	1.35	173.05	174.05	N	Y	-
43	1,4-Benzoquinone		$C_6H_4O_2$	1.07	109.02	108.02	Y	Y	-
44	Allophanic acid methyl ester	Fatty acid	$C_3H_6N_2O_3$	2.29	136.07	118.04	Y	N	-
45	1-(2,2-Diethoxyethyl)-3-ethyl		$C_9H_{20}N_2O_3$	14.24	203.13	204.15	N	Y	-
46	9-Octadecenamide		$C_{18}H_{35}NO$	21.18	282.28	281.27	Y	N	-
47	Methyl linolenate		$C_{19}H_{32}O_2$	17.56	293.25	292.24	N	Y	-
48	Tetradecanamide		$C_{20}H_{44}N_4O$	21.44	355.34	356.35	N	Y	-
49	3,4-Diethyltetradecane		$C_{18}H_{37}NO$	21.59	284.29	283.29	Y	N	-
50	Xuulanin	Flavonoid	$C_{22}H_{24}O_4$	1.16	387.13	352.16	Y	N	-
51	Hydroxyisouric acid	Alkaloid	$C_5H_4N_4O_4$	1.08	185.03	184.02	Y	N	-
52	Indolothiazole		$C_9H_6N_2S$	1.97	173.01	174.02	N	Y	-
53	Usambarensine		$C_{29}H_{28}N_4$	11.24	431.22	432.23	Y	N	-
54	Sucrose	Carbohydrate	$C_{12}H_{22}O_{11}$	1.15	381.08	342.11	Y	N	-
55	3-*O*-Methylglucose		$C_7H_{14}O_6$	0.86	212.11	194.07	N	Y	-
56	Deoxyribose		$C_6H_{10}O_5$	1.61	161.04	162.04	N	Y	-
57	1,4-D-Xylobiose		$C_{10}H_{18}O_9$	1.03	300.13	282.09	Y	Y	-
58	*D*-Glucose		$C_6H_{12}O_6$	0.63	219.03	180.06	Y	Y	-
59	Ketolactose		$C_{12}H_{20}O_{11}$	2.00	339.09	340.09	Y	Y	-

Continuation Table 2.

Serial Nr	Compounds name	Chemical group	Chemical formula	Retention time (min)	M/Z ratio	Mass	Plant beneficial Yes/Not	Health beneficial Yes/Not	Others
60	Succinamic acid	Organic acid	$C_4H_8N_2O_5$	2.28	182.07	164.04	Y	Y	-
61	1,3,7-Trimethyluric acid		$C_8H_{10}N_4O_3$	0.693	245.04	210.07	N	Y	-
62	Sorbitolamine		$C_6H_{15}NO_6$	1.09	198.09	197.08	Y	N	-
63	Abscisic acid		$C_{15}H_{20}O_4$	13.69	282.17	264.13	Y	N	-
64	Benzenesulfonic acid		$C_{17}H_{28}O_3S$	16.25	311.17	312.17	N	Y	-
65	Itaconic acid		$C_5H_6O_4$	1.87	129.02	130.03	N	Y	-
66	Fumaric acid		$C_4H_4O_4$	1.29	115.00	116.01	Y	Y	-
67	Sinalexin	Sulphur compound	$C_{10}H_8N_2OS$	1.57	203.02	204.03	Y	N	-
68	1,10-Phenanthroline	Heterocyclic compound	$C_{12}H_8N_2$	1.07	179.06	180.06	Y	N	-
69	*N*-(4-Azidobutyl) methanesulfonamide		$C_5H_{12}N_4O_2S$	1.33	191.06	192.06	N	Y	-
70	*N*-(1-Phenylethylideneamino) ethanethioamide	Acetamide	$C_{10}H_{12}N_2S$	1.11	191.06	192.07	N	Y	-
71	Gadusol	Alcohol	$C_8H_{12}O_6$	7.80	203.05	204.06	N	Y	-
72	1-(3-Methylbutanoyl)-6-apiosylglucose	Glycosides	$C_{16}H_{28}O_{11}$	1.16	431.13	396.16	Y	N	-
73	Adenosine		$C_{10}H_{13}N_5O_4$	0.97	268.10	267.09	N	Y	-
74	2-(Isopropyl sulfonyl) naphthalene	Naphthalene	$C_{13}H_{14}O_2S$	0.85	235.08	234.07	N	Y	-
75	Ethyl 6-hydrazinonicotinate	Vitamin	$C_8H_{11}N_3O_2$	2.32	180.07	181.08	N	Y	-
76	Octanediamide	Enzyme	$C_8H_{16}N_2O_2$	14.24	171.11	172.11	N	Y	-
77	Methyl *N*-[6-(hexylsulfamoyl)-1(*H*)-benzimidazol-2-l] carbamate		$C_{15}H_{22}N_4O_4S$	1.33	353.12	354.13	N	Y	-
78	Hydroxythiopental	Steroid	$C_{11}H_{18}N_2O_3S$	1.83	257.09	258.10	N	Y	-
79	5-Methyl-thiazolo[3,2-d]tetrazole	Triazole	$C_4H_4N_4S$	1.41	139.00	140.01	N	Y	-
80	1-(2-Hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole	Nitro-imidazole	$C_{15}H_{22}N_4O_4S$	1.33	353.12	354.13	N	Y	-
81	1-Hexylthiourea	Thiourea	$C_{18}H_{38}N_2OS$	17.04	329.26	330.27	N	Y	-
82	Protionamide	Thioamides	$C_9H_{12}N_2S$	1.08	215.04	180.07	N	Y	-
83	2-Butylsulfinylacetonitrile	Unknown	$C_6H_{11}NOS$	12.65	144.05	145.05	N	Y	-
84	1-(Diaminomethylidene)-3-tetradecylurea		$C_{16}H_{34}N_4O$	20.07	297.26	298.27	N	Y	-
85	Diadamantyldiazomethane		$C_{21}H_{30}N_2$	17.92	309.23	310.24	N	Y	-
86	4-Piperidinobutyl sulfide		$C_{18}H_{36}N_2S$	18.40	311.25	312.26	N	Y	-

Figure 1. Total positively charged ions chromatogram of banana pseudostem sap obtained by liquid chromatography mass spectrometry (LC-MS) through methanolic extraction.

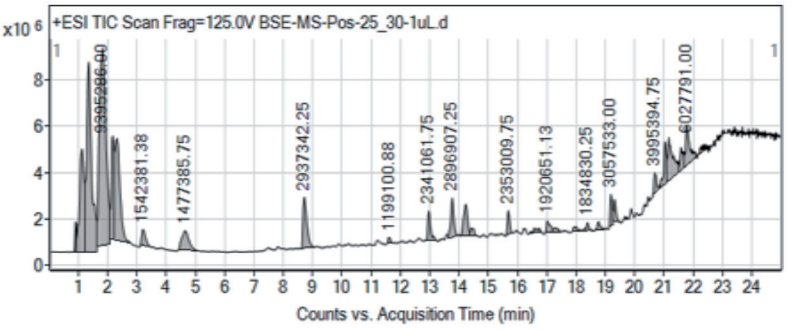
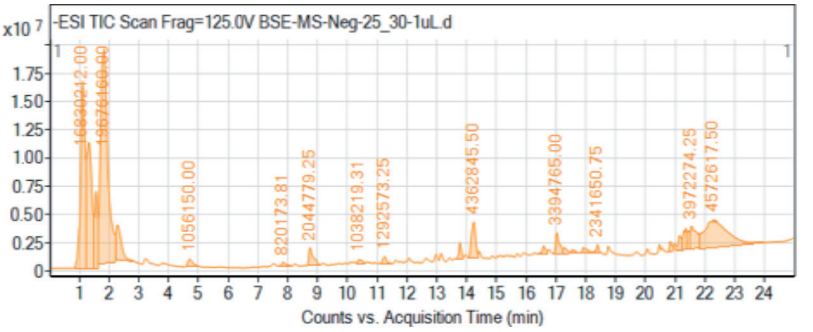


Figure 2. Total negatively charged ions chromatogram of banana pseudostem sap obtained by liquid chromatography mass spectrometry (LC-MS) through methanolic extraction.



Similarly, the shoot growth was significantly affected by the application of low concentration aqueous banana pseudostem sap (5%-15%) over the control. However, 10% aqueous sap indicated the highest shoot length (16.0 cm) followed by 5% and 15% aqueous sap while the lowest 2.16 cm was recorded in 100% raw sap. Aqueous solution with 10% and 15% sap had 19.6% and 15.8% higher shoot length than the control, respectively. On the other hand, aqueous sap with higher sap content had an inhibitory effect on shoot length (Table 3, Figure 3). A similar trend was found in fresh weight. The seed treated with 10% aqueous sap had 21.7% higher fresh weight than the fresh weight recorded in the control. The increase in fresh weight was recorded at 19.9% in aqueous solution with 15% sap. However, the lowest values of fresh weight were recorded in the 100% raw banana pseudostem sap. Referring to the dry weight of corn seedling, seeds treated with 10% and 15% aqueous sap increased dry weight by 31.1% and 21.6%, respectively, when compared to the dry weight in control. An increase in sap concentration in the aqueous solution resulted in diminishing dry weight, yet the lowest dry weight was recorded in the 100% raw sap treatment (0.09 g).

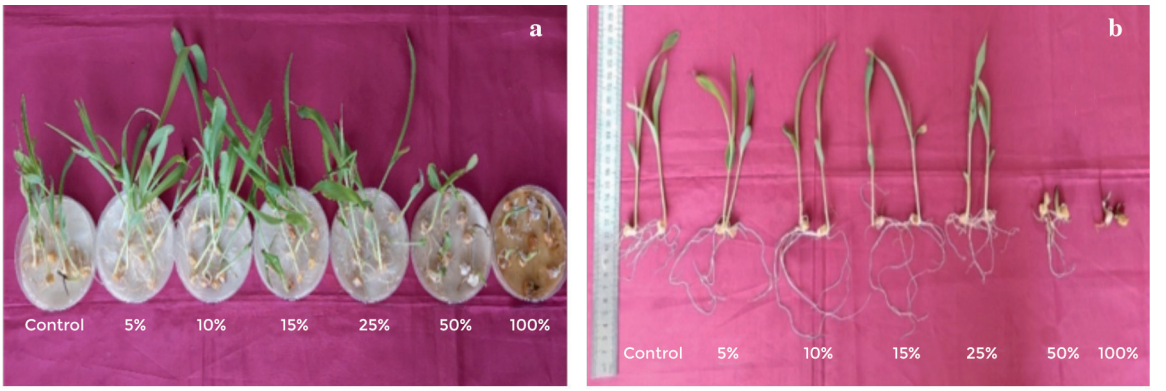
The SPAD value in the seedling also varied due to the sap concentration in the aqueous solution. The greenness tended to increase as the sap concentration rose up to 15% in the aqueous solution. The seed treated with 15% aqueous sap had 29.1%, more greenness than the control sample. However, the second highest amount of SPAD value was recorded in aqueous sap with 10% sap, which was significantly similar to aqueous solution with 15% sap (Table 3). The Petri dish also visually (to the naked eye) showed the development of fungal colonies at the higher concentration (50%-100%) sap treatments (Figure 3) while the lower concentration (5%-15%) of sap was free from any external growth.

Table 3. Effect of different concentrations of fresh banana pseudostem sap on seedling growth of sweet corn.

Treatments	Germination	Root length	Shoot length	Fresh weight	Dry weight	SPAD value
	%	cm		g		
Control (water)	90.0 ± 2.88a	12.8 ± 0.25b	13.3 ± 0.55b	2.21 ± 0.07c	0.19 ± 0.005b	22.3 ± 0.92c
5% Aqueous sap	92.3 ± 1.50a	14.7 ± 0.34a	15.2 ± 0.42a	2.40 ± 0.06b	0.20 ± 0.01b	23.9 ± 0.59bc
10% Aqueous sap	91.7 ± 4.40a	15.5 ± 0.53a	16.0 ± 0.18a	2.69 ± 0.04a	0.25 ± 0.005a	26.1 ± 1.04ab
15% Aqueous sap	90.0 ± 2.88a	15.0 ± 1.04a	15.4 ± 0.60a	2.65 ± 0.09a	0.23 ± 0.01ab	28.8 ± 1.08a
25% Aqueous sap	80.1 ± 3.05b	13.0 ± 0.34b	12.3 ± 1.20b	2.37 ± 0.03bc	0.20 ± 0.01b	22.9 ± 0.44c
50% Aqueous sap	70.0 ± 2.88c	6.83 ± 0.08c	3.26 ± 0.40c	1.92 ± 0.05d	0.14 ± 0.01c	22.6 ± 1.83c
100% Raw sap	57.7 ± 1.45d	1.83 ± 0.16d	2.16 ± 0.17c	0.64 ± 0.02e	0.09 ± 0.01d	-
LSD (5%)	8.73	1.49	1.81	0.17	0.03	3.03

Values are mean ± standard error of three replicates in the same column following the same letter are not significantly different according to LSD test ($p \geq 0.05$).

Figure 3. Pictorial presentation of the effects of different concentrations of banana pseudostem saps on shoot (a) and root length (b).



DISCUSSION

Phytochemicals in banana pseudostem sap

Phytocompounds are organic molecules produced in plants that are actively involved in plant growth, development, and other metabolic processes (Erb and Kliebenstein, 2020). Results identified 86 secondary metabolites in banana pseudostem sap that could be categorized into diverse groups (Table 2), which were less than the identified compounds in banana pseudostem by Deng et al. (2020). This might be due to solid-liquid extraction or employed LC-MS/MS analytical procedure. In the present study, we implemented the liquid-liquid extraction procedure where only water soluble phytocompounds may have appeared in the collected sap. This may reduce the number of identified compounds. Despite the limited number of phytocompounds, we did identify several important phenolic compounds *viz*. aminobenzoic acid, 1,4-benzoquinone, 2,4-dihydroxybenzoic, 3-amino-2-naphthoic acid and *P*-*O*-methyl-piscidic acid. These are all related to plant growth and development. Previously, Sumalan et al. (2020) verified the influential role of these phenolic compounds on growth, photosynthesis, nutrient uptake and productivity of plants. Results showed that a remarkable number of amino acids, such as glutamic acid, tryptophan, proline betaine, etc., were present in banana pseudostem sap (Table 2). Similar amino acids were identified by Deng et al. (2020) and these were reported as wielding significant effects on plant growth and development in studies by Qiu et al. (2020). Alkaloids are vital secondary metabolites related to both plants and humans (War et al., 2012). In our investigation, very negligible numbers of alkaloids were identified from the fresh sap and these were less than those identified by Deng et al. (2020). Among the identified compounds 5-hydroxysouric acid and usambarensine were especially beneficial to plants wielded significant influence on N assimilation, photosynthesis and plant protection (War et al., 2012). Likewise, the indolothiazole found in fresh banana pseudostem sap may inhibit obesity by controlling the blood glucose, total cholesterol, and alanine aminotransferase given that many people in both developed and developing nations have fat-rich diets.

Carbohydrates are an important component of the banana pseudostem, and they are necessary for providing energy for plants and animals' proper functioning. Several forms of carbohydrates such as glucose, sucrose, ketolactose, and deoxyribose, exist in banana pseudostem sap (Table 2) and they were mostly involved in the functioning of metabolic pathways, providing energy and structural resistance (Stein and Granot, 2019). It is recognized as a signaling metabolite, helping to form chlorophyll, rubisco, and the synthesis of various photo-protective pigments. It was proved that exogenous application of sugar in plants increases the sweetness of fruits, and provides plants with structural support (Siddiqui et al., 2020). However, excess glucose and blood sugar can lead to heart attacks, diabetes-related diseases and obesity.

Moreover, organic acids play an important role in plant metabolism. They are early photosynthetic products and act as a precursor to the synthesis of many other compounds. Generally, banana fruit flavor and ripening are controlled by the nonvolatile organic acids (Wyman and Palmer, 1964). We identified a larger number of organic acids (isopropyl malic acid, succinic acid, dihydroxybenzoic acid, and benzenesulfonic acid) from fresh banana pseudostem sap, which be critical in plant and human health. Based on the above information, the identified compounds in banana pseudostem sap may contribute to more efficient agriculture in terms of plant protection, growth, and development.

Application of banana pseudostem sap on sweet corn seedling growth

The present study showed that banana pseudostem sap is a good source of beneficial secondary metabolites and mineral nutrients for plants, and they greatly influence how well sweet corn seedlings grow (Tables 1, 2, and 3). The smaller concentrations of sap (5% to 25%) showed a higher percentage of seed germination compared to the control. The higher concentrations inhibited any expansion in germination. The pseudostem sap may contain some unknown allelo-compounds (abscisic acid) and pathogenic microorganisms which could inhibit and/or affect the germination rate (Talukder et al., 2015) in higher concentrations (50%-100%). Aslam et al. (2016) reported that smaller concentrations of plant extract promote seed germination. However, 5%-25% aqueous sap-treated seed was significantly identical to the control in terms of germination rate (Table 2). This finding corroborates the results documented by Roy et al. (2006), who observed 93% germination of lettuce seed by 15% aqueous banana pseudostem sap. Banana pseudostem sap (as the stock solution) was less concentrated than other plant extracts due to containing more than 85% water. In this context, 5%-15% aqueous sap had a very lower concentration and consequently, the germination percentage did not differ significantly from the control.

The root length, shoot length, dry biomass, and chlorophyll content were superior in 10% aqueous fresh sap compared to the others. This might be due to the contribution of water extractable plant beneficial phytochemicals (*viz*. 1-amino-1-carboxycyclopropane, 4-aminobenzoic acid, tetramethyl glutaric acid, proline betaine, butanoic acid, 1,4-benzoquinone, 5-hydroxybutyric acid and aluminum acetate) in the sap (Table 1) which could be absorbed by the seedling for better growth. In contrast, Kirillova et al. (2016) observed a 50% increase in biomass of amaranth plants when soaked in 1 μM aminobenzoic acid solution compared to the control. Moreover, the identified amino acids in the present study are water soluble (glutamic acid, phenylalanine, and tryptophan), are influential in the growth and development of plants, and these corroborates other study' findings (Tadros et al., 2019). Teixeira et al. (2018) reported that seed treatment and foliar application of glutamic acid, phenylalanine, cysteine and glycine individually or jointly stimulated root development and increased soybean seedlings' N uptake. This could lead to higher shoot length, biomass and more chlorophyll. Added to this, benzoquinone acts as a plant growth regulator and according to Ranade and David (1985) the smaller concentration (10^{-5} M) may increase 73% fresh weight and 46% biomass of mung bean. Moreover, 2,4-dihydroxybenzoic acid influences auxin activities (Mucciarelli et al., 2000). In another study, *P-O*-methyl-piscidic acid promoted P availability and this helped pigeon-pea and maize to experience better root growth (Miranda et al., 2015). Soluble nutrients such as N, P, K, Ca, Mg, Cu, Zn and B in the banana pseudostem sap (Table 1) contributed to the increased root length, shoot length, biomass, and the chlorophyll content of the seedlings compared to the control. This finding agreed with research documented in Bahtiar et al. (2017). Thus, the banana pseudostem sap (5%-15% concentration) has great potential for improving crop production practices and outputs.

CONCLUSIONS

The study qualitatively identified phytochemicals in banana pseudostem in 21 categories. A good number of identified phytocompounds and soluble nutrients are linked to the growth and development of plants. Application of banana pseudostem sap, especially at lower concentrations (5%-15%) significantly influenced sweet corn seedling growth and confirmed the stimulatory role of identified phytochemicals and mineral nutrients in banana pseudostem sap. Thus, the combined role of plant phytochemicals and native mineral nutrients in the banana pseudostem sap will help to create a good-to-high quality organic liquid fertilizer. Furthermore, the identified compounds in banana pseudostem sap that will benefit human health can be useful for pharmacological research. Although the quantification of secondary metabolites has not yet been completed, future research on the quantification of beneficial phytocompounds will lead to a better understanding of how banana pseudostem sap, for example if its application to agriculture is economical, efficient and environmentally friendly.

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