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Secondary metabolite analysis of the pith of selected local banana varieties as a constituent in bio-batteries

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Abstract

Innovative solutions are needed as a pre-requisite to address the challenges in bio-batteries where electrolytic materials play a major role in its performance. Therefore, the current investigation had focused to study the banana pith applicability as an electrolyte with an intention to find the best local banana variety among three selected varieties [Musa AAB Group (Plantain Subgroup) 'alukesel', Musa AAB Group (Mysore Subgroup) 'ambul' and Musa AAA Group (Cavendish Subgroup) 'ambun']. This was conducted by qualitative and quantitative phytochemical analysis on pith of 3 banana varieties. The trunks of three banana varieties were chopped using an electric blender and were kept on a hot plate set at 120 ^{o}C for 30 minutes which were used for phytochemical screening and thin layer chromatography (TLC) afterwards. The presence of saponins, tannins, flavonoids and phenolics were revealed from the preliminary phytochemical screening while terpenoids, alkaloids and steroids were absent in all 3 varieties. Among the three varieties ambun variety exhibited highest amount of total phenol (2.27 ± 0.09 mg gallic acid equivalents/g of extract) and total flavonoid (1.50 ± 0.04 mg quercetin equivalents/g of extract) contents. Although the pith of ambun headed among the selected varieties from its galvanic battery performance indicators in an acidic medium, the secondary metabolites do not seem to facilitate the efficacy of the bio-battery with a profound relationship. Hence, additional research work is required to elucidate the possible mechanism of action of these found secondary metabolites with further quantification methods.

Keywords: Bio-batteries, electrolyte, galvanic cell, phytochemical screening, thin layer chromatography.

Introduction

Portable source of energy storage devices such as batteries have evolved continuously into currently available metal and chemical batteries due to the rapid technological advances of science over the time. Yet, the existing commercialized battery designs still must cope many challenges which are evident from the manufacturing process to the disposal procedure without contaminating the environment. As a result, the research and development attention has been shifted to harvest energy using bio-batteries during the past few years as they provide low cost, simple and environmentally friendly solutions in comparison. Hence, after the initial work of research[1], bio-battery design has undergone many transformations due to structural modifications[2-3] and usage of new alternative materials[4-5] including banana varieties[6] as constituents.

Moreover, there is a need for bio-batteries which promote advantageous traits such as less expensive, less weight, increased battery life, reusability and environmentally safe disposable practices. After initial investigations[7-8], the current investigation was conducted to investigate the types of phytochemicals that are present in the pith, along with the determination of total polyphenolic and flavonoid contents of the selected banana variety with best bio-battery performance which was fabricated using the pith of three local banana varieties [*Musa* AAB Group (Plantain Subgroup) 'alu kesel', *Musa* AAB Group (Mysore Subgroup) 'ambul' and *Musa* AAA Group (Cavendish Subgroup) 'ambun'] as an electrolyte.

Material and methods

Plant material: The trunks of three banana varieties were cut into small cubes (1 x 1 x 1 cm^3) and were chopped using an electric blender separately and kept on a hot plate set at 120^{o}C for 30 minutes to concentrate the liquid content. Liquid extracts (Figure-1) of three banana varieties were subjected for Thin Layer Chromatography (TLC) and their freeze-dried samples were subjected for phytochemical screening (Figure-2).

Thin Layer Chromatography (TLC): Dichloromethane (5 ml) was added to a separatory funnel which contained liquid extract (10 ml) from each banana variety and shaken well respectively. After 15 min, dichloromethane layer was separated out. This procedure was done in three repetitions and combined extracts were added to a round bottom and dried using a rotary evaporator. Finally, dichloromethane (2 ml) was added to each dried extract and mixed well. Then 5 µl from each banana variety was spotted on a TLC plate. As the mobile phase methanol, dichloromethane and cyclohexane (0.1:1:1 v/v) was

used. TLC fingerprint profiles were observed under UV light using wavelengths of 254nm and 366nm. Vanillin sulphate was used as the spray reagent and heated the TLC plate at 105°C to visualize the spots. R_f values were calculated before and after using the spray reagent.

Phytochemical Screening: Weight of 5g from each freeze-dried sample was dissolved in 20ml of water and subjected for phytochemical screening[9-10].

Test for Phenolics: i. Ferric Chloride Test: Few drops of $FeCl_3$ were added to a few drops of extract and mixed well. The presence of water soluble phenolics are indicated by green or blue colour. ii. Vanillin Test: Few drops of 10% vanillin in ethanol and concentrated HCl were added to a few drops of extract. The presence of phenolic acid is indicated by pink whereas the indication of phenyl propene is given by red pink. iii. Lead Acetate Test: Few drops of lead acetate were added to a few drops of extract. The presence of flavones and flavonols are indicated by a yellow precipitate.

Test for Flavonoids: To dilute ammonia solution (5ml), 3ml of aqueous filtrate was added and mixed well. Then concentrated H_2SO_4 was added drop wise. If yellow colour is formed and then disappears on standing, it is an indication for flavonoids.

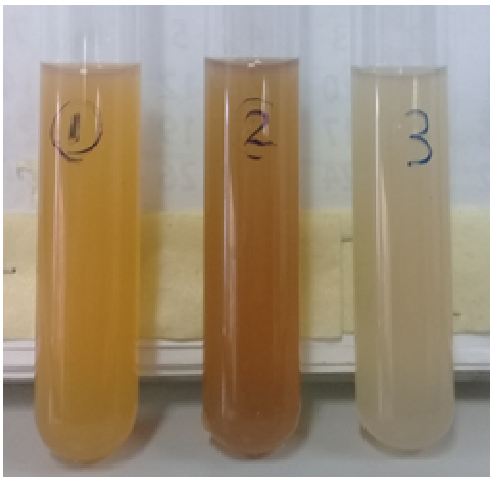
Test for Alkaloids: To extract (2ml), 6 drops of Mayer's regent and 1% HCl were added. Orange or cream brown red precipitate is an indication for alkaloids.

Test for Saponins: Frothing Test: To extract (5ml), water (5 ml) was added and shaken vigorously. Froth persistency for at least 10 minutes is an indication for saponins. With 3 drops of olive oil, the froth was mixed and shaken vigorously. The emulsion formation is an indication for saponins.

Test for Tannins: i. Each extract (200mg) was diluted in 10 ml distilled water and filtered. $FeCl_3$ solution (2 ml of 0.1%) was added to 2ml of the filtrate. Tannins are indicated by a blue-black precipitate. ii. Vanillin Test: Few drops of concentrated HCl and 10% vanillin in ethanol were added to a few drops of extract. Red colour is the indication for tannins.

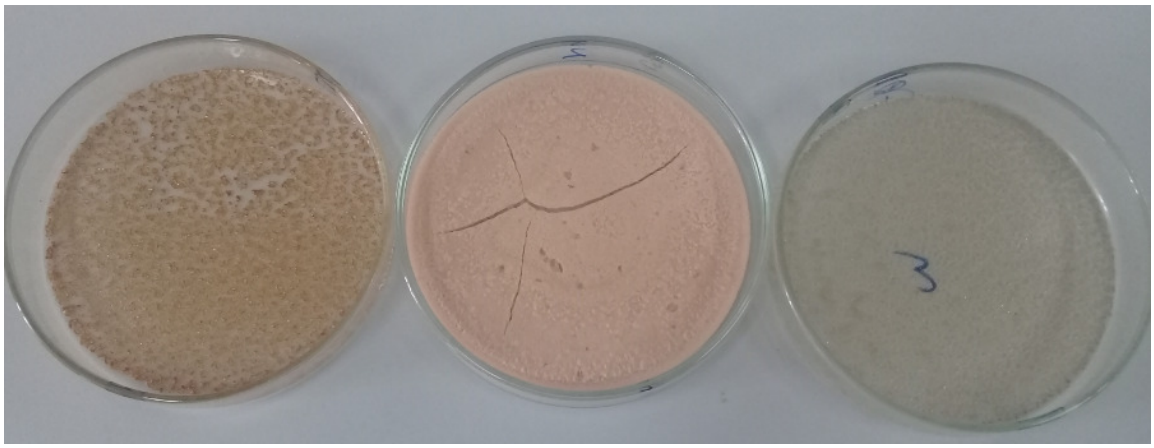
Tests for Steroids: To 2ml of extract, 2ml of acetic anhydride was added and mixed. Then H_2SO_4 (2ml) was added along the wall of the test tube. Violet to green or blue colour change is an indication for steroids.

Test for Terpenoids: i. Salkowski Test: 2ml of chloroform was mixed with 5ml of extract. Then, 3ml of concentrated H_2SO_4 was added along the sides for the formation of a layer. The presence of terpenoids is indicated by a reddish brown colour. ii. Test for Monoterpenes: Few drops of 10% vanillin in ethanol were added to a few drops of extract. Concentrated H_2SO_4 was then added. A red colour is an indication for terpenoids.



(a) (b) (c)

Figure-1: Liquid extracts of (a) Alu Kesel, (b) Ambul and (c) Ambun.



(a) (b) (c)

Figure-2: Freeze dried extracts of (a) Alu Kesel, (b) Ambul and (c) Ambun.

Total Polyphenolic Content (TPC): Total polyphenolic content of each freeze-dried extract was determined by the Folin-Ciocalteu reagent[11] with the usage of 96-well micro-plates. In this method, 20µl of 0.25 and 0.125mg/ml of water extract were added to 110µl of 10 times diluted freshly prepared Folin-Ciocalteu reagent. Afterwards, the pre-plate reading was taken. Then 70µl of sodium carbonate solution was added to the mixture which was then incubated at room temperature (25 ± 2^o C) for 30 minutes. At 765 nm, the absorbance was recorded.

To construct the standard curve, gallic acid of five different concentrations (1, 0.5, 0.25, 0.12 and 0.06 mg/ml) were used. TPC of water extract was mentioned as mg gallic acid equivalents/g of extract on dry weight basis.

Total Flavonoid Content (TFC): Aluminium chloride ($AlCl_3$) method with 96 well micro-plates[12] was used to determine the total flavonoid content of water extract. To 100 µl of 0.25 mg/ml water extract, $AlCl_3$ (100 µl of 2%) in methanol solution was added. Then the mixture was incubated at room temperature (25 ± 2^o C) for 10 minutes. At 415 nm, the absorbance was recorded. Before adding the $AlCl_3$ solution, pre-plating reading was recorded. To construct the standard curve, five different concentrations of quercetin (125, 62.5, 31.25, 15.62 and 7.81 mg/ml) were used. TFC of water extract was mentioned as mg quercetin equivalents/g of extract on dry weight basis.

Results and discussion

TLC technique was used to compare the phytochemical profiles of three varieties of *Musa* spp grown in Sri Lanka (Table-1). Slight differences were observed in the TLC fingerprint profiles of 3 varieties (Figure-3 and 4). Furthermore, many researchers have investigated the phytochemicals present in many parts including fruit[13], stem[14] and fruit peel[15] of *Musa* spp. The presence of saponins, tannins, flavonoids and phenolics were revealed from the preliminary phytochemical screening while terpenoids, alkaloids and steroids were absent in all 3 varieties. Similar results were observed with Onyenekwe and co-workers[14]. Ambun variety contained less amounts of tested phytochemicals when compared to other two varieties. The quantitative phytochemical analysis of TPC and TFC were found to be 1.10 ± 0.06, 0.58 ± 0.11, 2.27 ± 0.09 mg gallic acid equivalents/g of extract and 0.96 ± 0.05, 0.40 ± 0.10, 1.50 ± 0.04 mg quercetin equivalents/g of extract respectively in the pith juice of Alu Kesel, Ambul and Ambun.

As an electrolytic media for bio-batteries, pith of Ambun headed among the selected varieties due to its reported higher short circuit current and open circuit voltage with a less pH in its electrolytic pith[7]. Yet, a profound connection between secondary metabolites was not evident with the cell performance. Hence, additional research work is required to elucidate the possible mechanism of action of these found secondary metabolites with further quantification methods.

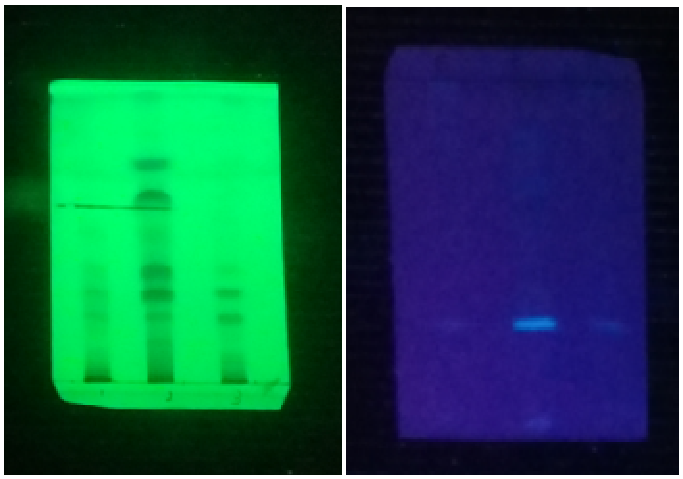
Conclusion

Even overall battery performance parameters such as the cell geometry, weight, electrode separation, different electrode combinations etc. should be further investigated for optimum performance of the proposed environmentally friendly bio-batteries with the goal of high energy and low-cost manufacturing process. In conclusion, this is the first report on phytochemical constituents of pith juice of *Musa* spp grown in Sri Lanka.

Conflict of interest: The authors do hereby declare that there is no conflict of interest.

Table-1: R_f values observed for Thin Layer Fingerprint profiles of Alu Kesel, Ambul and Ambun (after spraying with vanillin sulphate).

Alu Kesel	Ambul	Ambun
0.05 (dark brown)	0.04 (dark brown)	0.05 (dark brown)
0.08 (light brown	0.07 (light brown)	0.07 (light brown)
0.11 (yellow)	0.10 (pink)	0.08 (orange)
0.14 (light brown)	0.21 (yellow)	0.10 (orange)
0.21 (yellow)	0.42 (yellow)	0.14 (purple)
0.26 (red)	0.47 (purple)	0.18 (yellow)
0.42 (light yellow)	0.60 (yellow)	0.37 (yellow)
0.48 (light purple)	0.90 (purple)	0.44 (yellow)
0.59 (pink)		0.49 (purple)
0.86 (purple)		0.66 (yellow)
		0.90 (purple)



(a) (b)

Figure-3: Thin Layer Fingerprint profiles Alu Kesel, Ambul and Ambun at (a) 254 nm and (b) 366 nm.

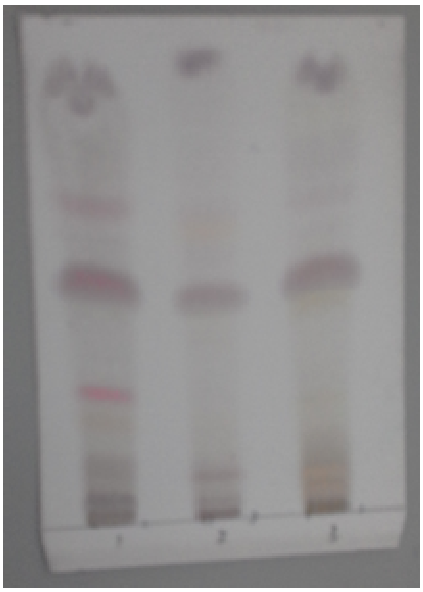


Figure-4: Thin Layer Fingerprint profiles Alu Kesel, Ambul and Ambun after spraying with vanillin sulphate.

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