Effects of Solvent System on the Polyphenol Extraction from Banana (*Musa spp. Var.* Ambul kesel) Pseudo-stem

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***Abstract* — This research was conducted to investigate the effects of extraction solvent system on the best recovery of polyphenolic compounds from banana (Musa spp. Var. Ambul kesel) pseudo-stem. The investigated extraction conditions were: different methanol proportions as the solvent (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 %, methanol/water), using the maceration technique with a water bath. Shaking speed was the 200 rpm for three hours in two repetitions and the solvent/solid ratio was 20 ml/g. This research was conducted at pH 5.0 at the reaction temperature of 60 ^{0}C as these were the optimum extraction conditions to extract polyphenols from this banana (Musa spp. Var. Ambul kesel) pseudo-stem according to the results obtained from the previous research work. The present study revealed that the best delivery of total phenolic compounds from banana (Musa spp. Var. Ambul kesel) pseudo-stem could be reached by utilizing 70% methanol as the extraction solvent system. The maximum yield of 1.35±0.18 g GAE/100g extract was recorded for total phenolic compounds using the optimum extraction solvent system.**

Keywords- banana pseudo-stem, polyphenols, optimization

I. INTRODUCTION

Plant polyphenolic compounds are synthesized in plants partly as a response to ecological and physiological pressures such as pathogen and insect attack, UV radiation and wounding [1]. As well as plant polyphenolics are contributing to plant's colors. Polyphenolics are secondary metabolites and are derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants [2]. So far, there are more than 8000 polyphenolic compounds that have been identified and characterized in different plant species ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins [3]. In the last two decades, there has been more interest in the potential health benefits of dietary polyphenols as antioxidant. Several epidemiological studies and associated meta-analyses strongly showed that the consumption of these polyphenols offered better protection against chronic diseases such as cancers, cardiovascular diseases, cerebrovascular diseases, diabetes, ageing and neurodegenerative diseases [4]. The main classes include flavonoids, phenolic acids, stilbenes and lignin and tannins [5]. The total phenolic and total flavonoids in various solvent extracts of pseudo-stem of different banana cultivars varies from 7.58 to 291 mg gallic acid equivalent and from 4 to 80 mg catechin equivalent, respectively [6,27]. In reverse phase HPLC analysis of phenolic compounds from the banana pseudo-stem extracts indicated the presence phenolic acids such as tannic, pyrocatechol, catechol, gentisic, (+)-catechin, protocatechuic, gallic, caffeic, chlorogenic, ferulic, and cinnamic acids [26]. Literature shows that the polyphenolic content and flavonoid content was about four times higher than in banana pseudo-stem flour (only the pseudo-stem sheaths without the tender core) than the flour of boiled tender core of the banana pseudo-stem [7]. It has been reported that banana pseudo-stem has 2.06% of tannins [8].

Solvent extractions are the most commonly used procedures to prepare polyphenolic extracts from plant materials due to their ease of use, efficiency, and wide applicability [28]. It is generally known that the yield of chemical extraction depends on the type of solvents with varying polarities, extraction time and temperature, pH value of the extraction medium, sample-to-solvent ratio as well as on the chemical composition and physical characteristics of the samples [29]. Depending on the solvent system used during exaction, a mixture of phenolics soluble in the solvent will be extracted from plant materials [2,9,29]. It may also contain some non-phenolic substances such as sugar, organic acids and fats. As a result, additional steps may be required to remove those unwanted components. Solvents, such as methanol, ethanol, acetone, ethyl acetate, and their combinations have been used for the extraction of phenolics from plant materials, often with different proportions of water [1,9]. Selecting the right solvent affects the amount and rate of polyphenols extracted [3,9]. In particular, methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols while the higher molecular weight flavanols are better extracted with aqueous acetone [10,28, 29]. The methanolic extracts of different parts of banana (*Musa paradisiaca*) registered higher phenolic contents [11], while the 90% ethanolic extract of banana (*Musa acuminata*) pseudostem shows the maximum phenolic content [12]. To obtain the highest phenolic compounds from banana pulp and peel from 15 banana cultivars, 80% methanol has been used as the best extraction solvent [13]. It has been reported that the best solvent to extract tannin contained D-catechin and gallic acid related phenolic compounds from Aralu was the 80% methanol [14]. One research study reported the antioxidant properties of three banana cultivars (*Musa acuminata* 'Berangan', 'Mas' and 'Raja' and found out the type of solvent used had a significant effect ($p<0.05$) on the extraction of antioxidant compounds from banana fruits and further concluded that 70% acetone was the best solvent to extract polyphenols from the used banana cultivars [15]. Sometimes, the polyphenolics bind with other plant components, such as carbohydrates and proteins and these interactions may lead to the development of some complexes that may be difficult to solubilize in organic solvents

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[1,2,3,9,10]. Thus it is difficult to develop a general protocol for the phenolic extraction from plant materials and needs close screening strategies to establish a viable analytical method. In one study on the extraction of phenolic compounds from peanut hulls using methanol and ethanol, methanolic extracts gave higher amounts of phenolic compounds than ethanolic extracts [16]. The use of organic solvents, in mixtures with water, contributes to the creation of a moderately polar medium that enhances the extraction of polyphenols. In particular, methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols [9,10].

II. Objectives

Present study is aimed to investigate the solvent effect (effect of methanol concentration) on the best recovery of polyphenols from banana (*Musa spp.* Var. Ambul kesel) pseudo-stem.

III. Methodology

Out of more than 40 different cultivars of Banana (*Musa spp.*) in Sri Lanka, Ambul kesel cultivar was selected for this research. Ambul kesel was harvested fresh from Veyangoda area, Sri Lanka. After fruit bunch harvesting, the pseudo-stem was separated from the plant at ground level and transferred to the laboratory. Blanched banana pseudo-stem flour (BBPF) was processed [6,7,11,17] by peeling off the epidermis (pseudo-stem sheaths) manually from a sterile knife and sliced into small pieces and blanching the sliced pieces [steam blanching the samples for 1 minute followed by cooling in cold water (15 ^{0}C, 3 min) to prevent enzymatic browning] before dried in a hot air ventilated oven (Ueshima, model AG-1110, Japan) at 45 ^{0}C for 24 hours. The dried banana pseudo was ground in a blender (Panasonic, model MX-GX1511, Taiwan) and further sieved through a 600-µm mesh sieve. Then it was kept in an airtight plastic container wrapped with Aluminium foils and stored in a cool room (Lae Electronic, model CDC 80, Italy) at -10 ^{0}C.

Extraction of polyphenolic compounds from banana (*Musa spp.* Var. Ambul kesel) pseudo-stem using maceration technique by using a water bath shaker [17,18]

From the prepared banana pseudo-stem powder (BBPF), 5 g was extracted for 3 hours with 100 ml of *n*-hexane in two repetitions, in a water bath shaker (Clifton, model NE5-10D, UK) at ambient temperature for fat removal at 200 rpm. The extract was filtered using a Buchner funnel and the filtrate which contained the lipids was removed. The residue was re-extracted with 100 ml solvent (10% methanol/water) after adding approximately 2 cm^3 of 100 ppm sodium bisulphite and adjusting the pH value of the extraction medium to 5.0 with 1M HCl at 60 ^{0}C for 3 hours at 200 rpm in two repetitions. The new extract was filtered using a Buchner funnel and the filtrate was obtained. The extract was then centrifuged (Universal centrifuge, model Z306, Germany) at 4000 rpm for 20 minutes to remove pulp and the precipitate. Then the extract was concentrated using a rotary evaporator (Rotary vaccum evaporator, Roteva, India) under reduced pressure at 50 ^{0}C. Finally, the remaining water was removed by lyophilization (Freeze dryer, model FDS 8512, Korea). The crude extract obtained was kept in dark glass bottle, wrapped with aluminium foil and stored in a cool room (Lae Electronic, model CDC 80, Italy) at -10 ^{0}C. The total phenolic content was determined by Folin-Ciocalteau assay [22,23] using gallic acid as the standard. The same procedure was conducted by changing the methanol concentration of the solvent [20,30,40,50,60,70,80,90,100 % (methanol/water)].

IV. Results and Discussion

The yields of total phenolic compounds extracted from BBPF in relation to the methanol content in the extraction solvent are shown in Figure [8].

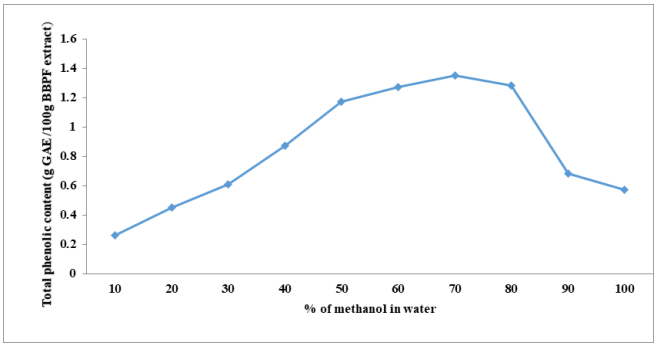

Fig. 1: Yield of total phenolic compounds extracted from blanched banana pseudo-stem flour (BBPF) in relation to the methanol content in the extraction solvent

The maximum yield of total phenolic compounds (1.35±0.18 g GAE/100 g extract) from blanched banana pseudo-stem flour (BBPF) has been extracted with the 70 % methanol solvent. Generally, the extraction yield of polyphenols is greatly depending on the solvent polarity. Amount and composition of phenolic compounds is observed to be diversified at sub-cellular level within the tissues [1,9,10]. The recovery of polyphenols from plant materials is reported to be influenced by the solubility of the phenolic compounds in the solvent used for the extraction process [2,3,10]. It is reported [6] that presence of phenolic acids such as tannic, pyrocatechol, catechol, gentisic, (+)-catechin, protocatechuic, gallic, caffeic, chlorogenic, ferulic, and cinnamic acids in the banana pseudo-stem. To extract these polar acids, moderately high polar solvent is needed [1,10]. Even though, the polarity of the extraction solvent increases after 70 % methanol, the extracted amount of phenolic compounds decreases. The reduction of the extraction of polyphenols after 70 % methanol occurs due to the incompatibility of the polarity of the solvent system with the polarities of the polyphenols in the BBPF. The use of methanol with water contributes to the creation of a moderately high polar medium that enhances the extraction of polyphenols. Relative polarities of methanol and water are respectively 0.762 and water 1.00. Hence, with the addition of water to methanol, the polarity of the solvent mixture increases continuously. Higher yield of polyphenolic compounds could be received with the matching of the polarity of the solvent mixture with the polarities of polyphenols in the BBPF. The results obey the general rule of "like dissolves like" principle. Another possible reason for the increased efficiency with the presence of some amount of water might be due to the increase in swelling of plant material by water, which increases the contact surface area between the plant matrix and the solvent. Aqueous methanol solvent can release the cell wall bound polyphenols from the

cells and neutralize the activity of polyphenol oxidase enzyme [10,20].

V. CONCLUSION

Solvent extractions are the most commonly used procedures to prepare extracts from plant materials due to their ease of use, efficiency and wide applicability. It is generally known that the yield of chemical extraction depends on the type of solvents with varying polarities, pH value of the extraction medium, extraction time, temperature, sample-to-solvent ratio and on the chemical composition and physical characteristics of the solutes [1,3,10]. The solubility of plant phenolics is governed by the chemical nature of the plant sample, as well as the polarity of the solvents used. Plant materials may contain phenolics varying from simple (e.g., phenolic acids, anthocyanins) to highly polymerized substances (e.g., tannins) in different quantities [1,9]. Moreover, phenolics may also be associated with other plant components such as carbohydrates and proteins [4,10]. These interactions may lead to the development of some complexes that may be difficult to solubilize in organic solvents alone. Therefore, there is no universal extraction procedure suitable for extraction of all the plant phenolics. Depending on the solvent system used during extraction, a mixture of phenolics soluble in the solvent will be extracted from plant materials. Aqueous methanol solvent has been used in extracting polyphenolic components from plant materials due to many advantages [2,4,28,29].

It is reported that presence of phenolic acids such as tannic, pyrocatechol, catechol, gentisic, (+)-catechin, protocatechuic, gallic, caffeic, chlorogenic, ferulic, and cinnamic acids in the banana pseudo-stem [26]. To extract these polar acids, moderately high polar solvent is needed. The use of methanol with water contributes to the creation of a moderately high polar medium that enhances the extraction of polyphenols [20,24,25]. Relative polarities of methanol and water are respectively 0.762 and water 1.00. Hence, with the addition of water to methanol, the polarity of the solvent mixture increases continuously [19]. Higher yield of polyphenolic compounds could be received with the matching of the polarity of the extraction solvent with the polarities of the polyphenols in the BBPF. The best recovery of polyphenolic compounds from banana (*Musa spp. Var.* Ambul kesel) pseudo-stem was obtained with the 70% methanol solvent and the optimum yield was recorded as 1.35±0.18 g GAE/100 g BBPF extract.

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