Screening of surfactants and glycols for polyphenol extraction from cacao bean shells

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Abstract: The cacao bean shell (CBS) is a by-product of the cacao industry and has been known to contain polyphenols that impart antioxidant activity. Surfactant-assisted extraction (SAE) offers a green alternative for extracting bio-functional compounds such as polyphenols. SAE was employed and investigated for the extraction of polyphenols from CBS. Surfactant (Tween 80, lecithin, and decyl glucoside) and glycol (Glycerol, polyethylene glycol, and propylene glycol) solutions at 10% were evaluated as alternative solvents for polyphenol extraction vis-à-vis 50% ethanol. The total phenolic content (TPC) yields of Tween 80 and polyethylene glycol (PEG) were 19.33 ± 0.80 and 18.28 ± 0.63 mg gallic acid equivalent (GAE) per g CBS, respectively. These TPC yields were comparable to 50% ethanol at 19.40 ± 0.59 mg GAE/g. The total flavonoid content (TFC) yield of 10% Tween 80 was significantly higher than that of the 50% ethanol sample ($p < 0.05$) at 17.16 ± 2.56 mg catechin equivalent (mg CE/g) vs 12.35 ± 0.56 mg CE/g, respectively while TFC yield from 10% PEG was at 13.35 ± 1.59 mg CE/g. This indicates that 10% Tween 80 is a viable alternative to organic solvents for extracting polyphenols from CBS.

Key Words: Cacao bean shell; surfactant-assisted extraction; polyphenols; total phenolic content; total flavonoid content

1. INTRODUCTION

The cacao fruit from the *Theobroma cacao* tree is the primary raw material used to produce chocolate. Around 10-20% (Rojo-Poveda et al., 2020) of the bean weight is the cacao bean shell (CBS) removed before grinding the kernel to edible cocoa mass, while 70-80% of the fruit is the cacao pod (Porto De Souza Vandenberghe et al., 2022). The extraction of bio-functional compounds from agricultural by-products could upcycle CBS. Both CBS and cacao pod husk have been reported to have bio-functional activities, including antimicrobial (Hutasoit et al., 2020), antioxidant (Martínez et al., 2012); antidiabetic (Indrianingsih et al., 2021), and wound healing (Chairunas & Abdat, 2022) properties. These activities are typically attributed to the phenolic and flavonoid compounds present in the fruit. Extraction of such compounds will not only valorize what would otherwise be waste but also supplement the income of cacao processors amidst the fluctuating prices of cacao. Cacao extracts have shown antimicrobial activity for food (Grassia et al., 2021; Kayaputri et al., 2020), dental (Percival et al., 2006), cosmetic (Hutasoit et al., 2020), wound healing (Chairunas & Abdat, 2022), and agricultural applications (Rachmawaty et al., 2018).

Polyphenols, flavonoids, and antioxidants contribute to the bio-functional activity of natural extracts. These compounds have different polarities, which limit the efficiency of water extraction. Amphiphiles such as surfactants have been proposed to modify the polarity of solvents (Hosseinzadeh et al., 2013). The hydrophobic tail captures non-polar compounds within the micelle structure, whereas the hydrophilic head extracts polar compounds and supports its applicability in an aqueous base. Surfactant-assisted extraction (SAE) offers a simple method for extracting both polar and non-polar compounds from cacao waste, which can enhance its biofunctional activity (Rangel-Yagui et al., 2005). The micellar structure can have spherical, cylindrical, or ellipsoidal shapes. These structural changes modify polarity at the microscopic level, which improves the solubility of non-polar compounds (Malysa-Pasko et al., 2018).

Current valorization methods for obtaining bio-functional compounds from CBS for food applications use either organic extraction (Hutasoit et al., 2020; Nsor-Atindana et al., 2012) or energy-intensive (Huynh et al., 2023; Jafari et al., 2023; A. C. Mellinas et al., 2020; C. Mellinas et al., 2019) methods, which are difficult to commercialize. Surfactant-assisted extraction explores surfactants that are typically incompatible with food (Gümüş Yılmaz et al., 2019; More & Arya, 2024). Extraction using food-safe surfactants has been reported (Leite et al., 2018; Sharma et al., 2015) but not on cacao. The effective valorization of agricultural waste for food applications must be coupled with a sustainable and scalable process. This study aims to screen and evaluate the effect of various surfactants and co-surfactants on the extraction of polyphenols from CBS. The findings from this study serve as a basis for developing an extraction protocol using a green solvent to sustainably valorize cacao bean shells. The application of nonvolatile, green solvents potentially allows for a process that can be commercialized with a responsible environmental footprint.

2. METHODOLOGY

2.1 Materials

Roasted cacao bean shell by-product was purchased from La Esquina de Marcelo farm (Bohol, Philippines). CBS was ground using a grinder (Biolomix BCG300). The CBS was ground until it passed through 40-mesh but retained on 80-mesh; the ground CBS was frozen until use to prevent oxidation. Decyl glucoside and propylene glycol were purchased from Asteria Apothecary. Glycerol, egg lecithin, hydrochloric acid, and sodium hydroxide were obtained from Theo-Pam Trading Corporation. Polyethylene glycol (PEG-400), catechin hydrate (HPLC grade) and aluminum chloride hexahydrate were purchased from DKL Laboratory. Sodium citrate, dipotassium phosphate and Tween 80 were obtained from Yana Chemodities. Citric acid, gallic acid monohydrate, Folin Ciocalteu's reagent, potassium dihydrogen phosphate, sodium bicarbonate, sodium carbonate and absolute ethanol were obtained from Belman laboratories. Sodium nitrite was obtained from Scharlab Inc. Decyl glucoside, propylene glycol and PEG-400 were technical grades. All other chemicals were of analytical grade.

2.2 SAE of polyphenols from CBS

Surfactant (Tween 80, decyl glucoside, lecithin) and co-surfactant (PEG 400, Glycerol, Propylene glycol) solutions were prepared at 10% concentration (Kiai et al., 2018) in 50 mM phosphate buffer. The final pH of the extraction solvent was adjusted to 7. The extraction was conducted in 125 mL Erlenmeyer flasks incubated in a water bath (DaiHan MaXturdy™ 45) at 50°C and 150 rpm for one hour. The samples were centrifuged at 6,000 rpm at 4°C (Hemle Z327K) for 5 minutes. The supernatant was filtered and adjusted to 20 mL with deionized water. The extracts were diluted to fall within the gallic acid and catechin calibration curves for TPC and TFC, respectively. Extraction of polyphenols was also performed using 50 mM phosphate buffer and 50% ethanol.

2.3 Evaluation of Total Phenolic Content

A standard curve was prepared using gallic acid as a reference. The absorbance was read using a microplate reader (SpectraMax Mini) at 760 nm. The total phenolic content (TPC) was evaluated as described in previous studies (Wanna et al., 2023; Yaowachai et al., 2023): 20 µL of diluted sample was combined with 100 µL of 10% Folin-Ciocalteu's reagent in a 96-well microplate. The mixture was incubated for 1 min before adding 80 µL of 7.5% sodium carbonate solution. The samples were then incubated in the dark for 90 minutes at room temperature before analysis. TPC of surfactant and co-surfactant solutions were also read as blanks. The samples were diluted with water to fall within the calibration curve. TPC was expressed as mg gallic acid equivalent (GAE) per gram CBS.

2.4 Evaluation of Total Flavonoid Content

The total flavonoid content (TFC) was evaluated using the $AlCl_3$-$NaNO_2$ method. The standard curve was prepared using catechin. The TFC assay was performed in a 96-well microplate as previously described, with minor modifications (Liew et al., 2018; Toro-Uribe et al., 2020): 25 µL of diluted sample was added to 7.5 µL of 5% $NaNO_2$ solution. The mixture was incubated for one minute before adding 7.5 µL 10% $AlCl_3$ (samples) or water (sample blanks). The microplate was allowed to stand for 10 min before adding 50 µL of 1M NaOH to induce a peach red color change. The volume was then filled to 250 µL and the absorbance was read at 510 nm. Sample blanks were prepared to account for the yellow-brown color of the cacao shell extracts. The TFC is reported as mg catechin equivalent (CE) per g CBS.

2.5 Statistical analysis

All experimental runs were randomized and conducted in duplicates. The results are reported as the mean. The screening experiments were performed using an OFAT (one-factor-at-a-time) design. MS Excel Analysis ToolPak was used for data analysis. Analysis of variance (ANOVA) was used to determine the significance of the factors, while a t-test was conducted to analyze the statistical significance of differences between groups.

3. RESULTS AND DISCUSSION

The surfactants were screened for their effect on TFC and TPC (Kiai et al., 2018) at a fixed concentration of 10%. The highest TPC extraction was obtained from Tween 80 at 19.33 ± 0.80 mg GAE/g which was significantly higher ($p < 0.05$) than the buffer-only, decyl glucoside, and lecithin-treated samples. ANOVA results also showed that the surfactant type significantly affected TPC extraction ($p < 0.01$). This corroborates the results obtained by Giovanoudis et al., where the polyphenol recovery from apricot cannery waste using Tween 80 was higher than that of lecithin (Giovanoudis et al., 2023). Skrypnik and Novikova reported that at 1% concentration and pH 4, Tween 80 performed better than 70% ethanol in terms of TPC extraction (Skrypnik & Novikova, 2020).

Surfactant treatment did not significantly affect the TFC extraction ($p > 0.05$). However, the TFC obtained by Tween 80 treatment was significantly higher($p < 0.05$) than that obtained by the buffer-only treatment, as shown in **Fig. 1**. Folin Ciocalteu's (FC) assay is the most common method used to determine the TPC. The principle relies on colorimetric changes that occur when phenolic compounds react with reducing compounds. However, this makes the FC assay susceptible to overestimation, hence accounting for other compounds such as sugars and amino acids (Dominguez-López et al., 2024). The inconsistency between the significance of surfactant treatment in TPC and TFC can be due to (1) the higher proportion of phenolic acids extracted by Tween 80 or (2) overestimating the TPC. Considering food compatibility and the significant difference between the buffer-only and the individual surfactant treatments, Tween 80 was selected as the best-performing surfactant.

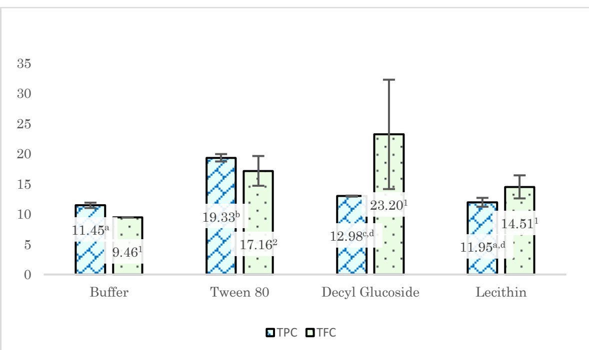

Fig. 1 Effect of 10% surfactant treatment on TPC and TFC extraction from CBS

The co-surfactants used in this study have been selected from solvents used previously: propylene glycol (PG) (Conceição et al., 2023; Erzsébet et al., 2020; Myo & Khat-udomkiri, 2022; Sangthong, 2024), glycerol (Abdoun et al., 2022; Anis & Ahmed, 2022; Huamán-Castilla et al., 2020; Kowalska et al., 2021; Lim et al., 2024; Manousaki et al., 2016; Michail et al., 2016) and PEG 400 (Donmez et al., 2020; Giovanoudis et al., 2023; Liang et al., 2009; Šuran et al., 2021; T. Zhou et al., 2011; X.-Y. Zhou et al., 2014). Polyol co-surfactant treatment significantly ($p < 0.01$) affected both TPC and TFC extraction, with PEG 400 outperforming ($p < 0.05$) as shown in **Fig. 2.** This corroborates the findings of Petchsomrit *et al.* where PEG 400 had higher TPC extraction than PG from marine alga (Petchsomrit *et al.*, 2024). However, they noted that PG extracts had slightly better antioxidant activity than that from PEG 400. Mai *et al.* (Mai *et al.*, 2020) coupled UAE with PEG-400 to obtain catechin from *Eunymus alatus.* Medeiros & Kanis also showed that the addition of just 5% PEG significantly improved coumarin and TFC extraction compared to just water, and is at par with ethanol at 30% (Medeiros & Kanis, 2010). Donmez *et al.* showed that PEG 400 significantly improved TPC and TFC extraction from propolis when compared with water (Donmez *et al.*, 2020). Hirano, Shiraki & Arakawa found that PEG behaves like an organic solvent in the solubilization of proteins (Hirano *et al.*, 2012).

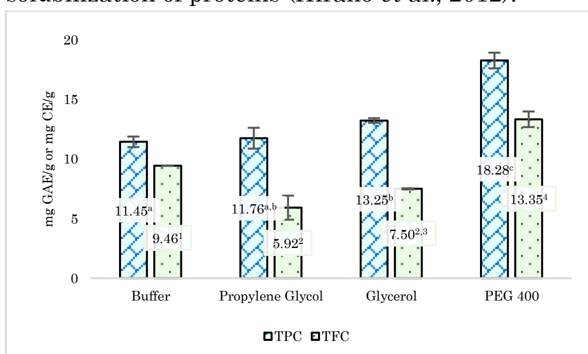

Fig 2. Effect of 10% glycol treatment on TPC and TFC extraction from CBS

The TPC and TFC obtained by the Tween 80 and PEG 400 extracts are at par with the 50% ethanolic extracts (see **Table 1**). PEG 400 treatment improved TPC and TFC extraction by 59.7% and 41.1%, respectively, relative to aqueous extraction, while Tween 80 allowed 68.8% and 81.4% improvement over the buffer-only sample for TPC and TFC, respectively. Propylene glycol extracts of CBS were previously studied to have comparable TPC and TFC with 50% ethanol extracts (Sangthong, 2024) .

This supports the hypothesis that green extraction using surfactants and polyols can be a viable alternative to organic extraction.

Table 1. Comparison of SAE with aqueous and ethanolic extraction

Solvent	TPC (mg GAE/g)	TFC (mg CE/g)
pH 7 Buffer	11.45 ± 0.55	9.46 ± 0.01
10% PEG 400	18.28 ± 0.78	13.35 ± 1.59
10% Tween 80	19.33 ± 0.98	17.16 ± 2.56
50% Ethanol	19.40 ± 0.72	12.35 ± 0.56

4. CONCLUSIONS

The potential of surfactants and co-surfactants as green solvents for the extraction of polyphenols and flavonoids from cacao bean shell waste was explored. Tween 80 and PEG 400 obtained the highest TPC and TFC among the surfactants and co-surfactants tested, respectively. Tween 80 was selected as the best surfactant with TPC at 19.33 mg GAE/g and 17.16 mg CE/g for flavonoids. On the other hand, PEG 400 obtained 18.28 mg GAE/g TPC and TFC of 13.35 mg CE/g. The values obtained from this study are at par with that of 50% ethanol.

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