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Isolation and analyses of polymeric polyphenol fractions from black tea

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Abstract

Polymeric polyphenols are the most abundant pigments in black tea. Very limited information is available on the isolation and chemical characterization of these compounds. The present paper reports the comparative yields, physicochemical properties and preliminary biological activities of five polymeric black tea polyphenol (PBP) fractions isolated by liquid–liquid and Soxhlet-based solid (tea powder)–liquid extraction methods. The two methods have also been comparatively evaluated as regards cost, time, labour and practicality of scale-up. Overall, results show that PBP fractions, isolated by both the procedures, were free of other known biologically active monomeric and oligomeric components, and have similar physicochemical properties and biological effect(s). Solid–liquid extraction results in a relatively greater yield of PBP-1 to -3, significant reduction in cost, time and labour and also makes scale-up relatively easier.

Keywords: Black tea; Extraction methods; Polymeric black tea polyphenol fractions; Yields; Physicochemical properties; Biological activity

1. Introduction

Tea is one of the most commonly consumed beverages throughout the world. Based on the manufacturing technique, teas can be classified as: green tea (20–22% of world tea consumption), oolong tea (2–3% of world tea consumption) and black tea (73–78% of world tea consumption). During the manufacture of black tea, a major proportion of monomeric free catechins [90% of total polyphenol content in green tea] in the fresh green tea leaf undergoes oxidative polymerization, to form oligomers – theaflavins (TFs) [13% of total polyphenol content in black tea] and polymers – polymeric black tea polyphenols (PBPs)/thearubigins (TRs) [47% of total polyphenol content in black tea] (Harbowy & Balentine, 1997). Despite the fact that both TFs and PBPs were discovered in the early 1960s (Roberts & Smith, 1961), information on the formation and chemical structures of TFs has been rapidly increasing, whereas that on PBPs, which are the most abundant of oxidatively polymerized products in black tea, has remained elusive or at the best speculative (Haslam, 2003; Menet, Sang, Yang, Ho, & Rosen, 2004).

Roberts and Smith (1961) could separate PBPs into two large groups, namely PBP SI and PBP SII, based on differences in chemical polarities. Brown, Eyton, Holmes, and Ollis (1969) modified the above method to obtain five fractions, namely ethyl acetate-soluble PBP-1 and water-soluble PBP-2, PBP-3, PBP-4 and PBP-5, and suggested that the PBPs isolated were polymeric in nature. Several standard chromatographic (Bailey, Nursten, & McDowell, 1992; Robertson &

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Bendal, 1983) and modern analytical techniques (Degenhardt, Engelhardt, Wendt, & Winterhalter, 2000; Degenhardt, Engelhardt, Winterhalter, & Ito, 2001) have not been successful in preparative fractionation of PBPs, due to the ability of PBPs to react strongly with active surfaces (Finger, Kuhr, & Engelhardt, 1992).

The health beneficial effects of green tea have been attributed to monomeric free catechins (Surh, 2003), while biological effect(s) and health benefits, if any, of two major group of pigments, theaflavins (TFs) and thearubigins (TRs)/polymeric black tea polyphenols (PBPs), from black tea, are not known. Recent results from our laboratory have indicated that PBPs retain the beneficial effects of their precursors (Krishnan & Maru, 2004). However, for in vivo biological evaluation, large quantities of PBPs are required. Hence, in the present communication, liquid–liquid solvent extraction procedure (Brown et al., 1969) for isolation of PBPs from black tea liquor has been compared with a solid (tea powder)–liquid extraction procedure, set up in our laboratory with respect to their yields, physicochemical properties, preliminary in vitro biological activities, cost, time, labour and possibility of scale-up.

2. Materials and methods

2.1. Sample and chemicals

A popular brand of black tea purchased from the local market in Mumbai, India was used for the entire study. Pre-coated silica gel plates, with or without 254 nm fluorescent indicator, (+)-catechin (C), (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCG) and caffeine, were purchased from Sigma (St. Louis, MO, USA). TF(s) were isolated in our laboratory (Morse et al., 1997). All organic solvents and other reagents were of AR or HPLC grade and purchased locally. A Soxhlet continuous extractor [Length (l) = 14 in. and Internal Diameter ((i.d.) = 4 in.) was employed for carrying out solid–liquid extraction of black tea.

2.2. Isolation of PBPs from black tea by liquid–liquid extraction (Method A)

Polymeric black tea polyphenol fractions (PBPs) were isolated from black tea as described (Brown et al., 1969). Isolation/purification of PBPs was carried out by successive extraction of an aqueous extract of black tea with chloroform, ethyl acetate and *n*-butanol, followed by acidification and further extraction with *n*-butanol. The chloroform extract yielded mainly caffeine. The residues from the other three extracts were purified by fractional precipitation, employing mixtures of acetone/chloroform, acetone/ether and methanol/ether. This process yielded five polymeric black tea polyphenol fractions, namely PBP-1, PBP-2, PBP-3, PBP-4 and PBP-5, which belonged to one general structural type (Brown et al., 1969).

The above method involved use of large volumes of solvents, which resulted in increased costs and increased time for processing. In addition, practical handling of large volumes of solvents was also difficult, which made scale-up impractical. Hence, black tea powder was extracted directly with solvents in a Soxhlet continuous extractor, wherein volumes of solvents for extraction may substantially be reduced, which in turn may decrease the costs, time of processing, labour and, most importantly, may make scale-up practically feasible.

2.3. Solid–liquid extraction (Method B)

Black tea powder (450 g) in a thimble was decaffeinated with chloroform (2.5 l) in a Soxhlet continuous extractor, until no colour was present in the solvent (∼24 h). Air-dried black tea powder (≈445 g) in a thimble was further Soxhlet-extracted with ethyl acetate (2.5 l, 24 h). The ethyl acetate extract was dried in vacuo, while air-dried black tea powder (≈415 g) in a thimble was further Soxhlet extracted with *n*-butyl alcohol (2.5 l, 24 h) to obtain *n*-butyl alcohol extract, which was dried in vacuo.

The dried ethyl acetate extract was dissolved in acetone (200 ml) and precipitated with 8 volumes of diethyl ether, thrice, to obtain a precipitate of PBP-1. Subsequently, the dried *n*-butyl alcohol extract was dissolved in methanol (130 ml) and precipitated with 10 volumes of diethyl ether, thrice, which yielded PBP-2 as a precipitate, while the filtrates were dried, dissolved in acetone (100 ml) and precipitated with 10 volumes of diethyl ether, thrice to get PBP-3.

Next, the residual air-dried black tea powder (≈351.8 g) was boiled with distilled water (1.5 l) for 20 min to obtain an aqueous solution which, after filtration, was acidified with 3.5 N sulfuric acid (75 ml). This acidified aqueous solution was then extracted with equal volumes of *n*-butyl alcohol, until the upper *n*-butyl alcohol layer became colourless (≈7 times), following which the *n*-butyl alcohol extracts were pooled, dried in vacuo and processed in same way as for PBP-2 and PBP-3, to obtain PBP-4 and PBP-5.

The above procedures were carried out in a chemical safety hood with necessary protective gadgets and precautions. To prevent any light-mediated effect(s), containers were covered with brown paper and/or aluminium foil during the course of extraction. All the PBP fractions were dried in vacuo, employing a rotary flash evaporator, to remove any residual organic solvent, and stored at −20 °C for later use. Yields of PBPs

by both methods presented are means ± SE of three independent extractions.

2.4. Yields of aqueous-extractable solids

To compare the yields of aqueous-extractable solids in both procedures, equal weights of tea powder-derived decaffeinated black tea liquor (Method A) and liquor obtained from decaffeinated black tea powder (Method B) were freeze-dried and weighed. Results are means ± SE of two independent extractions.

2.5. Evaluation of physicochemical parameters

2.5.1. Total polyphenol content

Total polyphenol content of each PBP fraction extracted by both methods was determined as gallic acid equivalents, employing Folin and Ciocalteau's phenol (FC) reagent (Ragazzi & Veronese, 1973). Results, expressed as mg gallic acid/g PBP, are means ± SE of two independent experiments carried out in duplicate.

2.5.2. Reactivity with ferric chloride ($FeCl_3$)

The PBP fraction (5 mg), isolated by both methods, was dissolved in acetone (1 ml) and 20 μl (≡100 μg) were spotted onto Whatman No.3 filter paper and reacted with 10 μl of $FeCl_3$ (1%) and potassium ferricyanide (1%) mixed in equal proportions. EGCG, a major green tea polyphenol, was used for comparison. Blue colour formation was indicative of a positive response.

2.5.3. pH of PBP solutions

An aqueous solution (1%) of each polymeric black tea polyphenol, obtained by both the methods, was prepared. PBP-1, -2 and -3 showed complete dissolution, whereas PBP-4 and -5 showed 90% and 80% solubilty, respectively. The pH of the resultant aqueous solutions was measured at 25 °C, using an APX 175E-type pH meter from Ingold, Germany. It may be noted that all PBP fractions were completely soluble in phosphate-buffered saline, pH 7.4, which was employed as the solvent in all biological assays.

2.5.4. Determination of contamination by other black tea components

To ascertain that the fractions obtained by both methods were free of other known biologically active mobile components present in black tea, such as caffeine, free catechins [C, EC, ECG, EGC, EGCG, GCG] or TFs, 100 μl of each PBP fraction (5 mg dissolved in 1 ml acetone) were spotted alongside standards of caffeine and free catechins and TFs (isolated in the laboratory) on pre-coated silica gel plates with 254 nm fluorescent indicator and developed in chloroform:ethyl acetate:formic acid (6:4:1) and visualized under UV light.

2.5.5. Proanthocyanidin test for PBPs

Each PBP fraction (25 mg), obtained by either method, was heated (100 °C, 15 min, sealed glass ampoule) in 1 ml of 0.35 N HCl in anhydrous isopropanol. The acid hydrolysate was spotted and the chromatogram was developed in D1 with formic acid:3 N HCl (1:1), followed by D2 in water:acetic acid:concentrated HCl (10:30:3). Pink spots were observed, indicating the proanthocyanidin nature of the fractions obtained (Brown et al., 1969).

2.5.6. UV absorption spectra

PBP fractions (1 mg/ml) were dissolved in 0.1 M sodium phosphate buffer, pH 7.4, and UV spectra recorded on a Hitachi U-2001 spectrophotometer.

2.5.7. Fourier-transformed infra-red and nuclear magnetic resonance analyses

PBP fractions were compared by Fourier-transformed infra-red (FT-IR) and nuclear magnetic resonance (NMR) analyses. FT-IR spectra were recorded on a Magna 550 Series infrared spectrophotometer, Nicolet, USA. Spectra were taken in transmittance mode at a resolution of 0.4 cm^{-1} and frequency of 32 scans over a range of 400–4000 cm^{-1}. ^1H NMR spectra were recorded on a Mercury Plus (300 MHz) instrument, Varian, USA at a rate of 318 completed transients (ct) over an acquisition time (at) of 2 s. DMSO was employed as solvent and also served as an internal standard.

2.5.8. Comparison of biological activity of PBP-2

Our earlier studies have shown that all PBPs isolated possessed biological activity (Krishnan & Maru, 2004). PBP-2 was found to be the most abundant of all PBP fractions isolated by both the methods and hence was used as a representative PBP for evaluating biological effects, employing in vitro model systems.

2.5.9. Effect of PBP-2 on [^3H]-B(a)P-derived DNA adducts in vitro

Various concentrations of chemopreventive agent (PBP-2 isolated by both methods) in 0.1 M phosphate buffered saline, pH 7.4, were compared for their effect on formation of [^{3}H]-B(a)P-derived DNA adducts in vitro, as reported earlier (Deshpande & Maru, 1995; Krishnan & Maru, 2004). Results, expressed as pmol [^{3}H]-B(a)P bound/mg DNA, are means ± SE of at least five observations.

2.5.10. Effect of PBP-2 on CYP 1A1 activity

PBP-2, isolated by both procedures, was dissolved in 0.1 M sodium phosphate buffer, pH 7.4, and tested for

its effect on the activity of isozyme of CYP-450, i.e., CYP 1A1 (Krishnan & Maru, 2004; Pohl & Fouts, 1980). Results, expressed as nmol resorufin formed/min/mg protein, are means ± SE of at least five observations.

All parameters investigated were statistically analyzed, employing the Student's *t*-test or ANOVA, followed by Bonferroni's test.

3. Results

3.1. Yield of PBPs and aqueous-extractable solids

Yields of various PBPs isolated by liquid–liquid extraction (Method A) and solid–liquid extraction (Method B) are presented in Fig. 1, along with yields of total aqueous-extractable solids in decaffeinated black tea liquor (Method A) and decaffeinated black tea powder (Method B), while keeping the brewing conditions identical.

The percentage yield of total PBPs isolated by method A, involving liquid–liquid extraction was 7.07%, with the yield of PBP-1 to PBP-5 being 1.07%, 2.93%, 0.75%, 2.00% and 0.30%, respectively. Method B, which was based on solid–liquid extraction of black tea powder, yielded 10.3% of total PBPs, of which 2.68%, 3.79%, 1.34%, 2.20% and 0.32% were the individual yields of PBP-1 to PBP-5, respectively (Fig. 1). There was a 1.5-fold increase in the yield of total PBP fractions in method B as compared to method A. This was mainly due to a 2.5-, 1.3- and 1.8-fold increase in the yields of PBP-1, PBP-2 and PBP-3, respectively, in method B as compared to method A, while the yields of PBP-4 and PBP-5 were comparable in both the methods. In method A, the percentage yield, in descending order, was, PBP-2 > PBP-4 > PBP-1 > PBP-3> PBP-5, whereas, for method B, the pattern observed was, PBP-2 > PBP-1 > PBP-4 > PBP-3 > PBP-5. Amongst all PBPs, the yield for PBP-2 was highest and that for PBP-5 was lowest in both the methods. The percentage yield of aqueous-extractable solids was found to be comparable in both the methods.

3.2. Evaluation of physicochemical properties

3.2.1. General

PBP fractions isolated by the two methods were compared for their physicochemical properties, such as colour and pH of aqueous solutions, TLC mobility, $FeCl_3$ reactivity, total polyphenol content, proanthocyanidin nature, UV, FT-IR and NMR spectra (Table 1).

3.2.2. Total polyphenol content

All PBPs, isolated by both methods, were compared for their total polyphenol contents, in terms of gallic acid equivalents. PBP-5 showed relatively greater reactivity to the FC reagent, while PBP-2 and PBP-4 were the least reactive. In both methods, the reactivity was in the order: PBP-5 > PBP-1 > PBP-3 > PBP-2 ⩾ PBP4, in terms of total polyphenol content. Total polyphenol contents of each PBP isolated by both methods were similar (Table 1).

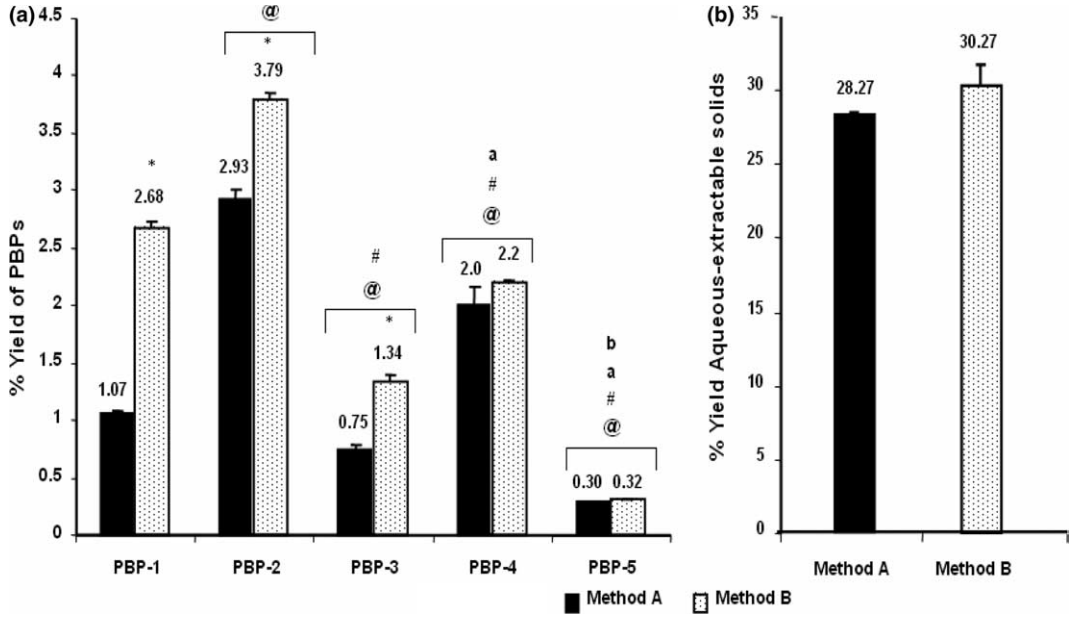

Fig. 1. Yield of (a) PBPs and (b) water-extractable solids (g% of black tea powder) from black tea powder. * = Significantly different as compared to respective PBP (Method A), $P < 0.05$ (Student's *t*-test), @ = Significantly different as compared to PBP-1, # = Significantly different as compared to PBP-2, a = Significantly different as compared to PBP-3, b = Significantly different as compared to PBP-4, $P < 0.05$ (ANOVA, followed by Bonferroni's test).

Table 1
Comparative physicochemical properties of PBPs isolated by two methods

Parameters	Method A					Method B				
	PBP-1	PBP-2	PBP-3	PBP-4	PBP-5	PBP-1	PBP-2	PBP-3	PBP-4	PBP-5
Colour	Light brown	Brown	Pale yellow	Dark brown	Dark brown	Light brown	Brown	Pale yellow	Dark brown	Dark brown
pH of 1% aq. solutions	5.53	5.55	5.40	4.26	4.09	5.53	5.55	5.40	4.26	4.09
TLC mobility[a]	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
$FeCl_3$ reactivity[a]	+	+	+	+	+	+	+	+	+	+
Total polyphenol measured as gallic acid (mg/g PBP)[a]	214 ± 1	114^{b} ± 1	$147^{b,c}$ ± 2	$113^{b,d}$ ± 1	$264^{b,c,d,e}$ ± 2	213 ± 1	117^{b} ± 2	$143^{b,c}$ ± 1	$116^{b,d}$ ± 1	$270^{b,c,d,e}$ ± 1
Proanthocyanidin test[a]	+	+	+	+	+	+	+	+	+	+
UV λ_{max1}	215	219	217	211	210	215	218	219	217	212
UV λ_{max2}	272	272	268	263	270	273	272	272	268	271
FT-IR			Suggestive of polymeric nature					Suggestive of polymeric nature		
NMR			Suggestive of polymeric nature					Suggestive of polymeric nature		

[a] For details, see text.
[b] Significantly different as compared to PBP-1.
[c] Significantly different as compared to PBP-2.
[d] Significantly different as compared to PBP-3.
[e] Significantly different as compared to PBP-4, $P < 0.05$ (ANOVA, followed by Bonferroni's test).

3.2.3. pH of aqueous solutions

The pH of PBP fractions was determined as mentioned above. The pH values of PBP fractions, isolated by both methods, were similar. All fractions had an acidic pH, ranging from 4.0 to 5.5 (Table 1).

3.2.4. TLC analysis

PBP fractions, isolated by both the methods, did not show any presence of other known biologically active, mobile components, such as free catechins, theaflavins or caffeine, known to be present in black tea, and PBPs were retained at the origin, showing a strong solid matrix reactivity (Table 1).

3.2.5. Proanthocyanidin test for PBPs

All PBP fractions isolated by both methods on acid hydrolysis and subsequent 2-D paper chromatography as mentioned in Section 2.5.5. showed pink spots characteristic of proanthocyanidins as reported (Brown et al., 1969). EGCG and solvent blank did not show the characteristic spots (Fig. 2; Table 1).

3.2.6. UV spectra

The UV spectra (190–340 nm) of PBP fractions, isolated by both methods, showed two absorption maxima, namely λ_{max1} and λ_{max2}, which were similar for each PBP. Overall, the range of λ_{max1} was 210–219 nm, whereas λ_{max2} ranged from 263–273 nm. UV spectra were identical for PBP-1, -3 and -4, isolated by Methods A and B, whereas some variation was observed for PBP-2 and -5 isolated by both methods (Fig. 3).

3.2.7. FT-IR analysis

Comparison of the FT-IR spectra of the monomeric free catechins (C, EC, ECG, EGC and EGCG, not shown) with those of PBPs isolated by methods A and B showed that, while the peaks in the fingerprint region (800–1600 cm^{-1}) were sharp for the free catechins, the corresponding peaks were poorly resolved for the PBPs. Additionally, the peaks in the 3300–3600 cm^{-1} region, assigned to the hydroxyl groups, were much broader when compared to the corresponding peaks for the free catechins. This confirms the polymeric nature of PBPs. Further comparison of the FT-IR spectra of PBPs isolated by Method A and Method B showed that PBP-1, PBP-3 and PBP-4, isolated by both the methods, were almost identical. However, considerable differences in the fingerprint region, especially in the 800–1000 cm^{-1} region, were discernible for PBP-2 and PBP-5 isolated by the two methods, thus suggesting appreciable dissimilarities in their structures (Fig. 4).

3.2.8. NMR analysis

The ^{1}H NMR spectra of PBP fractions showed poorly resolved peaks, making peak assignment

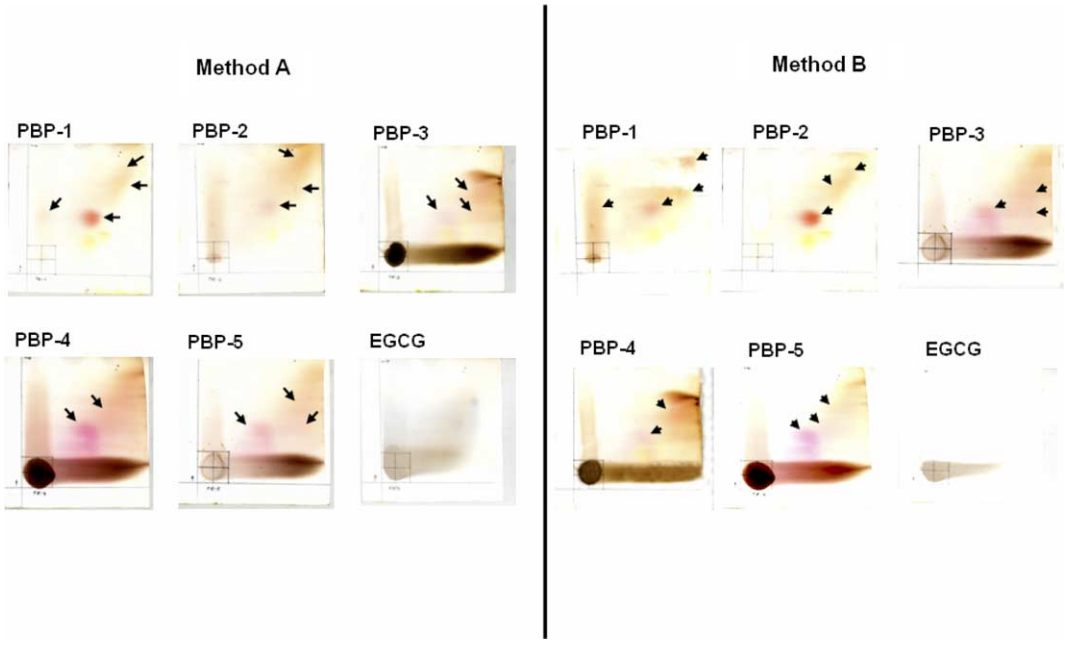

Fig. 2. Paper chromatograms (2-D) showing proanthocyanidin nature of PBPs isolated from black tea. Each PBP fraction (25 mg) was heated (100 °C, 15 min, sealed glass ampoule) in 1 ml of 0.35 N HCl in anhydrous isopropanol. The acid hydrolysate was spotted and the chromatogram was developed in D1 with formic acid:3 N HCl (1:1), followed by D2 in water:acetic acid:concentrated HCl (10:30:3).

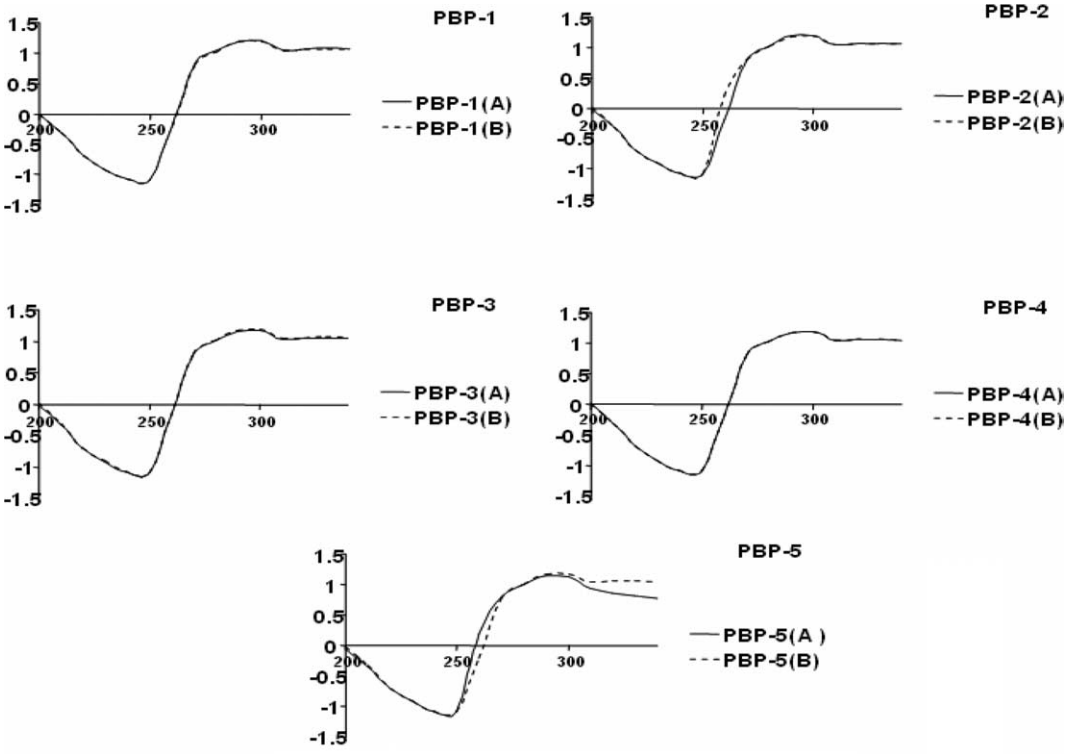

Fig. 3. UV spectra of PBPs. All PBP fractions were dissolved in 0.1 M sodium phosphate buffer, pH 7.4, and the resultant solutions employed for recording the UV spectra. Method A = Reported method of Brown et al. (1969), Method B = Solid–liquid extraction method.

difficult. However, the overall spectral characteristics are indicative of the polymeric nature of PBPs and their structural similarities. No major differences in the spectral pattern could be observed for individual PBP fractions isolated by the two different methods (Fig. 5).

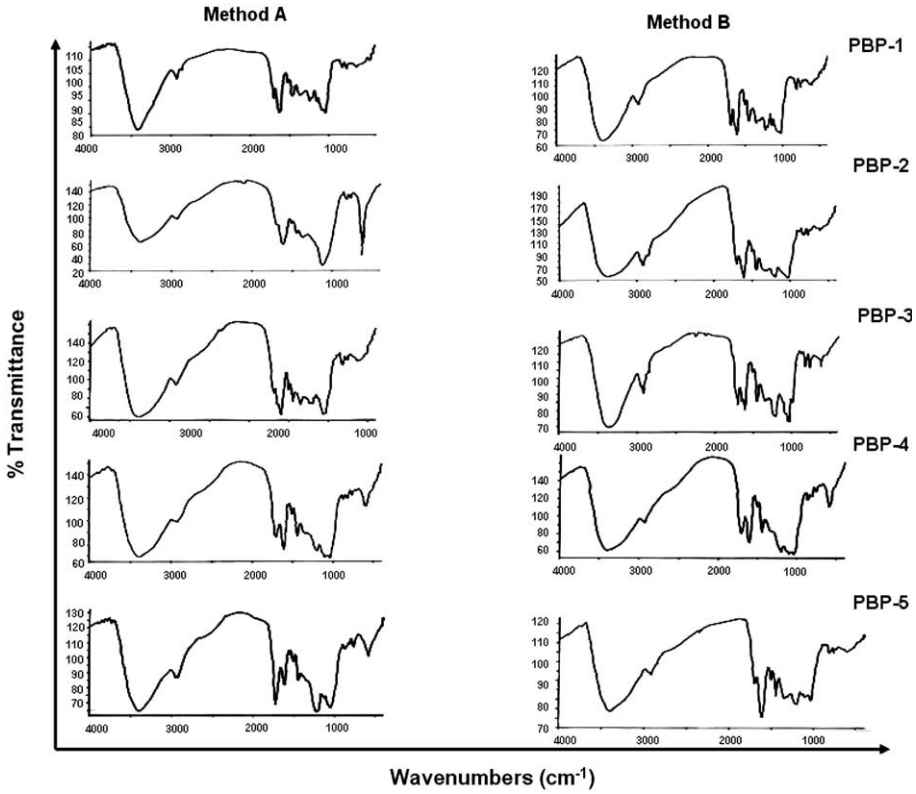

Fig. 4. Fourier-transformed infra-red (FT-IR) spectra of PBPs isolated by Method A (reported method of Brown et al., 1969) and Method B (solid–liquid extraction method). For details see Section 2.

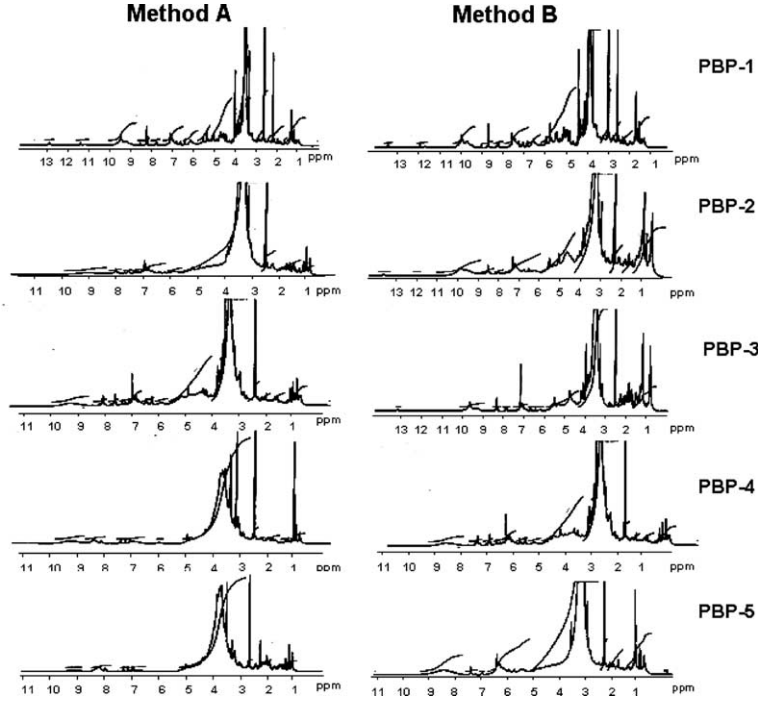

Fig. 5. Nuclear magnetic resonance (NMR) spectra of PBPs isolated by Method A (reported method of Brown et al. (1969)) and Method B (solid–liquid extraction method). For details see Section 2.

3.3. Comparison of volume of solvents required, cost, time and yield

As is clear from Fig. 6, solid–liquid extraction not only brought about a 45% increase in the yield of total PBPs, but also resulted in a 30-fold reduction in the volume of solvents required and cost, which reduced the time of isolation by 50%, when compared to the liquid–liquid extraction method of Brown et al. (1969).

3.4. Comparison of biological activity of PBP-2

Since PBPs were isolated by two different methods, similarities in physicochemical properties, overall increase in yields and reduction in cost, time and labour in Method B as compared to Method A, may necessitate comparative evaluation of their biological activity. Hence, the effect of PBP-2 (isolated by method A and method B) was studied on the microsome-catalyzed formation of (i) [^{3}H]-B(a)P-derived DNA adduct(s) and (ii) resorufin from ethoxyresorufin in vitro.

PBP-2, isolated by both the methods, inhibited the microsome-catalyzed formation of (i) [^{3}H]-B(a)P-derived DNA adduct(s) (Fig. 7) as well as (ii) that of resorufin from ethoxyresorufin (Fig. 7) in vitro.

PBP-2 (100–800 μg), isolated by both methods, showed a dose-dependent inhibition of [^{3}H]-B(a)P-derived DNA adduct(s) formation in vitro. The extent of inhibition ranged from 7–72% (Method A) and from 13–79% (Method B). This was supported by a similar dose-dependent inhibition of the CYP 1A1 activity in vitro, wherein the range of inhibition was 7–100% (Method A) and 12.5–100% (Method B) (Fig. 6). PBP-2 (200 μg, Method A) brought about a relatively greater inhibition of CYP 1A1 activity than did PBP-2 (200 μg, Method B). Overall, the extent of inhibition by PBP-2 was comparable in both the assays.

4. Discussion

The isolation and identification of the components of food/beverage of plant origin that may afford protection against major chronic diseases is an important area of research, worldwide. Several plant-derived antioxidants, including free catechins from green tea, have shown promise in this regard while negligible information is available on the biological effect(s) of polymeric polyphenols from widely consumed black tea. This is probably due to lack of well-established isolation procedures and poor chemical characterization of these compounds. The strong matrix reactivity of PBPs has made their isolation on solid supports difficult (Finger et al., 1992; Opie, Robertson, & Clifford, 1990). Attempts at methylating or acylating the phenolic groups of PBPs, which are responsible for their strong binding to active surfaces, have also met with little success (Wedzicha & Donovan, 1989). This has prompted researchers to focus their attention on isolation of PBPs by liquid–liquid partitioning (Brown et al., 1969; Degenhardt et al., 2000, 2001).

Our earlier observation on biological effect(s) of PBPs isolated by liquid–liquid extraction (Brown et al., 1969) has shown promising effects in an in vitro model system (Krishnan & Maru, 2004). However, isolation by the reported procedure is very laborious, time-consuming, costly and not practical for scale-up; hence, while maintaining essentially similar conditions of extractions, a logical and obvious approach, using the Soxhlet continuous extractor, was employed.

The solid (tea powder)–liquid extraction procedure by-passes the preparation procedure for tea liquor employed in reported procedures and thus the volumes of several solvents required for extraction, time, and ultimately cost and labour are significantly reduced. These advantages and ease of isolation would not be meaning-

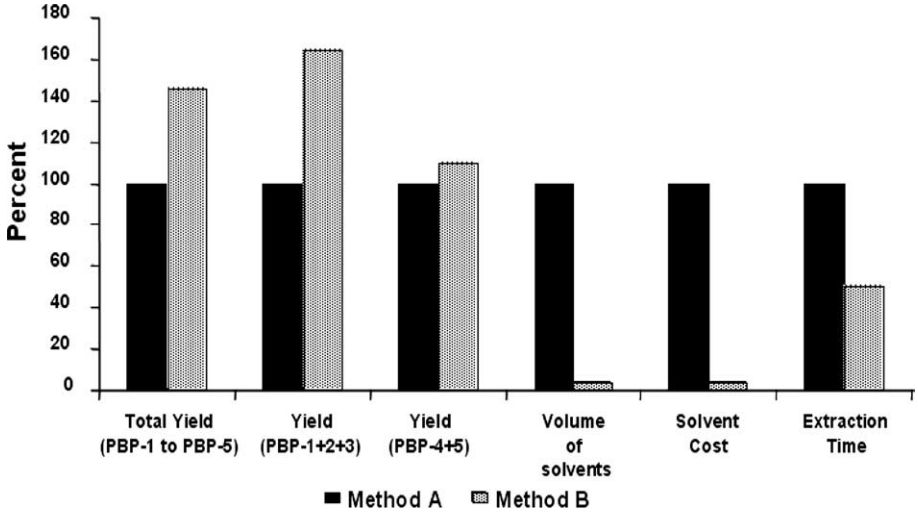

Fig. 6. Comparison of volume of solvents required, cost, time and yield of PBPs isolated by two methods.

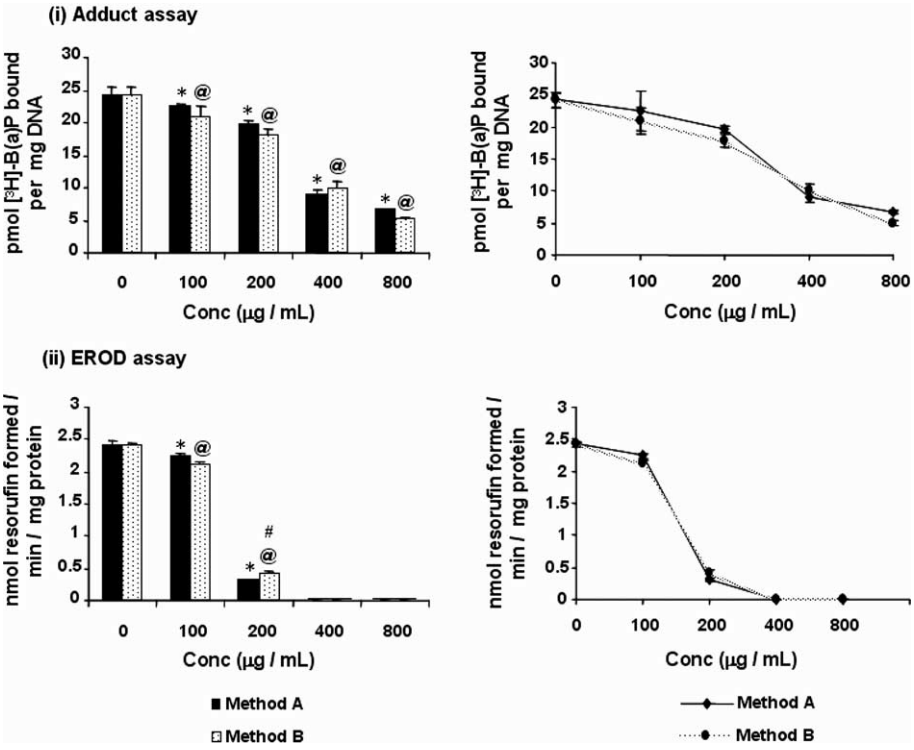

Fig. 7. Effect of PBP-2 on the microsome-catalyzed formation of (i) [^{3}H]-B(a)P-derived DNA adduct(s) and (ii) resorufin from ethoxyresorufin, in vitro. * = Significantly different as compared to 'Control' (Method A), @ = Significantly different as compared to 'Control' (Method B); $P < 0.05$ (ANOVA), # = Significantly different as compared to Method A; $P < 0.05$ (Student's *t*-test).

ful, or acceptable, had compounds not shown similar physicochemical characteristics and biological activities. Comparative evaluation of physicochemical properties suggest compounds isolated by both the procedures to be (a) free from other known biologically active monomeric and/or oligomeric components of black tea, (b) proanthocyanidin and polymeric in nature, and essentially with similar UV, FT-IR and NMR spectra. Likewise, the biological effects of PBP-2 isolated by both methods were similar to those observed in our earlier studies (Krishnan & Maru, 2004). Results also suggest that extended heating of compounds in the solvents in solid–liquid Soxhlet extraction, unlike the liquid–liquid extraction of Brown et al. (1969), does not appear to affect the stability, physicochemical characteristics (TLC mobility, UV, FT-IR and NMR spectra) or biological effects of the compounds isolated.

In addition to giving fractions with similar physicochemical and biological properties, solid–liquid extraction results in enhancement of yields of PBP-1, PBP-2 and PBP-3, which in turn increases the overall yields of PBPs by 45% as well as a 30-fold reduction in the requirement of solvents and cost and a 50% decrease in isolation time. In this procedure, scale-up is also feasible. Thus, our comparison of two procedures shows and records that the Soxhlet continuous extraction approach improves yield, and reduces cost, time and labour, without affecting the physicochemical and biological activities of these compounds. Results of this investigation are likely to help in enhancing progress on chemical characterization and evaluation of biological effect(s) of black tea-derived major pigments.

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