

Effect of Extraction Techniques on Phenolic Content, Antioxidant and Antimicrobial Activity of *Bauhinia purpurea*: HPTLC Determination of Antioxidants

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Abstract *Bauhinia purpurea* leaf was extracted by Soxhlet, ultrasonication and maceration extraction methods using ethanol (99.5%, v/v) to obtain Soxhlet (SBE), ultrasonicated (UBE) and macerated (MBE) *B. purpurea* leaf extract. The effects of different extracting methods on the polyphenolic content and antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), ferric-reducing antioxidant power (FRAP) and total antioxidant capacity (TAC) were investigated. Disc diffusion and broth dilution methods were also carried out to find the antibacterial activity of these extracts. Findings of this study showed that UBE possessed significant ($P < 0.05$) polyphenolic constituents followed by MBE and SBE. All the extracts exhibited good DPPH and ABTS radical scavenging as well as potential reducing ability in TAC and FRAP methods. UBE possessed significant ($P < 0.05$) radical scavenging activity and reducing ability followed by MBE and SBE. Even the results of antibacterial activity were similar to antioxidant activity, with UBE inhibiting most of the bacteria followed by MBE and SBE. All the extracts were subjected to thin layer chromatography (TLC) bioautography followed by high-performance TLC densitometric determination, and the results show that extraction using ultrasonication method yields the highest amount of antioxidant compounds among the three methods mentioned earlier. This study confirms ultrasonic extraction to be an ideal, simple and rapid method to obtain antioxidant- as well as antibacterial-enriched *B. purpurea* leaf extract. The HPTLC fingerprint profile can be used as a reference data for the standardisation of *B. purpurea* leaf.

Keywords Antioxidant · Antimicrobial · *B. purpurea* · Densitometry · Free radical · HPTLC

Introduction

Extraction is an important step involved in the discovery of bioactive components from plant materials. Currently, conventional extraction techniques such as maceration and Soxhlet extraction have been used along with several novel extraction techniques, such as ultrasonic extraction, accelerated solvent extraction and microwave-assisted extraction, to accelerate the extraction of bioactive compounds from plants (Wang and Weller 2006). Several studies have been published regarding the variation in the biological activities of extracts obtained from different extraction techniques, emphasizing the importance of selecting the suitable extraction method (Hayouni et al. 2007). The presence of several antioxidant components such as flavonoids, flavonols, vitamin C and tannins in plants makes them an attractive new source of antioxidants to replace the synthetic ones. These compounds also exhibited a wide range of biological activities, including anticancer and antimicrobial activities, in addition to their antioxidant activity (Penna et al. 2001; Ren et al. 2003).

Several methods are developed for the identification, separation and purification of bioactive compounds. TLC bioautography is an ideal, convenient and simple method for the rapid detection and separation of antioxidants as well as antimicrobials from complicated plant extract (Gu et al. 2009; Soberon et al. 2010). Herbal drug standardisation is an important area which is gaining more attention because of the indiscriminate use of herbal medicine. With regard to this, chromatographic fingerprint profiles have been shown to be very simple, more convenient and

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effective for the quality assessment of herbal medicine (Chen et al. 2006). The advantages of low operating cost, analysis time and the unique feature of image capture coupled with digital scanning profile make preparing the chromatographic fingerprint of herbs by means of HPTLC more convenient (Di et al. 2003).

Bauhinia purpurea L. is a flowering plant species belonging to the family *Leguminosae*, commonly found in Southeast Asia. Different parts of this plant have been used in traditional medicine to cure a host of illness such as body pain, rheumatism, fever, dropsy, stomach tumour, skin diseases, septicemia and diarrhoea (Asolker et al. 2000; Kirthikar and Basu 2001). The leaf, bark and root of this plant have shown analgesic, anti-inflammatory, antipyretic (Zakaria et al. 2007; Shreedhara et al. 2009), antidiabetic (Muralikrishna et al. 2008), antimalarial, antifungal, cytotoxic (Boonphong et al. 2007; Pettit et al. 2006), free radical scavenging (Zakaria 2007) and thyroid hormone-stimulating activities (Panda and Kar 1999). Young seeds and pods of this plant were used for edible purposes by certain tribal people of India after cooking (Rajaram and Janardhanan 1991). Several plant constituents including foliar flavonoids (Salatino et al. 1999), flavone (Yadava and Tripathi 2000), flavonone glycosides (Kuo et al. 1998), pacharin, bauhinias-tatins (Pettit et al. 2006), tannins, phytic acid and several fatty acids (Vijayakumari et al. 2007) were reported from the aerial parts of *B. purpurea*. Certain flavanones, dihydrodibenzoxepins and bibenzyls were also reported from the root of this plant (Boonphong et al. 2007).

Though different parts of this plant possess good medicinal properties with edible benefit, there is no published study particularly on the polyphenolic content, antioxidant and antimicrobial activities of the leaves of this plant. Hence, the main objective of the present study was to extract *B. purpurea* leaf using different extraction methods followed by the evaluation of the polyphenolic content and antioxidant and antimicrobial activities. Furthermore, TLC bioautography followed by high-performance thin layer chromatography (HPTLC) densitometric determination was also carried out to find and quantify the antioxidant components in these extracts. This HPTLC fingerprint profile can be used as a quality control tool for the standardisation of this plant extract. However, this method can also be used to distinguish the closely related species of this plant.

Materials and Methods

Plant Material

Fresh leaves of *B. purpurea* were collected from the campus of University Sains Malaysia (USM), Penang,

Malaysia, in November 2009. The plant was authenticated by certified botanist and the voucher specimen (no. 11047) was deposited in Herbarium, School of Biological Sciences, USM. Collected leaves were then washed with running water to remove the dirt, dried and ground to obtain powder of 40 mesh size.

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Folin–Ciocalteu reagent, potassium persulphate, *p*-iodonitrotetrazolium (*p*-INT) vanillin, sodium carbonate, aluminium chloride hexahydrate, ferric chloride, sodium acetate, ascorbic acid, gallic acid and catechin were purchased from Sigma Chemicals, Germany. Mueller–Hinton agar and broth were from HiMedia Laboratories (Mumbai, India). Silica gel 60 GF₂₅₄ TLC plates (cat no.1.055 and 0.2-mm thickness) were from E Merck KgaA (Darmstadt, Germany). All the other solvents used in this study were of analytical grade and purchased from Fisher Scientific Sdn. Bhd, Malaysia.

Extraction Methods

Soxhlet Extraction

Fifty grams of the ground sample was refluxed with 500 mL of ethanol (99.5%, v/v) at 70 °C for 48 h using Soxhlet apparatus. After 48 h, the liquid extract was separated from solid residue by vacuum filtration then concentrated using a rotary evaporator (Buchi Rotavapor R-215, Switzerland) followed by a drying process in a freeze dryer (LABCONCO, Free Zone 6 Liter, USA) to obtain constant mass of dried Soxhlet extract of *B. purpurea* (SBE). This was kept in an airtight container at 4 °C until further analysis.

Ultrasonic Extraction

Ultrasonic extraction was carried out using BRANSON 5510 ultrasonic cleaner for 1 h. The power and frequency were set at 135 W and 40 kHz, respectively. Five hundred millilitres of ethanol (99.5%, v/v) was added to 50 g of the ground sample in a 2-L conical flask and sonicated for 1 h at room temperature in the dark. Water in the ultrasonic bath was circulated and regulated at constant room temperature (25±1 °C) to avoid the rise in water temperature caused by ultrasonics. After the extraction, the sample residue was dissolved in ethanol (100 mL) and extracted repeatedly until the extract became clear. Separation and further treatment of the liquid extracts

were done in a similar way as described in Soxhlet extraction to get the dried ultrasonicated extract of *B. purpurea* (UBE).

Maceration

Fifty grams of the ground sample was extracted with 500 mL of ethanol (99.5%, v/v) at room temperature in the dark with occasional stirring for 3 days. After 3 days of maceration, the liquid extract was separated from the solid residue by vacuum filtration and the marc left out was once again washed with 200 mL of fresh ethanol. Separation and further treatment of the liquid extracts were done in a similar way as described in Soxhlet extraction to get the dried macerated extract of *B. purpurea* (MBE).

Determination of Total Phenolics, Total Flavonoids and Total Tannins

The colorimetric method described by Singleton and Rossi (1965) was used to determine the total phenolic (TP) content of the *B. purpurea* leaf extracts obtained by different methods of extraction, and the values were expressed in terms of milligrams of gallic acid equivalents (mg GAE) per gram extract. Aluminium chloride colorimetric method described by Sakanaka et al. (2005) was followed to find out the total flavonoid (TF) content in the extracts, and the values were expressed in terms of milligrams of catechin equivalents (mg CAE) per gram extract. Vanillin–HCl method with slight modifications was used to measure the total tannin (TT) content in the extracts, and the values were expressed in terms of milligrams of catechin equivalents (mg CAE) per gram extract (Burns 1971). All the measurements were done in triplicate.

DPPH and ABTS Radical Scavenging Assays

DPPH radical scavenging activity of SBE, UBE, MBE, vitamin C and BHT at different concentrations (5–60 µg/mL) was determined according to the method described by Blois (1958). The method described by Re et al. (1999) was followed to determine the ABTS radical scavenging activity of the same samples at varying concentrations (2.5–40 µg/mL). All the measurements were done in triplicate, and the percentage inhibition of the samples was calculated using the following formula:

$$\text{Percentage inhibition of samples} = [(A_0 - A_1/A_0) \times 100]$$

where A_0 is the absorbance of the control and A_1 is the absorbance of samples.

TAC and FRAP Assays

The reducing ability of antioxidants present in SBE, UBE and MBE were evaluated by ferric-reducing antioxidant potency (FRAP) and total antioxidant capacity assays. Phosphomolybdenum(VI) will reduce to phosphomolybdenum(V) and ferric TPTZ will be reduced to ferrous TPTZ by antioxidants in total antioxidant capacity and FRAP assays, respectively (Prieto et al. 1999; Benzie and Strain 1996). Phosphomolybdenum method described by Prieto et al. (1999) was followed to measure the total antioxidant capacity of SBE, UBE and MBE. Vitamin C at various concentrations (5–100 µg/mL) was used to prepare the calibration curve, and the antioxidant activity was expressed as milligrams of vitamin C equivalent antioxidant capacity (mg VCEAC) per gram extract. FRAP assay for SBE, UBE and MBE was carried out according to the modified method of Benzie and Strain (1996). Ferrous sulphate solution in various concentrations (0.1–1 µM) was used for the preparation of the standard calibration curve. The FRAP values was expressed as millimolar ferrous equivalents per gram of dried extract.

HPTLC Densitometric Determination and Bioautography

Six microlitres of SBE, UBE and MBE solution (10 mg/mL) prepared in methanol (HPLC grade) was applied band-wise in duplicate (band length, 6.0 mm; distance between bands, 11.0 mm; distance from left edge, 20.0 mm; distance from lower edge, 8.0 mm) on a precoated silica gel G 60 F₂₅₄ plate using Linomat IV automated TLC applicator (Camag, Muttenz, Switzerland). TLC plates were developed to a distance of 8.0 mm beyond the origin using the mobile phase toluene/acetone/formic acid (4:4:2, v/v/v) in a Camag HPTLC twin-trough chamber. After the development time of 20 min, the plate was air-dried for 10 min. These plates were then observed under UV at 254 and 366 nm and the images were documented.

Separated bands were quantified by HPTLC densitometric scanning using Camag TLC Scanner III in the remission-absorption mode at 254 and 366 nm operated by Wincats software (version 3.1) to obtain the R_f value and peak areas of the chromatogram. The slit dimension of 2 × 0.10 mm and the scanning speed of 20 mm/s were maintained to record the peak areas of the resolved bands. The bands with antioxidant property were confirmed by spraying the same plate with 2.54 mM DPPH reagent (Gu et al. 2009) and comparing its R_f value with the R_f value obtained by HPTLC densitometric determination. The amount of antioxidants in the separated bands of SBE, UBE and MBE was evaluated via peak areas.

Determination of Antimicrobial Activity

Antimicrobial activity of UBE, MBE and SBE against three Gram positive (*Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*) and four Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Shigella*) bacteria was evaluated. Bacteria selected for this study were obtained from the School of Biological Sciences, USM, Penang, Malaysia. The bacterial cell suspension prepared in Mueller–Hinton (MH) broth from 24-h culture with concentration McFarland 0.5 was used for this study.

Agar diffusion method described by Bauer et al. (1966) was followed to determine the zone of inhibition (ZOI). Sterile blank discs (6-mm diameter, Oxoid, UK) were impregnated with 20 μ L of crude plant extracts (equivalent to 4 mg of the dried extract) prepared in methanol. Amoxicillin (10 μ g, Oxoid) and chloramphenicol (30 μ g, Oxoid) antimicrobial susceptibility test discs were used as positive controls and methanol as negative control. These discs impregnated with sample were dried then aseptically placed on Mueller–Hinton agar plates seeded with the bacteria (200 μ L of bacterial cell suspension in 20 mL of MH agar medium). These plates were incubated for 24 h at 37 °C. After the incubation time, ZOI was measured and recorded as mean diameter (in millimetres).

Minimum inhibitory concentration of these extracts was determined by a microtitre plate broth dilution method described by Elof (1998) with slight modifications. Serial twofold dilution of plant extracts (0.625–20 mg/mL) in DMSO and chloramphenicol (0.00625–0.2 mg/mL) was prepared in a sterile 96-well microtitre plate. Bacterial suspension (100 μ L) with 0.5 McFarland turbidity was added to each well and incubated for 24 h at 37 °C. After the incubation time, 50 μ L of *p*-INT (0.2 mg/mL) was added to all the wells, including negative control (DMSO), and again incubated for 1 h. These plates were then observed for any changes in colour. Wells with yellow colour were interpreted as no growth, whilst purple colour was interpreted as growth. The minimum inhibitory concentration (MIC) values calculated represent the lower concentration of samples which inhibit any visible growth of bacteria.

Statistical Analysis

The results of the present study are presented as mean \pm SD ($n=3$). Analysis of variance was performed (one-way ANOVA) and the significant differences between mean values were determined by Tukey's pairwise test at a level of significance of $P<0.05$. The statistical analysis was carried out using SPSS 17 (SPSS Inc., USA).

Results and Discussion

Extraction Yield, TP Content, TF Content and TT Content

The extraction yield, TP content, TF content and TT content of extracts (SBE, UBE and MBE) obtained from different extraction techniques are presented in Table 1. The extraction yield (grams extract/100 g dried leaves powder) was in the order of SBE (13.5)>UBE (8.8)>MBE (8.3). This higher yield of Soxhlet extraction might be because of its exhaustive extraction ability. Total phenolic content of SBE, UBE and MBE were 117.0 ± 0.7 , 196.8 ± 0.4 and 130.4 ± 1.6 mg GAE/g extract, respectively (Table 1). Our results showed that 1 h of sonication extracted out a significant amount of phenolic content ($P<0.05$) in comparison with 48 h of Soxhlet extraction and 72 h of maceration. Total phenolics method gives an idea about plant phenolic hydroxyl groups reacting with Folin–Ciocalteu reagent; hence, we also used total flavonoid and tannin methods to determine the quantity of their presence in these plant extracts. The results of total flavonoids and tannins also showed that UBE possessed significant ($P<0.05$) flavonoid and tannin content followed by MBE and SBE with values of 23.6 ± 0.8 , 13.0 ± 0.3 , 10.9 ± 0.4 and 66.4 ± 0.5 , 27.1 ± 0.3 and 10.4 ± 0.1 mg CAE/g, respectively. These findings showed that UBE possessed a significant amount of total flavonoids which was almost twice that of SBE and MBE. Moreover, the total tannin content of UBE was two times that of MBE and six times that of SBE. In brief, sonication of *B. purpurea* leaf resulted in the extraction of a high amount of phenolic constituents followed by maceration and Soxhlet extraction. A similar finding of increase in the phenolic compounds by sonication was reported by Proestos and Komaitis (2006) from aromatic plants.

DPPH and ABTS Radical Scavenging Assays

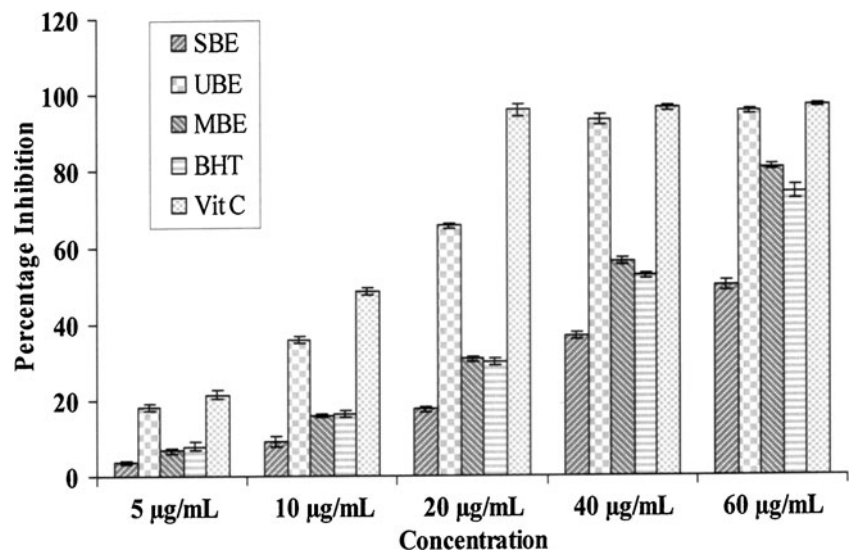
In the DPPH assay, percentage inhibition of DPPH free radical was recorded at different concentrations (5–60 μ g/mL). As

Table 1 Extraction yield, TP content, TF content and TT content in *B. purpurea* extracts obtained from different extraction techniques

Samples	Extraction yield (g dry extract/100 g dried leaves powder)	TP content (mg GAE/ g extract)	TF content (mg CAE/ g extract)	TT content (mg CAE/ g extract)
SBE	13.5	117.0 ± 0.7 a	10.9 ± 0.4 a	10.4 ± 0.1 a
UBE	8.8	196.8 ± 0.4 c	23.6 ± 0.8 c	66.4 ± 0.5 c
MBE	8.3	130.4 ± 1.6 b	13.0 ± 0.3 b	27.1 ± 0.3 b

Values were expressed as the mean \pm standard deviation ($n=3$). Values followed by different letters in the same column were significantly different ($P<0.05$) from each other

Fig. 1 DPPH radical scavenging activity of different extracts and standards



shown in Fig. 1, all the extracts showed a concentration-dependent radical scavenging activity. At all the concentrations tested, the DPPH radical scavenging activity of UBE and MBE was better than that of BHT. Moreover, at a concentration of 40 µg/mL, the DPPH radical scavenging activity of UBE (93.3 ± 1.4) was similar to that of vitamin C (96.4 ± 0.6). Thus, the DPPH radical scavenging activity of all the tested samples were in the order of vitamin C > UBE > MBE > BHT > SBE. Figure 2 shows the ABTS radical scavenging activity of SBE, UBE, MBE, vitamin C and BHT. Almost a similar trend of DPPH assay was observed here; the plateau was achieved at a concentration of 20 µg/mL for UBE (98.7 ± 0.5) and vitamin C (98.4 ± 2.2), whereas

at a concentration of 40 µg/mL macerated extract, BHT and Soxhlet showed percentage inhibition of 88.6 ± 3.0 , 83.5 ± 2.1 and 71.4 ± 0.9 , respectively. At all the concentrations evaluated, UBE possessed the highest ABTS radical scavenging activity in comparison with other extracts and standard samples.

Total Antioxidant Capacity and Ferric-Reducing Antioxidant Potency Assays

Antioxidants present in the sample which caused the reduction of Mo(VI) to Mo(V) result in the formation of green phosphomolybdenum complex which was measured

Fig. 2 ABTS radical scavenging activity of different extracts and standards

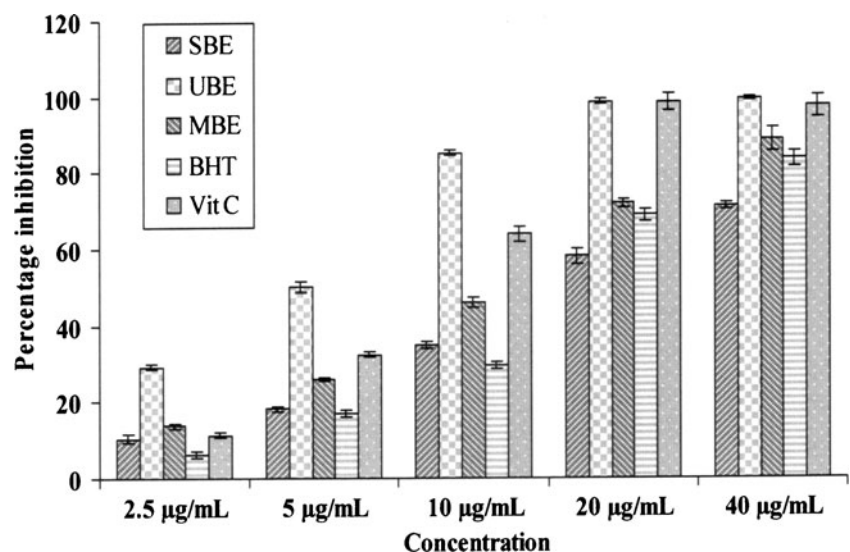


Table 2 TAC and FRAP values of *B. purpurea* extracts obtained from different extraction techniques

Samples	TAC value (mg VCEAC/g extract)	FRAP value (mM Fe ²⁺ /g extract)
SBE	219.9±7.04a	2.7±0.04a
UBE	377.5±7.53c	4.3±0.04c
MBE	241.6±3.21b	3.1±0.01b

Values were expressed as the mean±standard deviation ($n=3$). Values followed by different letters in the same column were significantly different ($P<0.05$) from each other

at 695 nm (Prieto et al. 1999). The higher the absorbance of the tested samples, the stronger is the antioxidant activity. The results of total antioxidant capacity assay showed that UBE had significant antioxidant activity ($P<0.05$) followed by MBE and SBE, with values of 377.5 ± 7.53 , 241.6 ± 3.21 and 219.9 ± 7.04 mg VCEAC/g extract, respectively (Table 2). The reduction of ferric-TPTZ by antioxidants present in the samples to ferrous-TPTZ was monitored at 593 nm. The higher the absorbance value, the better is the reduction ability and the antioxidant activity (Benzie and Strain 1996). As shown in Table 2, a similar pattern of total antioxidant capacity (TAC) was observed even in this assay with UBE possessing a significant reducing ability ($P<0.05$) followed by MBE and SBE extracts with values of 4.3 ± 0.04 , 3.1 ± 0.01 and 2.7 ± 0.04 mmol ferric/g extract, respectively. Thus, the reducing capability of tested samples from these two assays was in the order of UBE>MBE>SBE.

HPTLC Densitometric Determination and Bioautography

The composition of mobile phase, the amount of sample to be applied and the scan speed were optimised to obtain

precise, compact and reproducible bands. HPTLC fingerprint obtained from HPTLC image and the peak intensities were in accordance with fluorescent band and their brightness, so it can be evaluated by a quantifiable comparison of the peak area or intensity. As seen from Fig. 3, qualitatively slight different HPTLC fingerprints were obtained for the extracts obtained by different extraction techniques. Separated compounds appeared as peaks in the densitogram, and the peak areas of these compounds correspond to their concentrations in the sample. The R_f value and the peak areas of the antioxidant bands confirmed from TLC bioautography were shown in Table 3. It is evident from the table that UBE have seven and six antioxidant bands with high peak area at 254 and 366 nm, respectively. MBE possessed six bands each, whereas SBE contained five and six antioxidant bands at 254 and 366 nm, respectively. Moreover, some of the antioxidant bands found in UBE were absent in the SBE and were present in less quantity. In brief, UBE yields more number of antioxidant components in short extraction time (1 h) with higher peak area in comparison with MBE and SBE. Hence, the present HPTLC fingerprint method was found to be easy for the comparison of extracts obtained from different extraction techniques.

Antimicrobial Activity

The antibacterial activity of SBE, UBE and MBE by disc diffusion method was determined against seven strains of pathogenic bacteria and shown in Table 4. All the extracts exhibited good antibacterial activity against all bacteria except *S. aureus* and *K. pneumoniae*. Among all these extracts evaluated, UBE possessed potent antibacterial activity followed by MBE and SBE. Furthermore, to find out the sensitivity of the bacterial strains for these extracts,

Fig. 3 HPTLC densitogram of extracts obtained by different extraction techniques at 366 nm

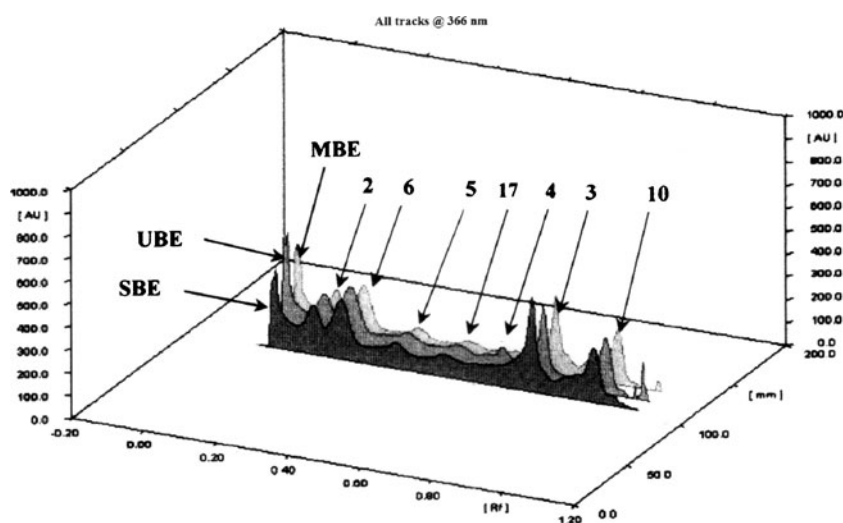


Table 3 R_f value and peak area of antioxidants present in HPTLC densitogram at 254 and 366 nm

Peak no.	R_f value	Peak area at 254 nm			Peak area at 366 nm		
		SBE	UBE	MBE	SBE	UBE	MBE
2	0.15	7,365	9,307	9,016	9,860	10,117	8,910
6	0.22	14,008	15,307	13,744	14,148	16,199	13,828
5	0.39	3,030	6,491	3,008	7,299	8,802	6,158
17	0.52	0	0	0	7,339	6,490	5,222
4	0.65	0	3,108	0	0	5,004	0
3	0.76	14,936	14,969	12,667	18,089	14,428	14,201
9	0.90	0	3,466	3,368	0	0	0
10	0.94	4,040	5,025	4,930	10,976	11,492	10,982

they were evaluated for the broth dilution method (MIC values). Table 4 summarises the MIC values of the tested extracts. There were substantial differences between the MIC values of these extracts. In this method, all bacterial strains were sensitive to UBE (MIC=0.625 mg/mL) except *S. aureus* and *K. pneumoniae*. Among all the bacterial strains, *B. cereus* and *E. coli* showed to be more sensitive to MBE (MIC=0.625 mg/mL) and SBE (MIC=2.5 mg/mL). Negative controls (methanol in disc diffusion and DMSO in broth dilution method) showed no antibacterial activity, whereas the antibacterial activity of positive controls was better than the extracts tested in both the methods tested (Table 4).

Similar to the results of antioxidant activity, UBE with high polyphenolic content exhibited potent antibacterial activity. Moreover, many studies have also reported that phenolic compounds are responsible for antimicrobial properties (Penna et al. 2001; Zhou et al. 2007). Thus, the obtained antibacterial activity could be due to the polyphenolic constituents present in the extracts.

Conclusions

In this study, different extraction techniques significantly affected the extraction yield, polyphenolic content

and antioxidant and antibacterial activities. The ultrasonic extraction method was simple, rapid and a better method for the extraction of antioxidant and antibacterial substances compared to Soxhlet as well as maceration method by providing a high amount of polyphenolics with potent antioxidant and antibacterial activities. These results also indicate that the selection of correct extraction method is important for obtaining the extracts with the required pharmacological activity. Knowledge about the polyphenolic content and antioxidant properties of *B. purpurea* leaf would assist in the efforts to achieve commercial utilisation of this plant as a new source of antioxidant after evaluating its complete toxicity profile.

The HPTLC method used in this study enables the development of chromatographic fingerprint profile and quantification of antioxidants present in terms of peak area for extracts obtained by different extraction techniques. This HPTLC fingerprint profile can be used as a reference data for the standardisation of *B. purpurea* leaf extract and the formulations containing *B. purpurea* as one of the main ingredients. The TLC bioautographic method described here is very simple, fast and can be utilised to differentiate the biological activity of extracts obtained by different extraction techniques or solvents or different conditions.

Table 4 ZOI and MIC values of *B. purpurea* leaf extracts obtained by different extraction techniques

Name of bacteria	SBE		UBE		MBE		CLMPL		AMXN	
	ZOI	MIC	ZOI	MIC	ZOI	MIC	ZOI	MIC	ZOI	MIC
<i>B. cereus</i>	7	5	9	0.625	8	1.25	26.5	0.2	15.5	NT
<i>B. subtilis</i>	7	2.5	8.5	0.625	7.5	0.625	28	0.025	17.5	NT
<i>S. aureus</i>	6.5	5	7.5	1.25	6.5	2.5	20.5	0.1	17.5	NT
<i>P. aeruginosa</i>	7	2.5	9	0.625	7.5	1.25	28.5	0.025	17.5	NT
<i>K. pneumoniae</i>	7	5	7.5	2.5	7	5	25.5	0.2	18	NT
<i>E. coli</i>	7	2.5	9	0.625	7.5	0.625	25	0.025	NT	NT
<i>Shigella</i>	6.5	10	8	0.625	7.5	2.5	26	0.05	17	NT

ZOI zone of inhibition (in millimetres), MIC minimum inhibitory concentration, NT not tested, CLMPL chloramphenicol, AMXN amoxicillin

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