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AN EFFICIENT DETERMINATION OF PHENOLIC COMPOUNDS BY HPLC-DAD AND THEIR BIOACTIVITY ASSAY FROM AERIAL PARTS OF *EUCALYPTUS TERETICORNIS* PLANT

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Abstract

Current study is based on the extraction of free and bound phenolic compounds (PC) in aerial parts *i.e.*, stem, skin, leaves, and seeds of *E. tereticornis* (local name: Sufeda) plant. The two different extraction procedures (Sonication and ultrasonic assisted base hydrolysis) and their antimicrobial activity against two bacteria i.e. *Escherichia coli* (*E. coli)* and *Staphylococcus aureus* (*S. aureus)* were analyzed. The RP-HPLC-DAD was applied for separation and detection of phenolic compounds (PC). The HPLC profiling revealed the existence of 13 PC in which 11 were phenolic acids and 2 aldehydes derivatives, while bound PC possessing greater fraction than free ones. The results from ultrasonic assisted extraction revealed the gallic (23.47 mg/g), *p*-coumaric (16.66 mg/g), *p*-hyroxybenzoic (11.03 mg/g) and ferulic (8.71 mg/g) acids. While Sonication extraction method shows the chlorogenic (8.14 mg/g), vanillic (11.47 mg/g), sinapic (4.68 mg/g) and caffeic (5.38 mg/g) acids as main constituents in skin, seed, stem and leaves respectively. Four PC were identified as bound phenolic acids i.e., *p*-coumaric, cinnamic, naringenin and catechin, while other of the compounds were found in both extracts. The free radical scavenging activity (RSA) and total phenolic contents (TPC) were present in greater amounts in seed (164.61 Mm/g) and skin (118.96 Mm/g) while total flavonoids and total tannins were higher in stem (42.77 Mm/g) and (28.13 Mm/g), respectively. *E. Tereticornis* leaves, stem, seeds and skin extracts were observed to be highly effective for *E coli* and *S. aureus*. The antimicrobial action of *E. Tereticornis* extracts was higher against *S. aureus* (MIC value 125 μg/mL) than *E. coli* (MIC value 250 μg/mL). It is concluded that the potential health benefits of *E. tereticornis* highlight its significance in the field of natural product chemistry and pharmacology.

Key words: *Eucalyptus tereticornis*; Phenolic compounds; Antibacterial activity; HPLC-DAD.

Introduction

Plants are essential for life. Humans have used them as a source of food, biofuel and medicines (Rathore *et al*., 2012; Bernabé-Antonio *et al*., 2015). According to world health organization (WHO), 80% of world's population depends upon traditional medicines for their primary health care, which involves plant extract (Mushtaq *et al*., 2014; Laghari *et al*., 2023). They have gained very effective defense mechanisms that make sure their survival under adverse conditions (Silva *et al*., 2016). Natural products obtained from plants are abundant in nature, these chemicals known as phytochemicals which are secondary metabolites. The natural products exhibit numerous biological activities and are generally used in the food, pharmaceutical, chemical, agriculture and cosmetic industries. These phytochemicals are very efficient in their properties like protection, adaptation and pollination (Luis *et al*., 2012). They occur in both free and bound forms with acids, sugars and other biomolecules and can be classified among various groups like flavonoids, tannins, phenolic acids and coumarins (Luthria *et al*., 2006; Li *et al*., 2016). According to the literature, PC identified from plant-based materials is more than 8000 (Memon *et al*., 2010). The acidic, basic or enzymatic hydrolysis is required for accurate estimation of total phenolic compounds in a plant (Memon *et al*., 2013; Sidhu *et al*., 2022). Phenolic acids and flavonoids have gained more attention from researchers in comparison to other phytochemicals due to their health beneficial properties (Memon *et al*., 2012). They attract more attention from researchers due to their physiological activities, including their ability to scavenge free radicals, reduce inflammation, fight against microbes, prevent blood clotting, protect against cardiovascular diseases, alleviate allergies, and promote vasodilatation (Siddiqui *et al*., 2017; Kubola *et al*., 2008). PC refers to secondary metabolites found in plants (Jani *et al*., 2015), they possess the ability to scavenge free radicals, protect against coronary heart diseases, and exhibit anti-carcinogenic activity (Sulaiman *et al*., 2014).

There are more than 700 species in the varied genus *Eucalyptus*, most of which are native to Australia. It is a member of the *Myrtaceae* family (local name: Sufeda), and a fast-growing tree that may reach 30 to 45 m in height and 1 to 2 m in diameter (Fawad *et al*., 2012)**.** It was grown from the seeds imported from the Congo to China in the 1970s (Ghaffar *et al*., 2015; Zhang *et al*., 2010). Eucalyptus has been used in traditional medicine around the world as an anti-inflammatory, anti-analgesic, and antipyretic treatment for respiratory infection symptoms like the flu, the common cold, and sinus congestion (Maurya & Srivastava, 2012). Eucalyptus contains many PC which possess antioxidant, anti-proliferative, anti-inflammatory, anti-hyperglycemic, and anti-thrombotic properties

(Bhuyan *et al*., 2016). Therefore, we aim to investigate the free and bound PC in *E. tereticornis* by two different extraction methods. Besides this, these extracts were also used to investigate the total phenolic contents, radical scavenging activity (RSA), total flavonoid, and total tannin contents by using a UV-visible spectrophotometer.

Material and Methods

Collection and standards: *E. tereticornis* stem, skin, leaves, and seeds were obtained in June 2021 from district Mirpurkhas, Sindh, Pakistan. Species identified by taxonomist, Institute of Plant Sciences, University of Sindh, Jamshoro, Pakistan. A voucher specimen of this plant is 1231215 and deposited in the herbarium of the same Institute. Highly purified flavonoid and PC standards, analytical or HPLC grade reagents were used throughout the study and purchased from (Merck Darmstadt, Germany).

Preparation of plant extracts: The parts of the *E. tereticornis*, including stem, skin, leaves, and seeds were washed carefully with tap water and pursued with de-ionized water, then shade-dried for about 2 weeks. The dried samples were crumbled and pulverized with an electric grinder and stored at room temperature in sealed containers.

Extraction

Extraction of free phenolic compounds: Briefly 0.5 g powder of each extract (stem, skin, leaves, and seeds) mixed in 25 mL of 80% aqueous methanolic solution and then sonicated for 30 min at room temperature by using a sonicator (XUB Series Digital Ultrasonic Baths) (Luthria *et al*., 2006). The extracts were centrifuged and then filtered with 0.22 μm PVDF nylon filters for further analysis.

Extraction of bound phenolic compounds: Briefly, 0.5 g power of each part of *E. tereticornis* was hydrolyzed in 10 mL of base hydrolysis solution, 10 mM EDTA, and 1% ascorbic acid in 100 mL of polypropylene tubes and purging with nitrogen gas (N_2). The tubes were vortexed and sonicated for approximately 30 minutes at 50°C. After sonication, the reaction mixture was cooled, and the pH was adjusted to 2.5 using 6 N HCl. The bound phenolic compounds were then extracted with 5 mL of ethyl acetate, vortexed for 30 seconds, and centrifuged for 10 minutes at 5000 rpm (Luthria *et al*., 2006). Subsequent analysis was also conducted using these extracts.

Analysis

Determination of total phenolic content: The total phenolic content (TPC) of *E. Tereticornis*extracts was evaluated by already reported method with slightly variations (Iqbal *et al*., 2005).

Evaluation of total flavonoid and total tannin contents: Total flavonoid and total tannin contents were evaluated by already reported method with slight variations (Laghari *et al*., 2011; Siddiqui *et al*., 2017).

Radical-scavenging activity (RSA): The stable, 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) was used to determine the free radical scavenging activities of extracts of different parts of *E. Tereticornis*. Briefly, 2 mL of each plant extract was mixed with 2 mL of 0.1 mmol/L DPPH solution and placed in the dark for 30 min (Iqbal *et al*., 2005; Mangi *et al*., 2021), after that, the absorbance was taken on at 517 nm. The quercetin standards in the concentration range of 1–10 μmol were prepared and used as a standard to estimate the free-radical scavenging action of all extracts. The standard curve plotted after obtaining the constant absorbance with a 3 mins time space and quercetin amount was calculated from the standard curve and expressed as mM/g of the sample.

Determination of antimicrobial activities: The antibacterial activity of *E. Tereticornis* (stem, skin, leaves, and seeds) extracts were evaluated by already reported method with slightly variations (Rahman *et al*., 2017; Bouchekrit *et al*., 2016; Memon *et al*., 2017).

Statistical analysis

The results were evaluated using Microsoft Excel 2013 as mean ± standard deviation and Minitab Software (version16.1.1) was operated for data analysis.

Results

HPLC assessment of phenolic compounds: Table 1 shows the data about standards of phenolic compounds. Each standard was run three times by using the reverse-phase liquid chromatography coupled with a diode array detector (HPLC-DAD) instrument examined at 325 nm, 310 nm, and 270 nm, data included the retention time (tR), regression coefficient (R^2), the linear equation between concentration and peak area, and maximum absorption wavelength of 17 phenolic acids and three aldehydes.

The HPLC showed the existence of 13 different phenolic compounds, which included 11 phenolic acids and 2 derivatives of aldehydes, acquiring a higher portion of bound phenolic compounds in comparison to free ones (Table 2). *p*-hydroxybenzoic, gallic, *p*-Coumaric, and ferulic acids were dominant constituents in the stem, skin, leaves, and seeds respectively, by Ultrasonic-assisted base hydrolysis extraction method. In contrast, on the other hand, the sonication extraction method revealed chlorogenic, sinapic, vanillic, and caffeic acid as the main constituent in the skin, seed, stem, and leaves, respectively. Moreover, the four phenolic compounds, *i.e*., naringenin, *p*-coumaric, cinnamic, and catechin, were analyzed as bound phenolic compounds only. However, the remaining phenolic compounds were observed in both (free and bound) extracts.

Leaves extracts: 7 BPA use abbreviations were analyzed as (1) Gallic acid, (2) naringenin, (3) sinapic acid, (4) caffeic acid, (5) *p*-HBA (*para*-hydroxybenzoic acid), (6) *p*-coumaric acid, (7) ferulic acid (Fig. 1a), and 6 FPA were identified as (1) gallic acid, (2) vanillin, (3) vanillic acid, (4) chlorogenic acid, (5) caffeic acid, (6) *p*-HBA (Fig. 1b).

Table 1. Identification and separation of standard phenolic compounds with their retention time and linearity.

S. No.	Standards	t_R	R^2	Regression equation	λ max (min)
1.	Gallic acid	8.70	0.999	y=305726x-249684	227, 272
2.	2,4,6-THBA	9.51	0.998	y = 49119x+29082	216, 255, 292
3.	Protocatechuic acid	13.16	0.997	y=530511x+112990	228, 259,294
4.	Pyrogallol aldehyde	14.18	0.999	y=337860x+147020	234, 291
5.	Protocatechuic aldehyde	14.35	0.998	y=548015x+303632	234, 281
6.	Gentisic acid	14.92	0.999	y= 13444x-1829.4	232, 327
7.	Naringrin	15.75	0.999	y=533000x+78590	216, 232, 278
8.	β-resorcinolic acid	18.99	0.998	y=200138x+46398	255, 294
9.	Hypogallic acid	19.61	0.998	y = 82657x – 14787	232, 314
10.	Vanilline	20.18	0.999	Y=626260x-138097	233, 281, 307
11.	Sinapic acid	23.71	0.991	y=643555x–1E+06	255,294
12.	Vanillic acid	25.18	0.999	y= 289390x-82077	223, 260, 294
13.	Catechein	27.39	0.999	y=337860x+147020	234, 291
14.	Chlorogenic acid	29.34	0.998	y = 97008x-33773	217,233, 327
15.	Caffeic acid	32.98	0.995	y=169059x-140031	233, 323
16.	PHBA	35.18	0.999	y=88856x-14995	234, 308
17.	P-Coumaric aicd	45.50	0.995	y=213962x-333316	232, 309
18.	m-Coumaric acid	47.59	0.999	y=533000x+78590	216, 232, 278
19.	Cinamic acid	49.05	0.992	y=568487x+305505	230, 280, 330
20.	Ferulic acid	51.15	0.998	y=174006x+127640	235, 322

Table 2. The bound (BPA) and free (FPA) phenolic compounds identified and quantified from four different parts of *E. tereticornis.*

S. No.	Phenolic compounds	$t_R^{\#}$ (min)	Leaves mg/g ± RSD*		Stem mg/g ± RSD		Seed mg/g ± RSD		Skin mg/g ± RSD	
			BPA[a]	FPA[b]	BPA	FPA	BPA	FPA	BPA	FPA
1.	Gallic acid	8.7	3.53 ± 0.13	0.57 ± 0.11	3.53 ± 0.12	0.26 ± 0.04	1.36 ± 0.05	3.98 ± 0.26	23.47 ± 1.25	2.34 ± 0.27
2.	Naringrin	15.75	4.29 ± 0.12	--	2.10 ± 0.11	--	4.53 ± 0.14	--	3.10 ± 0.12	--
3.	Vanilline	20.18	--	0.89 ± 0.13	3.83 ± 0.13	--	--	--	5.71 ± 0.21	0.60 ± 0.08
4.	Sinapic acid	23.71	2.95 ± 0.02	--	4.68 ± 0.11	4.68 ± 0.22	0.62 ± 0.01	0.61 ± 0.14	6.05 ± 0.31	0.54 ± 0.07
5.	Vanillic acid	25.18	--	1.09 ± 0.12	1.57 ± 0.09	--	--	11.47 ± 0.41	0.44 ± 0.01	3.89 ± 0.14
6.	Catechin	27.39	--	--	--	--	3.23 ± 0.12	--	--	--
7.	Chlorogenic aid	29.34	--	0.58 ± 0.11	--	--	--	--	0.80 ± 0.15	8.14 ± 0.22
8.	Caffeic acid	32.98	4.47 ± 0.12	5.38 ± 0.21	--	--	6.49 ± 0.16	1.63 ± 0.08	1.15 ± 0.11	--
9.	*p*-HBA	35.18	1.43 ± 0.01	0.42 ± 0.09	11.03± 1.02	--	--	--	1.43 ± 0.13	--
10.	*p*-Coumaric acid	45.50	3.21 ± 0.11	--	--	--	16.66±1.21	--	--	--
11.	*m*-Coumaric acid	47.59	--	--	1.45 ± 0.04	1.45 ± 0.21	--	--	--	--
12.	Cinnamic acid	49.05	--	--	--	--	--	--	1.61 ± 0.15	--
13.	Ferulic acid	51.16	8.71 ± 0.23	--	--	--	8.14 ± 1.11	0.82 ± 0.17	2.50 ± 0.17	--
Total phenolic acids			**28.62**	**8.96**	**28.22**	**6.40**	**41.05**	**18.54**	**46.30**	**15.53**

[a] BPA = Bound phenolic acid, [b]FPA = Free phenolic acid, $^{\#}t_R$ = Retention time, *RSD = Relative standard deviation

Seed extracts: 7 BPA were identified as (1) gallic acid, (2) naringenin, (3) sinapic acid, (4) catechein, (5) caffeic acid, (6) *p*-coumaric acid, (7) ferulic acid (Fig. 2a), and 5 FPA were identified as (1) gallic acid, (2) sinapic acid, (3) vanillic acid, (4) caffeic acid, (5) *m*-coumaric acid (Fig. 2b).

Skin extracts: The 10 bound phenolic compounds were identified in the extract of skin, as (1) gallic acid, (2) naringenin, (3) vanillin, (4) sinapic acid, (5) vanillic acid, (6) chlorogenic acid, (7) caffeic acid, (8) *p*-HBA, (9) sinamic acid, (10) ferulic acid (Fig. 3a), while 5 free phenolic compounds were identified as (1) gallic acid, (2) vanillin, (3) sinapic acid, (4) vanillic acid, (5) chlorogenic acid (Fig. 3b).

Stem extracts: The seven bound phenolic compounds were identified in stem extract as (1) gallic acid, (2) naringenin, (3) vanilline, (4) sinapic acid, (5) vanillic acid, (6) *p*-HBA, (7) *m*-coumaric acid (Fig. 4a), while three free phenolic compounds were identified as (1) gallic acid, (2) sinapic acid, (3) *m*-coumaric acid (Fig. 4b).

Total phenolic, total flavonoid, total tannin, and free radical scavenging activity of *E. Tereticornis* are given in (Table 3). The total phenolic content, total flavonoid, total tannin and antioxidant activity in *E. Tereticornis* were determined in the order of 39.03-80.56 mg/g, 25.62-42.77 mg/g, 15.30-28.13 mg/g and 29.33-164.61 mM/g dry weight of leaves, stem, seed, and skin, respectively.

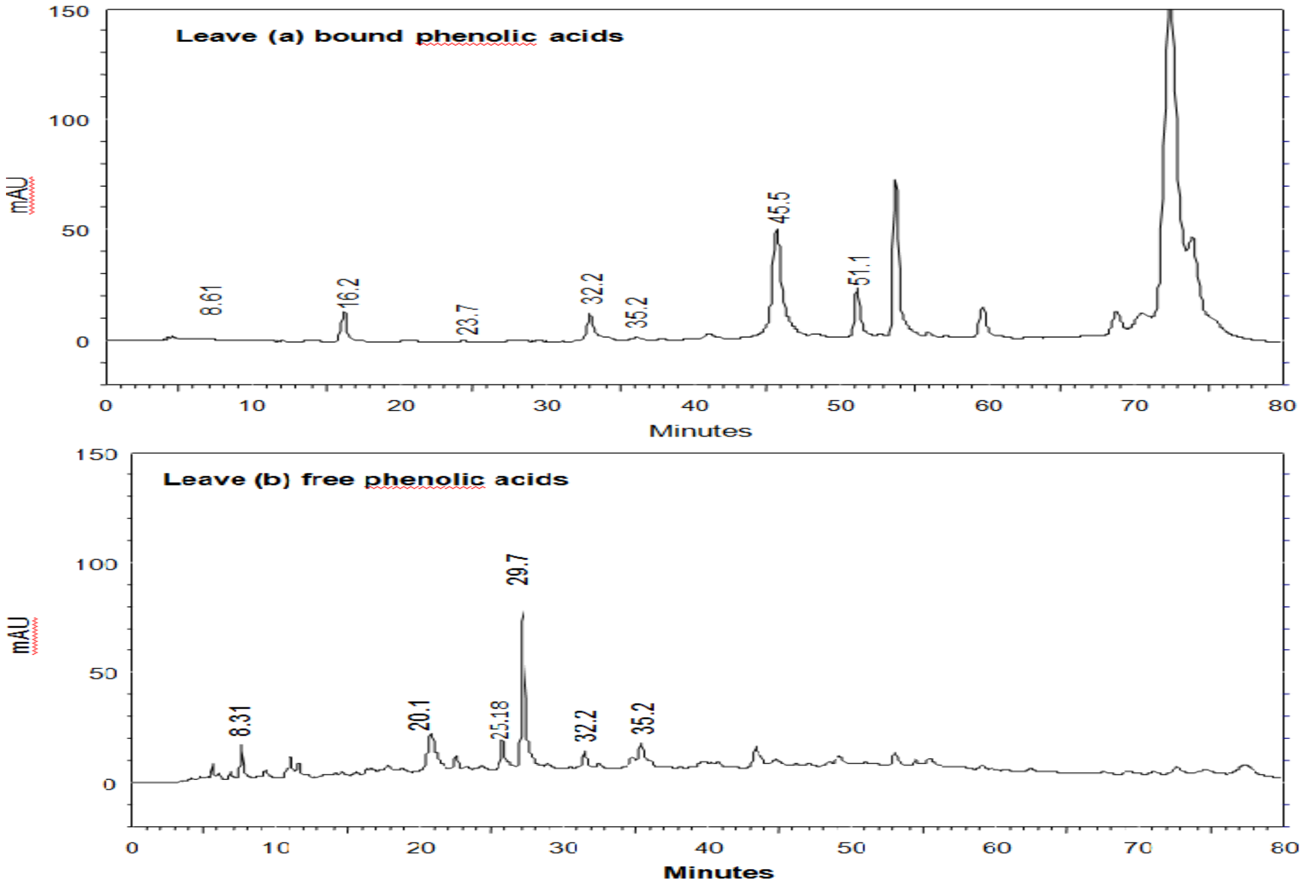

Fig. 1. HPLC chromatogram of *E. tereticornis* leaves (a) BFA and (b) FPA.

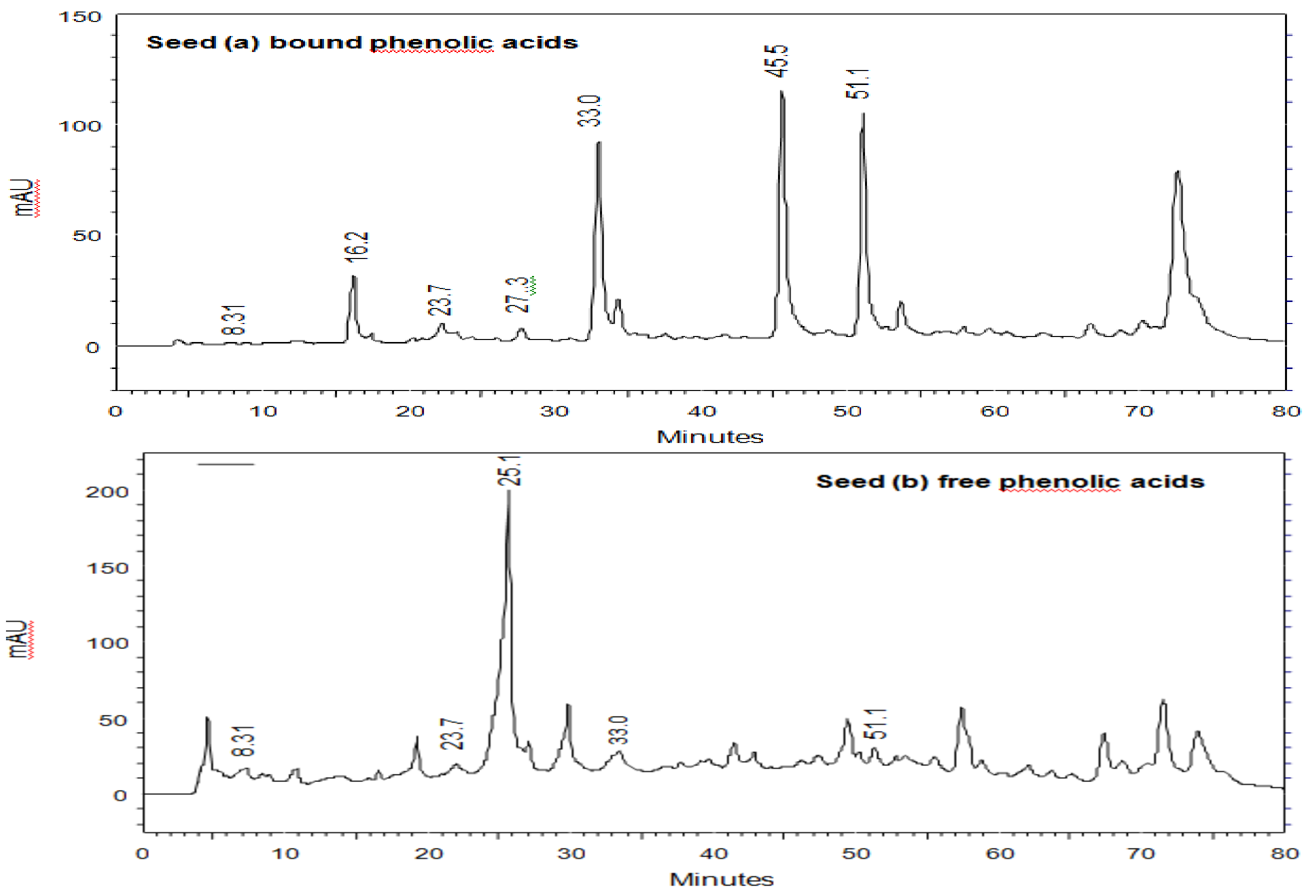

Fig. 2. HPLC chromatogram of *E. tereticornis* seed (a) BFA and (b) FPA.

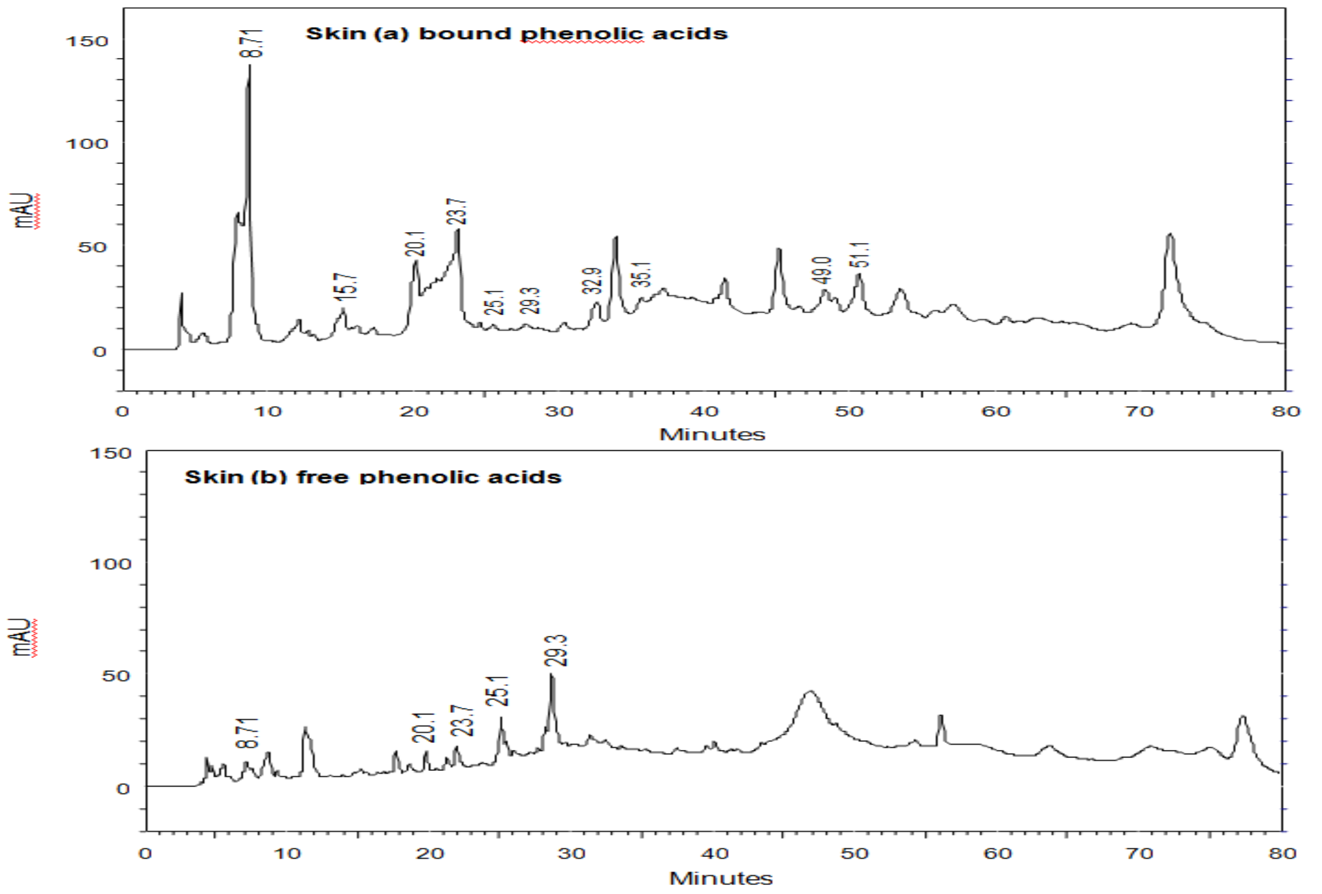

Fig. 3. HPLC chromatogram of *E. tereticornis* skin (a) BFA and (b) FPA.

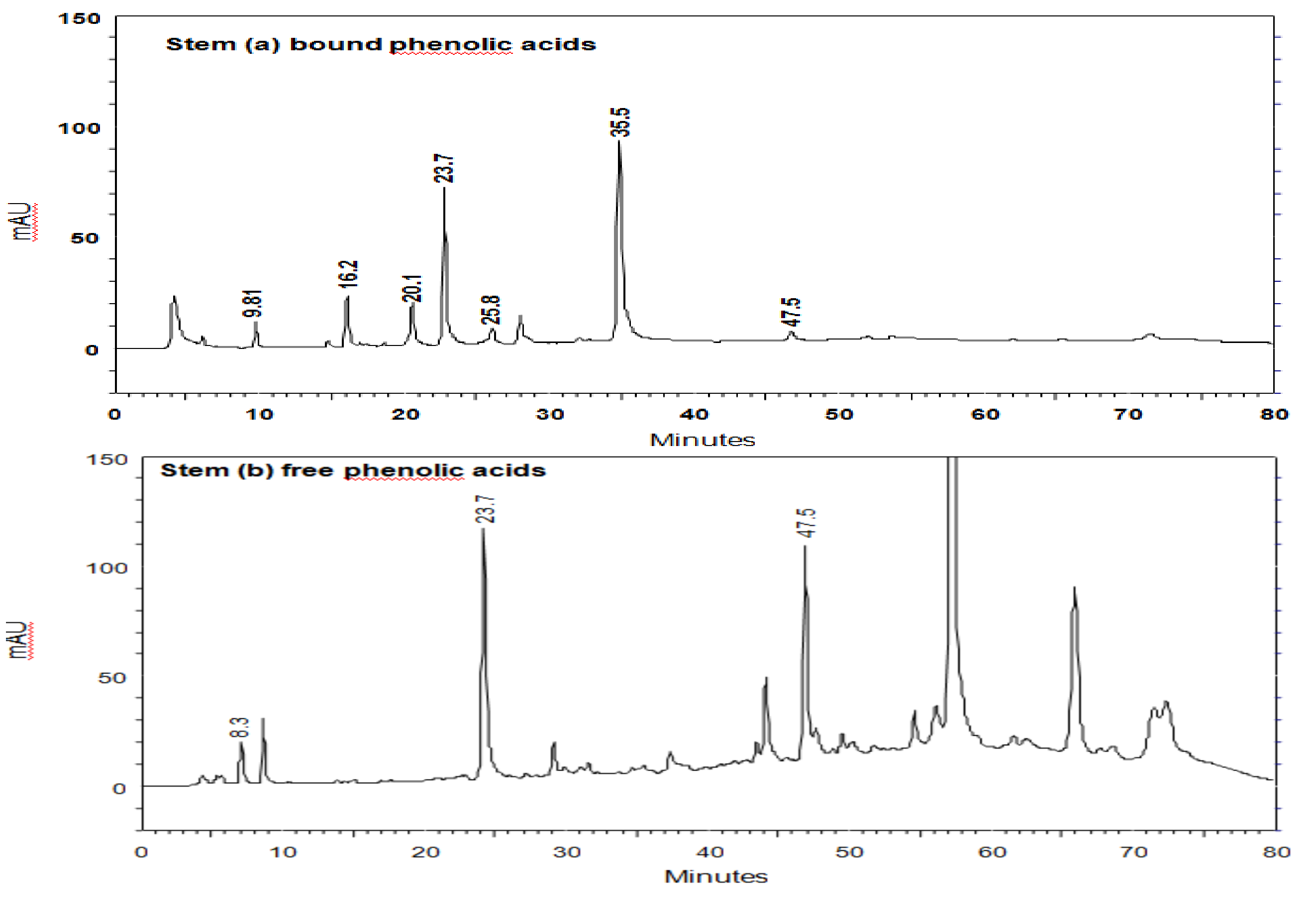

Fig. 4. HPLC chromatogram of *E. tereticornis* stem (a) BFA and (b) FPA.

Table 3. List of phenolic compounds and their biological activities.

S. No.	Sample	Total phenolics content by FC method as gallic acid eq. (mg/g ± RSD)	Total flavonoids content as rutin eq. (mg/g ± RSD)	Total tannins content as catechin hydrate eq. (mg/g ± RSD)	Total phenolic acids by HPLC-DAD (mg/g ± RSD)	DPPH radical scavenging activity as quercetin eq. (Mm/g ± RSD)
1	Leaves	56.58 ± 0.17	38.00 ± 0.22	22.17 ± 0.16	28.62	81.94 ± 0.83
2	Stem	39.03 ± 0.14	42.77 ± 0.26	28.13 ± 0.35	28.22	29.33 ± 0.11
3	Seed	80.56 ± 0.88	25.62 ± 0.21	15.30 ± 0.25	41.05	164.61 ± 1.22
4	Skin	62.10 ± 0.72	34.32 ± 0.17	19.40 ± 0.12	46.30	118.96 ± 1.03

Table 4. Zone of Inhibition (mm) for antibacterial activities of *E. Tereticornis*extracts.

Concentration (µg/ml)	Zones of inhibition							
	E. coli MIC 250 µg/ml				*S. aureus* MIC 125 µg/ml			
	Leave	Stem	Seed	Skin	Leave	Stem	Seed	Skin
1000	10 ± 0.04	9 ± 0.04	14 ± 0.05	12 ± 0.04	11 ± 0.05	11 ± 0.04	15 ± 0.06	13 ± 0.05
500	5 ± 0.02	4 ± 0.03	7 ± 0.02	6 ± 0.02	6 ± 0.03	5 ± 0.02	8 ± 0.04	6 ± 0.03
250	2 ± 0.01	2 ± 0.01	3 ± 0.02	2 ± 0.01	3 ± 0.01	3 ± 0.01	4 ± 0.03	3 ± 0.01
125	0	0	0	0	1 ± 0.01	1 ± 0.00	2 ± 0.01	1 ± 0.01

Table 5. Comparative analysis of current study findings and literature on phenolic compounds.

Plant	Part of plant (origin)	Phenolic compounds identified by HPLC	TPC (mg/g)	DPPH (mM/g)	TFC (mg/g)	TTC (mg/g)	Antimicrobial activity (zone of diameter in mm)		Reference
							S. aureus	*E. coli*	
E. hybrid	Leaves (Congo)	Gallic acid Protocatechuic acid *p*-hyroxybenzoic acid Gentisic acid *p*-coumaric acid Caffeic acid Chlorogenic acid Ferulic acid *p*-hydroxybenzaldehyde Hydroquinone Vanillin	137.8±1.072	_	_	_	_	_	(Chapuis-Lardy *et al.*, 2002)
E. citriodora	Plant (Pakistan)	–	–	12.0–52.8	_	_	31 ± 0.83	15 ± 0.835	(Ferreira *et al.*, 2016)
E. camaldulensis		–	–		_	_	21 ± 0.851	10 ± 0.835	
E. crebra		–	–		_	_	23 ± 0.836	12 ± 0.835	
E. globules		–	–		_	_	28 ± 0.835	13 ± 0.83	
E. melanophloia		–	–		_	_	26 ± 0.836	16 ± 0.833	
E. microtheca		–	–		_	_	16 ± 0.831	11 ± 0.835	
E. globulus	Leave (Algeria)	_	12.98 ±0.01	2.92	_	_	_	_	(Ghaffar *et al.*, 2015)
E. tereticornis	Plant (Pakistan)	Gallic acid Sinapic Acid Vanillic Acid Chlorogenic Acid Caffeic Acid*p*-hyroxybenzoic acid*p*-Coumaric A cid*m*-Coumaric Acid Cinamic Acid Ferulic Acid Naringrin Vanilline Catechein	80.56 ± 0.88	164.61 ± 1.22	42.77 ± 0.26	28.13± 0.35	15 ± 0.06	14 ± 0.05	Current study

Determination of antibacterial activities: The antibacterial activity of *E. Tereticornis*leaves, stem, seed and skin extracts against *E. coli* and *S. aureus* are tabulated in (Table 4). The results illustrate that against *E. coli*; leave extract shows the maximum zone of inhibition 10±0.04, 6±0.02, 3±0.01 mm, Stem extracts exhibit 9±0.04, 5±0.03, 2±0.01 mm, Seed extracts exhibit 14±0.05, 7±0.02, 3±0.02 mm and Skin extracts exhibit 12±0.04, 6±0.02, 2±0.01 mm, at the concentrations 1000, 500, 250 μg/mL respectively. While no zone of inhibition was shown at 125 μg/mL in four different extracts, hence against *E. coli E. Tereticornis*leaves, stem, seed and skin extracts showed the MIC value 250 μg/mL. Similarly against *S. aureus*; the leaves extract exhibited the zone of inhibition 11±0.05, 6 ±0.03, 3±0.01 and 1±0.01, stem extracts exhibited 11±0.04, 5±0.02, 3±0.01 and 1±0.01 mm, seed extracts showed 15±0.06, 8±0.04, 4±0.03 and 2±0.01 mm, and skin extracts exhibit 13±0.05, 6±0.03, 3±0.01 and 1±0.01 zone of inhibition at concentrations 1000, 500, 250 and 125 μg/ml respectively, hence showed the MIC value 125 μg/mL against *S. aureus*. Result revealed that *E. tereticornis* leaves, stem, seeds and skin extracts were found to be highly effective against *S. aureus* (MIC value 125 μg/ml) than *E. coli* (MIC value 250 μg/ml). Hence, control DMSO did not show the antimicrobial activity against both types of bacterial strains.

In (Table 5), the available data for PC identified by HPLC, total phenolic contents, antioxidant activity and antimicrobial activity from various parts of the plant is compiled. The data samples here are compared with the reported values for Congo, Algeria and Pakistan. The reported values for phenolic contents are higher in Congo. The correlation analysis indicates that phenolic compounds play a significant role in scavenging activity within Eucalyptus species. In contrast, the antimicrobial activity is higher in Pakistan. This difference could be attributed to various factors, such as different assays (including genetically modified ones) and varied growing conditions, such as distinct chemical compositions of soil and other ecological factors.

Discussion

The chemical compounds derived from plants are referred to as phytochemicals, and they exert various effects on the human body (Ameh *et al*., 2010; R. Yadav *et al*., 2011). These bioactive phytochemical constituents include alkaloids, terpenoids, tannins, steroids, phenolic acids, amino acids, saponins, glycosides, flavonoids, and carbohydrates, among others (Pavithra *et al*., 2009). Due to their extremely high molecular weight, phenolic compounds can exist in a variety of forms in plants, including free or solvent-extractable forms that can be extracted using aqueous methanol (CH_3OH) and aqueous acetone, bound forms that can be extracted by acids, bases, or enzymes, or forms that are covalently linked to other plant components (Memon *et al*., 2017). The total phenolic contents, BPC, and antimicrobial activity of *E. tereticornis* leaves, seeds, stems, and skin were assessed using ultrasonic-assisted base hydrolyzed extracts, as opposed to the FPC, total flavonoids, and total tannin contents, which were assessed using sonicated extracts. Methanol was employed as a solvent for the extraction of free phenolic compounds in order to lower the possibility of extracting other chemicals. However, precautions should be taken when extracting phenolic compounds since they are isomerizes in sunlight (trans-cis conversion), react with oxygen in basic solution to form quinines, and react with methanol at normal pH and temperature (Muthee *et al*., 2016; Tiwari *et al*., 2016).

The total phenolic contents of *E. tereticornis*in stem, skin, leaves, and seeds, revealed that the seed had the highest total phenolic contents. As a result, it is concluded that *E. tereticornis* is a rich source of phenolic compounds; therefore, HPLC-DAD is being used to further analyze the free and bound phenolic compounds. These substances belong to various classes of substances that have been demonstrated to have anti-microbial properties for a wide range of microorganisms *In vitro*. These substances may protect both people and animals from a wide range of diseases, including cancer, diabetes, cardiovascular issues, and others (Vasu *et al*., 2009). Numerous phytochemicals have healing qualities that guard against chronic disorders (Siddique *et al*., 2009). For instance, tannins and polyphenols have anti-diarrheal and anti-helminthic properties; coumarins have antiviral properties. Flavonoids also have anti-fungal, anti-inflammatory, anti-microbial, anti-bacterial, and anti-diarrheal properties. The results of the current research work show that the seed and skin have excellent free radical scavenging activity. Total flavonoid contents, total tannin contents, and radical scavenging activity (RSA) were also determined (Muthee *et al*., 2016). In stems and leaves, the total flavonoid and total tannin levels were found to be higher. When compared to *E. coli*, the antibacterial activity of ultrasonic-assisted base hydrolysis extracts is significantly more effective against *S. aureus*; the MIC (Minimum Inhibition concentration) value is 125 g/mL for *S. aureus* and 250 g/mL for *E. coli*. Phytomedicines, which can be produced from many plant components, including skin, leaves, root, flower, fruit, and seed, have long been used as remedies (Mojab *et al*., 2003). The knowledge of these plants' chemical components is crucial because it is necessary for the synthesis of biochemical components (Parekh & Chanda. 2007; Shrestha *et al*., 2015).

Conclusion

It is concluded that *E. tereticornis* leaves, stems, seed and skin extracts are very rich source of total phenolic, total flavonoid, total tannin contents and antiradical activity 39.03-80.56 mg/g, 25.62-42.77 mg/g, 15.30-28.13 mg/g and 29.33-164.61 mM/g, respectively. Furthermore, HPLC-DAD profiling conceded the existence of 13 compounds, of which 11 were phenolic compounds and 2 derivatives of aldehydes acquiring the larger fraction of bound phenolic compounds with respect to free ones. Gallic (23.47 mg/g), *p*-Coumaric (16.66 mg/g), *p*-hyroxybenzoic (11.03 mg/g) and ferulic (8.71 mg/g) acids were dominant constituents in skin, seeds, stem and leaves, respectively, by ultrasonic-assisted base hydrolysis extraction method. The antibacterial activity reveals that *E. tereticornis*leaves, stem, seeds and skin extracts are highly effective in action against *S. aureus* (MIC value 125 μg/ml) than *E. coli* (MIC value 250 μg/ml).

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