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Phytochemical analysis and *In-vitro* antioxidant activity of aerial parts of *Psiadia punctulata*

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Abstract
Psiadia punctulata (DC.) (Asteraceae) is a small shrub, which is native to Arabia and East and South Africa. The aim of the present study was to evaluate the chemical constitutions qualitatively and quantitatively, as well as the antioxidant activity of extracts obtained from the aerial parts of *Pisadia punctulata*. Phytochemical analysis of 80% methanol extract showed the presence of carbohydrates, flavonoids, steroids, triterpenoids, phenolic acids and saponins. The total phenolic and flavonoid contents of the 80% methanol extract ware 54.63±3.30 mg/g and 36.77±2.86 mg/g, respectively. The methanol extract and its fractions (ethyl acetate, chloroform and n-butanol) were exhibited antioxidant activity in rapid screening of antioxidant by dot-blot DPPH method and by TLC bioautography assay. Antioxidant activity ranged from 26±4.20% for chloroform fraction to 98±3.12% for n-butanol fraction. The aerial parts of *Pisadia punctulata* are a promising candidate for the production of naturally occurring antioxidant agents.

Keywords: Yemen, *Psiadia punctulata*, phytochemical**,** antioxidant

Introduction
Psiadia punctulata (DC.) Vatke (Asteraceae) is a small shrub, which is native to Arabia and East and South Africa (Mahadeo *et al.*, 2018) [25]. In African folk medicine, leaves of this plant are used for abdominal pain and colds. The Bedouin, an Arab seminomadic group, are known to apply leaves as a plaster cast for broken bones (Mahadeo *et al.*, 2018; Keriko *et al.*, 1997) [25, 23]. In Yemeni folk medicine the plant is also known to be used to treat fractures in animals (Hehmeyer *et al.*, 2012) [17] and its flowers and leaves are used in rheumatic diseases (Mothana *et al.*, 2011) [30]. Previous phytochemical studies on *Psiadia punctulata* collected in Saudi Arabia, have been reported the presence of flavonoids, terpenoids, phenylpropanoids and coumarins (Abou-Zaid *et al.*, 1991; Mossa *et al.*, 1992; Midiwo *et al.*, 2002) [1, 29, 28], as well as the antitrypanosomal, antileishmanial, antiplasmodial, and cytotoxic activities have been evaluated (Mahadeo *et al.*, 2018) [25]. Extracts of the aerial parts of *Psiadia punctulata* collected in Yemen was exhibited antimicrobial and cytotoxic activities (Mothana *et al.*, 2011) [30].
Yemen is characterized by its variations in the climate, which results in a wide variation of its flora. Up to date, very little research was done to investigate these traditionally used medicinal plants (El-Fiky *et al.*, 1995; Ali *et al.*, 2001; Mothana *et al.*, 2005) [12, 3, 31]. *Psiadia punctulata* that grows in south of Yemen is widely used by local people for treatment of many ailments, but lack of scientific information and knowledge of its chemical composition could not explain its therapeutic potential, so the aim of this work was to study the phytochemical composition and antioxidant activity of its aerial parts.

Materials and Methods
Collection and identification of plant material
The aerial parts (leaves, soft stems and flowers) of *Pisadia punctulata* were collected from Yafae area (south of Yemen) on July 2017, dried in the shaded area and then manually grinded and stored at room temperature for further analysis. The plant was identified by a taxonomist, professor Abdul Nasser Algifri, of the department of biology, of university of Aden, Yemen.

Extraction and fractionation
The extract was prepared and fractionated by standard methods (Harborne, 1998; Sass, 1940; Wagner and Bladt, 1996) [15, 40, 46]. The dried powdered leaves, soft stem and flowers (50 gm) were defatted with petroleum ether (boiling point 60-80 0C) in soxhlet extractor. The marc left after petroleum ether extract was dried completely and then packed well in soxhlet apparatus and extracted with 80% methanol (80-90°C), until the extraction was completed which was confirmed by the colour of the siphoned liquid. The methanol extract was concentrated by distilling off the solvent, evaporated to dryness and calculated the percentage yield in terms of air dried material. Part of methanol extract (80%) was suspended in water, extracted successively with chloroform, ethyl acetate and n butanol (6×300 ml each) and then resulting solutions were concentrated to provide chloroform, ethyl acetate, n butanol and water residue parts. The rest of methanol extract (20%) was used for further phytochemical analysis.

Qualitative phytochemical analysis
Different qualitative test were performed for establishing profiles of various extracts for their nature of chemical composition. Phytochemical screening of the extracts was carried out according to standard methods (Harborne, 2007; Trease and Evans, 2002; Peach and Tracey, 1956) [16, 44, 35].

Determination of total phenolic and flavonoid contents
The total phenolic content (TPC) of the aerial parts of *Psiadia punctulata* extract was determined using the modified Folin-Ciocalteu method (Ainsworth and Gillespie, 2007) [2], using gallic acid as the standard and expressing results as mg gallic acid equivalent (GAE) per gram of

sample. Different concentrations (30–120 µg/mL) of gallic acid were prepared in methanol. Aliquots of 0.5 ml of the test sample and each sample of the standard solution were mixed with 2 ml of Folin–Ciocalteu reagent and 4 ml of saturated solution of sodium carbonate (7.5% w/v). The tubes were covered with silver foils and incubate at room temperature for 30 minutes with intermittent shaking. The absorbance was taken at 765 nm using UV-VIS spectrophotometer. All the samples were analyzed in three replications. The total phenol was determined with the help of standard curve prepared from pure phenolic standard (Gallic acid). Determination performed in triplicates (n=3).

The total of flavonoid content (TFC) of the aerial parts of *Psiadia punctulata* extract was determined by aluminum chloride colorimetric method (Zhishen *et al.*, 1999) [48]. Different concentrations (30–120 µg/mL) of the extract and standard solution (quercetin) were added with 2 ml of distilled water and subsequently with 0.15 ml of sodium nitrite (5% $NaNO_2$, w/v) solution and mixed. After 6 minutes, 0.15 ml of (10% $AlCl_3$, w/v) solution was added. The solutions were allowed to stand for further 6 min and after that 2 ml of sodium hydroxide (4% NaOH, w/v) solution was added to the mixture. The final volume was adjusted to 5 ml with immediate addition of distilled water, mixed thoroughly and allowed to stand for another 15 min. The absorbance of each mixture was determined at 510 nm on UV spectrophotometer. TFC was determined as mg quercetin equivalent per gram of sample with the help of calibration curve of quercetin. Determination performed in triplicates (n=3).

Thin layer chromatography

Thin layer chromatography (silica gel 60 F254; Merck) of prepared extracts was performed to determine Rf values (Wagner and Bladt, 1996) [46]. Various solvent systems were tested to obtain best results. TLC plates were viewed in UV chamber and Rf of all were calculated. Different solvent systems were found to be effective to get maximum no. of spots for various extracts.

Antioxidant Studies

Rapid screening of antioxidant by dot-blot and DPPH staining

Rapid screening of antioxidant using Dot-Blot assay with DPPH staining was adopted with slight modification. Drops of each sample of 80% methanol extract and it's fractions (chloroform, ethylacetate, n-butanol)) were loaded onto a TLC layer (silica gel 60 F254; Merck) in order of decreasing concentration (0.5, 0.25, 0.125 and 0.0625 mg/ mL), along the row and dried for 10 min. The sheet bearing the dry spots was placed upside down for 10 s in a 0.05% 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution and the layer was dried. Stained silica gel layer revealed a purple background with yellow to white spots at the location where radical scavenging capacity observed. The intensity of the yellow color depends on the amount and nature of radical scavenger present in the sample (Soler-Rivas *et al.*, 2000) [42].

TLC bioautography assay

About 2 mcg of each extract/ fractions were loaded on TLC plate. The plates were developed in solvent system toluene: ethylformate: formic acid (50:40:10) to separate different constituents and various spraying reagents were used. One plate sprayed with 0.05% DPPH reagent to give antioxidant constituents in the deferent extracts/fractions. The antioxidant constituents were analyzed by DPPH technique (Kannan *et al.*, 2010; Chan *et al.*, 2007) [21, 8]. For this 0.05% of DPPH solution in methanol was sprayed on the surface of developed TLC plate and incubated for 10 min at room temperature. The active antioxidant constituents of the extracts were detected in sunlight as yellow spots produced via reduction of DPPH by resolved bands against purple back ground on the TLC plate. Second developed plate, after air-dried, the spots were visualized by spraying with Aluminum chloride (5% in methanol) and the third developed plate was sprayed with ferric chloride (2% in methanol) to identify the respective compounds. Chromatograms were evaluated in sunlight after derivatization with reagents. The colour of the spots was noted and Rf values were calculated (Wagner and Bladt, 1996; Waksmundzka-Hajnos *et al.*, 2008) [46, 47].

DPPH radical scavenging activity assay

The DPPH radical scavenging activity assay of 80% methanol extract and it's fractions was carried out according to the method of Chan *et al* with slight modification (Chan *et al.*, 2007) [8]. Different dilutions of the extract (30, 50, 70, 90, and 110 mcg/mL) were prepared. DPPH solution was also prepared by dissolving 6.0 mg of DPPH in 100 mL methanol. Then, 1 mL of extract from each dilution was added into the test tube containing 2 mL of DPPH solution. Control was prepared by adding 1 mL of methanol to 2 mL of DPPH solution. The mixture was shaken vigorously and was left to stand in the dark for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The scavenging activity of extract on DPPH radical was calculated using the following equation:

Inhibition % = [(A0 –A1) /A0] x 100;

A0 is the absorbance of control and A1is absorbance of test. Antioxidant activity of 80% methanolic extract and it's fractions (chloroform, ethyl acetate and n-butanol), expressed as IC50 values and compared with standard. The 50% inhibition (IC50) of antioxidant activity was calculated as the concentrations of samples that inhibited 50% of scavenging activity of DPPH radical's activity under these conditions (Huang *et al.*, 2007) [20]. The data were presented as mean values ± standard deviation (n = 3).

Result and Discussion

The percentage yield of 80% methanol extract of the aerial parts of *Psiadia puntulata* was 53.2% w/w of dried plant material. Phytochemical screening of the 80% methanol extract showed the presence of phytoconstituents such as carbohydrates, saponins, flavonoids, polyphenols, alkaloids, triterpenes, sterols and amino acid/ protein. Plant bioactive compounds have played a vital role worldwide in preventing and curing numerous human ailments. Moreover, information about different phyto-constituents of plants is a very important and advantageous because they are responsible for the therapeutic effectiveness of most medicinal plants, and may represent an alternative and economically feasible approach to new drugs (Chatterjee, 2015) [9].

The present study has been carried out for quantification of the total phenolic and flavonoid of 80% methanol extract of the aerial parts of *Psiadia puntulata*. The total phenolic content in the crude extract, was determined from regression

equation of calibration curve (y=0.0047x-0.1061, R2 = 0.9961) of gallic acid (30–120 µg/mL) and expressed in mg gallic acid equivalent (GAE) per gram dry extract. The result of total phenolic Content (TPC) was 54.63±3.30 mg/g of plant extract.

The standard calibration curve of gallic acid is shown in figure 1. The total flavonoid content of 80% methanol extract was also determined from regression equation of calibration curve (y=0.0061x+0,0388, R2=0.9994) of quercetin (30-120 µg/mL) and expressed in mg quercetin equivalent (RE) per gram dry extract. The result of total flavonoid Content (TFC) was 36.77±2.86 mg/g of plant extract. The standard calibration curve of quercetin is shown in figure 2. The present study indicated the presence of significant amounts of flavonoid and phenolic compounds in aerial parts of *Psiadia puntulata*. The phenolic and flavonoid compounds are potent antioxidants and free radical scavengers (Scalbert *et al.*, 2005; Eberhardt *et al.*, 2000; Trichopoulou *et al.*, 2003) [41, 11, 45] and have been reported to exhibit cardio-protective, hepatoprotective, anti-allergenic, anti-inflammatory, anti-microbial, antithrombotic, anti-atherogenic, and vaso-dilatory effects (Alpinar *et al.*, 2009; Middleton *et al.*, 2000; Tiwari, 2001) [4, 27, 43].

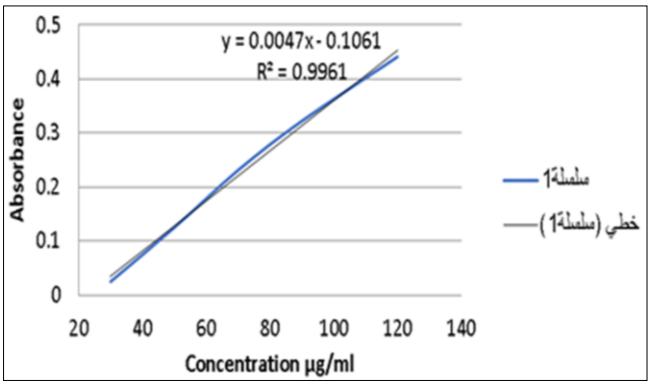

Fig 1: Standard calibration curve of gallic acid

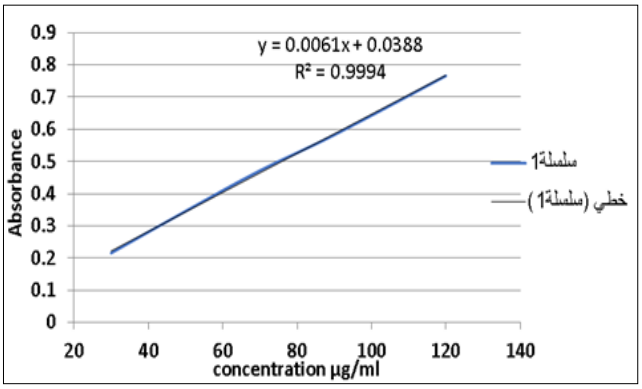

Fig 2: Standard calibration curve of quercetin

Thin layer chromatography

Chromatographic methods are excellent tools for monitoring the chemical composition of herbal drugs. TLC is the rapid and inexpensive method for the identification of number of compounds present in a crude sample and for the separation of plant extracts into different fractions (Sasidharan *et al.*, 2011) [39].

The TLC profiles reflect the phytochemical integrity of herbal medicines and can be easily used to provide a higher amount of data about the complex composition of such biological matrices (Patra *et al.*, 2010; Nicoletti, 2011; Braz *et al.*, 2012) [34, 32, 7]. TLC profile of 80% methanol extract and it's fractions was performed using a silica plate. Different solvent systems were investigated in order to obtain suitable elution in TLC plates. With solvent system toluene-ethyl formate-formic acid (50:40:10) were identified 12, 13, 9 and 9 spots in 80% methanol, ethyl acetate, chloroform and n-butanol respectively, also with chloroform-methanol-formic acid (44:3.5:2.5) were identified 7, 12, 17 and 2 pots in 80% methanol, ethyl acetate, chloroform and n-butanol respectively. Quercetin was identified in studied sample. Photos of the plates were taken in UV chamber then Rf values were calculated as well as the colour of spots were observed (Table 1), (figure 3).

Table 1: Observations of thin layer chromatographic studies of methanol 80% extract and it's fractions (ethyl acetate, chloroform and n-butanol) of *Psiadiapunculata* aerial parts.

Extracts/fractions	Mobile phase	No. of spots	Rf values	Colour of the spot at 365 nm
Methanol 80 %	Toleune-ethyl formate-formic acid (50:40:10)	12	0.35	Purple
			0.38	Light purple
			0.41	Reddish purple
			0.44	Reddish pink
			0.46	Reddish purple
			0.49	Pink
			0.54	Deep brown
			0.58	Light purple
			0.62	Pink
			0.67	Pink
			0.74	Pink
			0.85	Pink
	Chloroform-methanol-formic acid (44:3.5:2.5)	7	0.40	Light purple
			0.45	Light Green
			0.52	Deep purple
			0.62	Pink
			0.66	Purple
			0.78	Dark brown
			0.83	Dark brown

Ethyl acetate fraction	Toleune-ethyl formate-formic acid (50:40:10)	13	0.29	Light pink
			0.33	Light purple
			0.35	Dark green
			0.40	Pink
			0.43	Dark brown
			0.46	Pink
			0.48	Dark brown
			0.49	Pink
			0.54	Dark brown
			0.62	Pink
			0.66	Pink
			0.76	Pink
			0.82	Reddish pink
	Chloroform-methanol-formic acid (44:3.5:2.5)	12	0.22	Green
			0.26	Sky blue
			0.32	Purple
			0.40	Purple
			0.50	Purple
			0.56	Deep green
			0.67	Reddish purple
			0.71	Pink
			0.81	Deep brown
			0.86	Deep brown
			0.90	White
			0.93	Pink
Chloroform	Toleune-ethyl formate-formic acid (50:40:10)	9	0.08	Light pink
			0.18	Light pink
			0.25	Light pink
			0.30	Purple
			0.33	Pink
			0.40	Reddish brown
			0.46	Reddish brown
			0.50	Pink
			0.67	Pink
	Chloroform-Methanol-Formic acid (44:3.5:2.5)	17	0.08	Light blue
			0.22	Sky blue
			0.26	Sky blue
			0.28	Sky blue
			0.32	Sky blue
			0.37	Sky blue
			0.40	White
			0.42	Light purple
			0.46	Light green
			0.53	Reddish purple
			0.57	Light pink
			0.61	Light brown
			0.65	Light brown
			0.71	Light pink
			0.75	Light pink
			0.81	Dark brown
			0.87	Pink
N-Butanol	Toleune-Ethyl formate formic acid (50:40:10)	9	0.08	Sky blue
			0.18	Sky blue
			0.30	Sky blue
			0.35	Blue
			0.43	Blue
			0.46	Blue
			0.54	Blue
			0.65	Light pink
			0.85	Pink
	Chloroform-methanol-formic acid	2	0.12	Sky blue

	(44:3.5:2.5)		0.26	Sky blue
Quercetin	Toleune-ethyl formate-formic acid (50:40:10)		0.35	Dark green
	Chloroform-methanol-formic acid (44:3.5:2.5)		0.22	Green

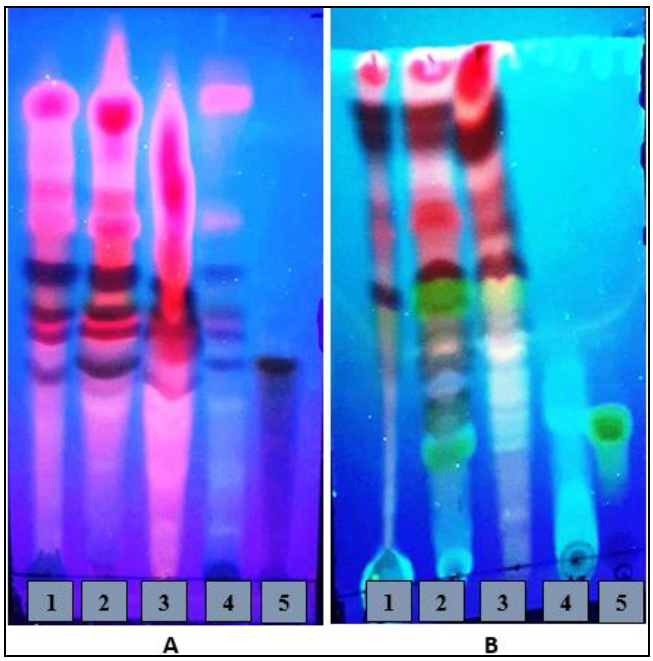

Fig 3: TLC plate of methanol 80% extract (1) and it's fractions (ethyl acetate (2), chloroform (3) and n-butanol (4)) and quercetin (5) obtained in: A- toluene-ethyl formate-formic acid (50:40:10), B-chloroform-methanol-formic acid (44:3.5:2.5) under UV 365 nm.

Antioxidant Studies

Rapid screening of antioxidant by dot-blot and DPPH staining

The use of DPPH radical provides an easy, rapid and convenient method to evaluate the antioxidants and radical scavengers (Rice-Evans *et al.*, 1996; Huang *et al.*, 2005) [36, 19].

The results of dot-blot assay showed colored spots where the aliquots of different extracts/ fractions were dropped. The purple area on the plate indicates no free radical scavenging (antioxidant) activity and the yellow area indicates free radical scavenger or antioxidant activity. The intensity of the yellow color depends upon the amount and nature of radical scavenger present in the sample (Maw *et al.*, 2011; El-Sayed *et al.*, 2011; Huang *et al.*, 2004) [26, 13, 18], Large diameter and high color intensity of the resulting dots (spots) indicate the high radical scavenging capacity of the tested extract (El-Sayed *et al.*, 2011) [13]. The results of dot-blot assay showed yellow coloured spot when stained with DPPH solution. All dots at concentration of 0.5mg/ml, 0.25 mg/ml, 0.125 mg/ml and 0.0625 of n-butanol fraction showed higher scavenging activity but methanol 80% extract, ethyl acetate and chloroform fractions only at concentrations of 0.5mg/ml and 0.25 mg/ml showed higher scavenging activity.

Dots at concentration of 0.125 mg/ml and 0.0625 of methanol 80% extract, ethyl acetate and chloroform fractions showed weak scavenging activity. The intensity of the yellow color of the dots was compared with those produced with quercetin (Figure 4).

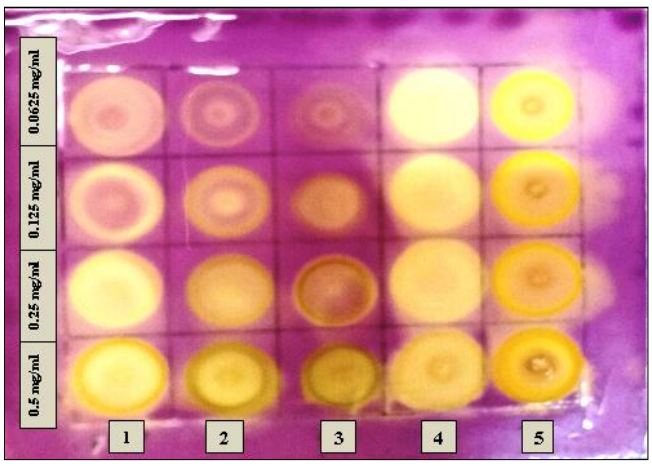

Fig 4: Dot blot assay of the methanol 80 % extract (1), ethyl acetate (2), chloroform (3), n-butanol (4) fractions of the aerial parts of *Psiadia punctulata* and quercetin (5) on a silica sheet stained with a DPPH solution in methanol.

TLC bioautography assay

In the screening of antioxidants, the TLC bioautography assay is the method of choice due to several advantages that include flexibility, simplicity and high throughput (Olech *et al.*, 2012; Cimpoiu, 2006; Badarinath *et al.*, 2010) [33, 10, 5]. To analysis of antioxidant constituents of *Psiadia punculata* aerial part, a TLC bioautography method was performed by spraying developed plates with 0.05% DPPH reagent, also identification of the respective compounds was performed by spraying other developed plates with specific reagents (ferric chloride and aluminum chloride).

Three TLC plates for methanol extract and it's fractions were developed in toluene-Ethyl formate -formic acid (50:40:10) to separate different constituents. One plate sprayed with 0.05% DPPH reagent to give antioxidant constituents, while second developed plate, the spots were visualized by spraying with ferric chloride (2% in methanol), and the third developed plate was sprayed with aluminum chloride (5% in methanol) to identify the respective compounds. Plate sprayed with 0.05% DPPH reagent showed the present of 5, 7, 3 and 4 spots, with yellow colour corresponding with antioxidant behavior in methanol extract, ethyl acetate, chloroform and n-butanol fractions respectively. Also all these spots were identified as phenolic compounds and flavonoids as showed in second and third plates. Quercetin was used as a reference compound. Photos of the plates were taken in dally light after derivatization with reagents then *Rf* values were calculated as well as the colour of spots were observed (Table 2), (figures 5). From the above results, studied plant is enlargement of phenolic compounds such as flavonoids with antioxidant activity. Phenolic acids and flavonoids are highly responsible of the health effect because, they play a key role as antioxidants due to the presence of hydroxyl substituents and their aromatic structure, which enables them to scavenge free radicals (Huang *et al.*, 2005; Bazzano *et al.*, 2002; Kefalas *et al.*, 2003) [19, 6, 22].

Table 2: Analyses of TLC plates of antioxidant constituents in extract/fractions obtained from *Psiadia punculata* aerial part developed in Toluene-Ethyl formate-formic acid (50:40:10).

Extract/ fractions	Rf values	Colour of the spots in daily light after derivatization with:		
		0.05% DPPH reagent	ferric chloride reagent	Aluminum chloride reagent
Methanol 80%	0.33	Yellow	Light brown	Brownish yellow
	0.34	Deep yellow	Brown	Brownish yellow
	0.46	Yellow	Blue	Yellow
	0.54	Deep yellow	Deep blue	Yellow
	0.58	Light yellow	Light brown	Light yellow
Ethylacetate	0.28	Light yellow	Light brown	Yellow
	0.34	Deep yellow	Brown	Yellow
	0.35	Deep yellow	Deep blue	Deep orange
	0.46	Yellow	Deep blue	Yellow
	0.54	Deep yellow	Deep blue	Yellow
	0.58	Deep yellow	Brown	Yellow
	0.62	Light yellow	Light brown	Yellow
Chloroform	0.34	Deep yellow	Light brown	Yellow
	0.46	Deep yellow	Deep blue	Brownish yellow
	54	Deep yellow	Brown	Brownish yellow
N-Butanol	0.34	Deep yellow	Light brown	Yellow
	0.35	Light yellow	Light brown	Light orange
	0.46	Light yellow	Light brown	Yellow
	0.54	Light yellow	Light brown	Brownish yellow
Quarcetin	0.35	Deep yellow	Brown	Deep orange

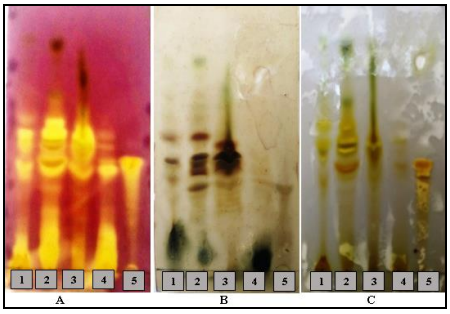

Fig 5: TLC plate of phenolic constituents of methanol 80% extract(1) and it's fractions (ethyl acetate (2), chloroform (3) and n-butanol (4)) and quercetin (5) in toleune-ethyl formate-formic acid (50:40:10) in daily light after derivatization with DPPH (A), FeCl3 (B) and Al Cl3 (C) reagents.

DPPH radical scavenging activity assay

Assessments of antioxidant properties of natural compounds are very important because of their uses in medicine, food and cosmetics (Liu, 2003; Sanchez-Moreno, 2002) [24, 38]. The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry (Maw *et al.*, 2011) [26], so it can be useful to assess various products at a time. The antioxidant activity of 80% methanol extract and it's fractions (chloroform, ethylacetate and n-butanol) of aerial part of *Psiadia punculata* was calculated according to the percentage inhibition in DPPH free radical scavenging assay. The experimental results (Table 3 and Figure 6) showed a dose-dependent increase in DPPH radicals scavenging activity as indicated by the discoloration of DPPH. Scavenging activity ranged from 11.1±3.35% for chloroform fraction at a concentration of 30 µg/ml to 80.2±3.15% for n-butanol fraction at a concentration of 110 µg/ml, while for quercetin (standard compound) it was from 40.1±2.21% at a concentration of 30 µg/ml to 85.0±2.29% at a concentration of 110 µg/ml.

The IC50 value is the concentration of an antioxidant to quench 50% of DPPH free radicals in the reaction mixture under the assay condition. IC50 of quercetin was 37.76 µg/mL, while IC50 values for n-butanol fraction, methanol extract, ethylacetate and chloroform fractions were 47.35 µg/mL, 68.16 µg/mL, 89.53 µg/mL and 96.59 µg/mL respectively. In the DPPH assay, the higher antioxidant activity is reflected in the lower IC50 value. It has been proposed that samples with IC50 lower than 50 µg/mL are very strong antioxidants, with 50-100 µg/mL are strong, with 101-150 µg/mL are moderate, and with IC50 greater than 150 µg/ mL are weak antioxidants (Fidrianny and Anggraeni, 2018) [14]. N-Butanol fraction showed very strong antioxidant because IC50 was 47.35 µg/mL. The concentration of antioxidant needed to decrease the initial DPPH concentration by 50% (IC50) is a parameter widely used to measure the antioxidant activity (Sanchez-Moreno *et al.*, 1998) [37].

Table 3: The DPPH free radical scavenging activity of the methanol 80% extract and it's fractions of studied plant and Quercetin.

Concentration µg/ml	Radical scavenging effect (%)				
	Quercetin	Methanol 80% extract	Ethyl acetate fraction	Chloroform fraction	n-Butanol fraction
30	40.1±2.21	27.4±1.35	14.3±3.35	11.1±3.35	38.1±3.35
50	58.2±2.00	38.3±3.15	30.3±3.23	24.6±2.65	53.2±3.33
70	76.4±3.04	53.4±2.45	44.2±2.75	34.5±2.35	64.6±2.85
90	81.2±1.29	62.1±4.05	50.2±2.65	42.1±3.25	72.5±2.65
110	85.0±2.29	74.2±3.25	58.3±3.45	52.1±4.11	80.2±3.15

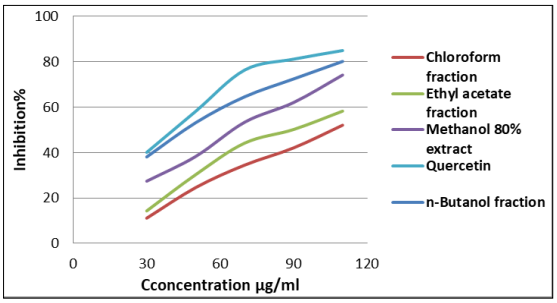

Fig 6: The DPPH free radical scavenging activity of the methanol 80% extract and it's fractions of studied plant and Quercetin

Conclusion

The present study revealed that the 80% methanol extract and it's fractions (chloroform, ethylacetate and n-butanol) of aerial part of *Psiadia punculata* have medicinally crucial bioactive agents, justifying its usage in the traditional medication for treating various illnesses. The study also identified and determined the chemical constituents and evaluated the antioxidant activity of the aerial part of this plant. Further work is required to isolate and characterize the individual bioactive and to identify active compounds in these plant extracts.

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