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Evaluation of antioxidant and DNA nicking potential along with HPTLC fingerprint analysis of different parts of *Pterospermum acerifolium* (L.) Willd

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ABSTRACT

Objective: The present study was designed to evaluate the *in vitro* antioxidant and DNA nicking potential of various extracts and fractions of *Pterospermum acerifolium* (L.) Willd (Family: Stericuliaceae).
Research design and methods: Antioxidant properties of the extracts and fractions was assayed by DPPH scavenging activity, non-site-specific and site-specific OH radical scavenging activity mediated 2-deoxy-D-ribose degradation, total antioxidant activity and lipid peroxidation assay by using rat liver homogenate. DNA nicking assay was studied by PT257R/T plasmid. Estimation of total phenolic content and total flavonoid content was done. Further HPTLC fingerprint of active fractions was performed.
Results: Ethyl acetate fractions of leaves, flowers and bark exhibited potent antioxidant property and DNA protective effect compare to all the other extracts and fractions. Total phenolic and flavonoid content determination was also showed ethyl acetate fractions were rich in phenolic and flavonoid contents. HPTLC fingerprint revealed the total number of peaks present in the active ethyl acetate fractions of leaves, flowers and bark.
Conclusion: The present study indicated that, ethyl acetate fractions of leaves, flowers and bark showed effective antioxidant and DNA protection activity and it could be the initiation for various other pharmacological studies on those fractions.

1. Introduction

Free radical is a chemical compound, which contains an unpaired electron spinning on the peripheral layer around the nucleus. The family of free radicals generated from the oxygen is called reactive oxygen species (ROS), which cause damage to other molecules by extracting electrons from them in order to attain stability.[1] Due to over-production of reactive species, induced by exposure to external oxidants or a failure in the defense mechanisms, damage to cell structures, DNA, lipids and proteins occur which increases the risk of different diseases.[2] Antioxidants thus play an important role to protect the human body against damage by reactive oxygen species.[3] The body possesses antioxidant defense mechanisms such as enzymatic antioxidant systems (superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) etc.) and non-enzymatic antioxidant systems (ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids, etc). Normal levels of antioxidant system in the body are not sufficient for the eradication of the free radical injury.[4] Thus the need of the body for antioxidants from external sources becomes mandatory. Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used have side effects and are also carcinogenic,[2] thus there is a need for more effective, less toxic and cost effective antioxidants. Medicinal plants appear to have desired advantages and hence there occurs a growing interest in natural antioxidants from plant sources.[1]

Pterospermum acerifolium (L.) Willd (Family: Sterculiaceae) commonly known as kanakchampa, is an evergreen large tree up to 24 m in height and 2.5 m in girth with smooth bark, greyish brown in color. It is found in the sub-Himalayan tract and outer valleys from Yamuna eastwards to West Bengal, Assam and Manipur, up to an altitude of 1200 m.[5] It has been traditionally used for the treatment of blood troubles, inflammation, ulcer, tumors, leprosy, small pox

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eruptions, general tonic, diabetes, gastrointestinal disorder, bronchitis, cough, cephalic pain, migraine and antimicrobial agent.[6,7] From leaves, kaempferol-3-0-β-D-galactoside (0.3%) as the major flavonoid and luteolin, luteolin-7-0-β-D-glucoside, luteolin 7-0-β-D glucuronide were isolated.[8] In flowers, 24 β-ethylcholest-5-en-3-beta-0-alpha-cellobiside, β-amyrin, β-sitosterol, n-triacontanol, n-hexacosane-1-26-diol dilignocerate and a mixture of acids and saturated hydrocarbon were isolated from the light petroleum ether extract of the dried flowers.[9] In addition to that, 4′-(2-methoxy-4-(1,2,3-trihydroxypropyl) phenoxy) luteolin, 5,7,3′-trihydroxy-6-O-β-D-glucopyranosyl flavone, 3,5-dihydroxyfuran-2(5H)-one[10] kaempferol and kaempferide-7-glucoside were isolated from the alcoholic extracts of the dried flowers.[11] From barks, D-galacturonic acid, D-galactose and alpha-rhamnose in the molar ratio 5:3:3 were isolated.[12] Pharmacological studies conducted in the plant are comparatively low. Hence this study is designed to evaluate the antioxidant and DNA nicking potential of the various extracts and fractions of leaves, flowers and bark of *P. acerifolium* along with their HPTLC fingerprint analysis of active fractions.

2. Materials and methods

2.1. Plant materials

Leaves, bark and flowers of *P. acerifolium* collected from the campus of BIT-Mesra, Ranchi in the month of August 2011. The plant material had been identified and authenticated from taxonomy department of National Botanical Research Institute (NBRI), Lucknow. The voucher specimens (NBRI/CIF/247/2011) were retained in the Department of Pharm. Sciences, BIT-Mesra, Ranchi for future reference.

2.2. Extraction and fractionation

Air dried coarsely powdered leaves (1 kg) was extracted with methanol for the period of 72 h, filtered, concentrated on rotary evaporator (Buchi, US) to get 96.4 g of extract PALME. The obtained extract (25 g) was dissolved in distilled water and successive extraction with hexane (PALHF), ethyl acetate (PALEF) and the remaining residue as aqueous fraction (PALAF) and concentrated to dryness to obtained respective fractions.

Powdered flowers (1 kg) and bark (1 kg) was extracted separately with 50% ethanol for the period of 72 h and concentrated to get 61.32 g of 50% ethanol extract of flower PAFEE and 74.61 g of 50% ethanol extract of bark PABEE. Both the obtained extract (25 g) was dissolved in distilled water and extracted successively with hexane (flower PAFHF and bark PABHF), chloroform (flower PAFCF and bark PABCF), ethyl acetate (flower PAFEF and bark PABEF) are concentrated to dryness to obtained respective fractions.

2.3. Free radical scavenging activity using DPPH

The ability of the samples to scavenge the free radicals was estimated by *in vitro* method using a stable nitrogen centered radical viz. DPPH. Scavenging of DPPH free radical determines the free radical scavenging capacity or antioxidant potential of the test sample, which shows its effectiveness, prevention, interception and repair mechanism against injury in a biological system. Extract 0.05 ml dissolved in methanol was added to a methanolic solution of DPPH (100 μM, 2.95 ml) at different concentration (200–1000 μg/ml) and the absorbance was recorded at 517 nm.[13]

$$\text{DPPH scavenging activity } (\%) = [(AC - AS)/AC] \times 100$$

where AC is the absorbance value of the control and AS is the absorbance value of the added test samples solution.

2.4. Hydroxyl radical scavenging activity

2.4.1. Non-site-specific OH radical scavenging activity mediated 2-deoxy-D-ribose degradation

The deoxyribose method was used for determining the scavenging effect on OH as describe by Halliwell.[14,15] The reaction mixture contained ascorbic acid (50 μM), $FeCl_3$ (20 μM), EDTA (2 μM), H_2O_2 (1.42 mM), deoxyribose (2.8 mM), with different concentrations of samples in a final volume of 1 ml in potassium phosphate buffer (10 mM, pH 7.4). It was incubated at 37 °C for 1 h and then 1 ml of 2.8% TCA and 1 ml of 1% TBA were added. The mixture was heated in a boiling water bath for 15 min. It was cooled and absorbance was measured at 532 nm with the spectrophotometer (Shimadzu UV 1700).

2.4.2. Site-specific OH radical scavenging activity mediated 2-deoxy-D-ribose degradation

The ability of the extract to inhibit site-specific OH mediated degradation was also carried out to understand its role as a metal chelator. The reaction mixture contained ascorbic acid (50 μM), $FeCl_3$ (20 μM), buffer (2 μM), H_2O_2 (1.42 mM), deoxyribose (2.8 mM), with different concentrations of samples in a final volume of 1 ml in potassium phosphate buffer (10 mM, pH 7.4). It was incubated at 37 °C for 1 h and then 1 ml of 2.8% TCA and 1 ml of 1% TBA were added. The mixture was heated in a boiling water bath for 15 min. It was cooled and absorbance was measured at 532 nm with the spectrophotometer (Shimadzu UV 1700).[14,15]

2.5. Total antioxidant capacity

Total antioxidant capacity was measured according to spectrophotometric method. 0.1 ml of the extract (1000 μg/ml) dissolved in water was added to an Eppendorf tube and combined with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95 °C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. Ascorbic acid was used as the standard.[13]

2.6. Assay of lipid peroxidation

Male Wistar rats (160–180 g) were procured from the animal house of BIT-Mesra, Ranchi, India. All animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature 24–27 °C and humidity 60–65% with 12:12 light:dark cycles). Food was provided in the form of dry pellets and water *ad libitum*. The animals were allowed to get acclimatized to the laboratory conditions for 7 days before the commencement of the experiment. All experiments involving animals complied with the ethical standards of animal handling and approved by Institutional Animal Ethics Committee (BIT/PH/IAEC/34/2011).

Randomly selected rats were fasted overnight and were sacrificed by cervical dislocation, dissected and abdominal cavity was perfused with 0.9% w/v saline. Whole liver was taken out and visible clots were removed and a weighed amount of liver was processed to get a clear homogenate in cold phosphate buffered saline, pH 7.4 using glass Teflon homogenizer and filtered to get a clear homogenate. The degree of lipid peroxidation was assayed by estimating the thiobarbituric acid reactive substances (TBARS) by using the standard method with minor modifications.[16] Briefly, different concentrations of samples (200–1000 μg/ml) were added

to the liver homogenate. Lipid peroxidation was initiated by adding 100 μl of this reaction mixture was taken in a tube containing 1.5 ml of 0.67% TBA in 50% acetic acid. The mixture was heated in a hot water bath at 85 °C for 30 min and in a boiling water bath to complete the reaction. The intensity of the pink colored complex formed was measured at 535 nm in a spectrophotometer (Shimadzu UV 1700). The percentage of inhibition of lipid peroxidation was calculated by comparing the results of test with those of controls not treated with the extracts.

2.7. DNA nicking assay

2.7.1. Isolation of DNA

Plasmid was isolated by alkaline lysis method[17] from overnight grown culture contain PT257R/T plasmid. Super coiled plasmid was eluted from the gel following the instruction of silica bead DNA gel extraction kit (Fermantas).

2.7.2. Nicking assay

DNA damage protective activity of extract was performed using super coiled PT257R/T DNA. A mixture of 10 μl of sample (40 μg/ml) and plasmid DNA (0.5 μg) was incubated for 10 min at room temperature followed by addition of 10 μl of Fenton's reagent (30 mM H_2O_2, 50 μM ascorbic acid and 80 μM of $FeCl_3$). The final volume of mixture was made up to 20 μl and incubated for 30 min at 37 °C. The DNA was analyzed on 1% agarose gel using ethidium bromide staining and photographed in Gel Doc (Gel doc, Syngene). Quercetin (50 μM) was used as positive control.[15,18]

2.8. Estimation of total phenolic content

About 0.1 ml of the sample (10 μg/ml) was mixed with 0.5 ml of folin–Ciocalteu reagent (diluted with 1:10 ratio with distilled water) and 1.5 ml of sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml with distilled water. The mixture was allowed to stand for 2 h. The absorbance was measured at 750 nm using spectrophotometer (Shimadzu UV 1700). Gallic acid was used as standard and results were expresses as mg/g gallic acid equivalent.[19]

2.9. Estimation of total flavonoid content

About 0.5 ml of the sample (100 μg/ml) was mixed with 1.5 ml of methanol (75% v/v), 0.1 ml of aluminum chloride (10% w/v), 0.1 ml of potassium acetate (1 M) and 2.8 ml of distilled water. The mixture was allowed to stand for 30 min in room temperature. The absorbance was measured at 435 nm using spectrophotometer (Shimadzu UV 1700). Quercetin was used as standard and results were expresses as mg/g quercetin equivalent.[19]

2.10. HPTLC analysis

A number of solvent systems were tried, and the satisfactory resolution was obtained in solvent system, dichloromethane–methanol (9:1) for leaf ethyl acetate fraction PALEF, dichloromethane–methanol (9.5:0.5) for flower (PAFEF) and bark (PABEF) ethyl acetate fraction.

Chromatographic separation of PALEF, PAFEF and PABEF was performed on 5 cm × 10 cm aluminum-backed HPTLC plates coated with 200 μM layers of silica gel 60GF254 (Merck, Darmstadt, Germany). Before use, the plates were prewashed with methanol and activated at 110 °C for 5 min. All the three samples (5 μL each) were applied on to HPTLC plate 13 mm apart from middle of bands by spray-on technique along with nitrogen gas supply for simultaneous drying of bands, by means of Camag (Switzer-land) Linomat V sample applicator fitted with a 100 μL syringe (Hamilton, Bonaduz, Switzerland). A constant spot application rate of 0.15 μL/s was used. Plates were developed with appropriate solvent system. Before development the chamber was saturated with mobile phase for 20 min at room temperature (32 ± 2 °C). Chromatography was performed in Camag's twin-trough chamber. Wavelength for detection was evaluated from complete UV spectrum. Scanning was performed with a Camag TLC scanner 3 under control of Camag winCATS planar chromatography manager software (version 1.4.2). The slit dimensions were 6 mm × 0.45 mm and the scanning speed was 20 mm/s.

2.11. Statistical analysis

All data on all antioxidant activity tests are the average of triplicate analysis. Linear regression analysis was used to calculate the IC_{50} values.

3. Results

The findings from the experimental work have been summarized under different subheadings.

3.1. Free radical scavenging activity using DPPH

The free radical scavenging activity of extracts was studied by its ability to reduce the DPPH. In leaves, the extract PALME scavenged the DPPH radical with IC_{50} value of 469.83 μg/ml (Table 1). Amongst the fractions, the ethyl acetate fraction PALEF showed higher activity with low IC_{50} value of 223.67 μg/ml (Table 1) followed by, PALAF showing scavenging activity with IC_{50} value of 431.78 μg/ml (Table 1). In flowers, the ethyl acetate fraction PAFEF showed highest DPPH radical scavenging activity with IC_{50} value of 569.50 μg/ml (Table 2) compared to other extracts and fractions. Subsequently, the extract PAFEE (569.50 μg/ml) and chloroform fraction PAFCF (619.02 μg/ml) showed significant activity (Table 2). In bark, the ethyl acetate fraction PABEF showed the highest scavenging activity with IC_{50} value of 450.4 μg/ml (Table 3).

3.2. Hydroxyl radical scavenging activity

As the hydroxyl radical scavenging activity has been carried out using site-specific and non-site-specific method. It has been found that, there has been a considerable difference in the scavenging activity between both the methods. In leaves, non-site-specific method – IC_{50} values of OH radical scavenging activity ranges from 322.08 (PALEF) to >1000 μg/ml (PALHF), site-specific method – 276.42 (PALEF) to 943.95 μg/ml (PALHF) (Table 1). In flowers, non-site-specific method – IC_{50} values of OH radical scavenging activity ranges from 396.30 (PAFEF) to >1000 μg/ml (PAFHF), site-specific

Table 1
Antioxidant activity of methanolic extract of *Pterospermum acerifolium* leaves and its various fractions.

Test	IC_{50} values (μg/ml)				Inhibition %	
	PALME	PALHF	PALAF	PALEF	Ascorbic acid	Quercetin
DPPH	469.83	>1000	431.78	223.67	92.13	–
LPO	431.82	>1000	523.33	335.75	–	86.48
NS-OH radical	363.82	>1000	644.69	322.08	–	83.71
S-OH radical	276.4	943.95	546.45	277.36	–	88.64

NS-OH radical – Non-site-specific OH radical scavenging activity; S-OH radical – Site-specific OH radical scavenging activity.

Table 2
Antioxidant activity of 50% ethanolic extract of *Pterospermum acerifolium* flowers and its various fractions.

Test	IC_{50} values (μg/ml)				Inhibition %	
	PAFEE	PAFHF	PAFCF	PAFEF	Ascorbic acid	Quercetin
DPPH	569.50	>1000	619.02	330.61	92.13	–
LPO	347.73	>1000	768.4	241.25	–	86.48
NS-OH radical	543.61	>1000	768.86	396.30	–	83.71
S-OH radical	455.41	>1000	694.56	340	–	88.64

NS-OH radical – Non-site-specific OH radical scavenging activity; S-OH radical – Site-specific OH radical scavenging activity.

Table 3
Antioxidant activity of 50% ethanolic extract of *Pterospermum acerifolium* bark and its various fractions.

Test	IC_{50} values (μg/ml)				Inhibition %	
	PABEE	PABHF	PABCF	PABEF	Ascorbic acid	Quercetin
DPPH	627.06	>1000	717.78	450.4	92.13	–
LPO	709.58	>1000	927.08	533.78	–	86.48
NS-OH radical	688.93	>1000	854.05	548.43	–	83.71
S-OH radical	595.86	>1000	773.95	452.04	–	88.64

NS-OH radical – Non-site-specific OH radical scavenging activity; S-OH radical – Site-specific OH radical scavenging activity.

method 340 (PAFEF) to >1000 μg/ml (PAFHF) (Table 2). In bark, non-site-specific method – IC_{50} values of OH radical scavenging activity ranges from 548.43 (PABEF) to >1000 μg/ml (PABHF), site-specific method – 452.04 (PABEF) to >1000 μg/ml (PABHF) (Table 3).

3.3. Total antioxidant capacity

In leaves, the total antioxidant percentage is highest in ethyl acetate fraction (PALEF – 81.63%) followed by PALME (72.61%), PALAF (68.21%) and PALHF (30.16%) in decreasing order. In flowers, the ethyl acetate fraction (PAFEF) showed the highest antioxidant percentage value (77.32%), which subsequently decreased in PAFEE (68.20%), PAFCF (51.71%) and PAFHF (35.33%). Similar observations have been noticed in the bark extracts. The total antioxidant percentage ranges from highest of 73.2% for PABEF followed by 66.32% for PABEE, 46.81% for PABCF to a lowest of 32.14% for PABHF.

3.4. Assay of lipid peroxidation

In leaves, the extract PALME inhibits the LPO with IC_{50} value of 431.82 μg/ml (Table 1). The ethyl acetate fraction PALEF showed highest LPO inhibition potential with IC_{50} value of 335.75 μg/ml (Table 1). In flowers also amongst the extract and fractions, the ethyl acetate fraction PAFEF showed highest LPO inhibition potential with IC_{50} value of 241.25 μg/ml (Table 2). In bark, the ethyl acetate fraction PABEF showed the highest activity with IC_{50} value of 533.78 μg/ml (Table 3).

3.5. DNA nicking assay

In DNA nicking assay, excluding n-hexane fraction of all the three parts of *P. acerifolium* offered protection against the damage of super coiled plasmid DNA induced by OH radical. The ethyl acetate fractions of leaf (PALEF) and flower (PAFEF) showed the most effective protection and the action was very close to that of quercetin (50 μM) (Fig. 1).

3.6. Total phenolic content

Total phenolic content (TPC) was estimated by using folin–Ciocalteu reagent and expressed as mg/g gallic acid equivalent (GAE). In leaves, ethyl acetate fraction PALEF showed high significant value of TPC about 377.50 GAE, followed by, extract PALME (305.00 GAE) and PALAF (236.25 GAE) which also showed the highest values. In flowers and bark, ethyl acetate fraction PAFEF (321.25 GAE), PABEF (257.50 GAE) and extract PAFEE (287.50 GAE), PABEE (230 GAE) showed significant values.

3.7. Total flavonoid content

Total flavonoid content (TFC) was estimated and expressed as mg/g quercetin equivalent. In leaves, ethyl acetate fraction PALEF showed the highest value of TFC (270 mg/g quercetin equivalent), followed by, extract PALME (208.3 mg/g quercetin equivalent). In flowers and bark, ethyl acetate fraction PAFEF (315 mg/g quercetin equivalent), PABEF (210 mg/g quercetin equivalent) and extract PAFEE (216 mg/g quercetin equivalent), PABEE (142 mg/g quercetin equivalent) showed high values as compared to other fractions.

3.8. HPTLC analysis

Based on the above results, HPTLC fingerprinting of ethyl acetate fractions (PALEF, PAFEF, PABEF) was taken. In HPTLC fingerprint observations, ethyl acetate fraction of leaves (PALEF), flowers

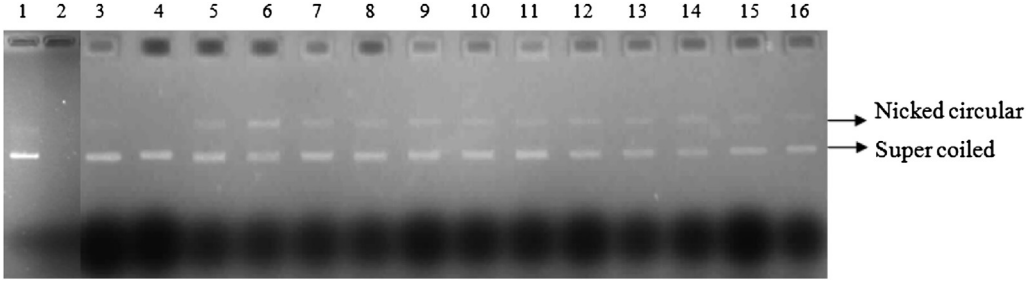

Fig. 1. DNA nicking activity of various extracts and fractions of different parts of *Pterospermum acerifolium*. Lane 1: Normal DNA; Lane 2: DNA + Fenton's reagent; Lane 3, 4: DNA + Fenton's reagent + Qurecitin (30, 50 μM); Lane 5: DNA + Fenton's reagent + PALME; Lane 6: DNA + Fenton's reagent + PALHF; Lane 7: DNA + Fenton's reagent + PALA; Lane 8: DNA + Fenton's reagent + PALEF; Lane 9: DNA + Fenton's reagent + PAFEE; Lane 10: DNA + Fenton's reagent + PAFHF; Lane 11: DNA + Fenton's reagent + PAFEF; Lane 12: DNA + Fenton's reagent + PAFCF; LANE 13: DNA + Fenton's reagent + PABEE; Lane 14: DNA + Fenton's reagent + PABHF; Lane 15: DNA + Fenton's reagent + PABCF; Lane 16: DNA + Fenton's reagent + PABEF.

Table 4
HPTLC profile of PALEF, PAFEF and PABEF.

Fraction	Peak	R_f	Max. height	Area	Area %
PALEF	1	0.64	142.9	7086.5	61.79
	2	0.85	120.9	4381.3	38.21
	3	0.77	100.5	3606.0	66.96
	4	0.59	82.1	1779.0	33.04
	5	0.87	52.0	940.4	39.69
	6	0.65	18.1	385.1	18.35
PAFEF	1	0.60	204.4	6902.0	58.42
	2	0.33	197.5	5546.8	43.85
	3	0.26	178.3	6653.4	36.35
	4	0.51	47.0	1086.4	8.59
	5	0.42	34.4	600.0	5.68
PABEF	1	0.39	27.2	636.5	25.78
	2	0.62	11.6	275.2	11.15
	3	0.26	186.1	8183.8	49.92
	4	0.53	126.5	8209.8	50.08

(PAFEF) and bark (PABEF) exposed several peaks (Table 4). The resultant chromatograms are showed in Figs. 2 and 3. Ethyl acetate fraction of leaves PALEF showed six peaks with R_f values in the range of 0.59–0.87 in the optimized solvent system of dichloromethane–methanol (9:1) (Table 4). The peak number 3 showed higher percentage of area of about 66.96% (Table 4 and Fig. 2). Ethyl acetate fraction of flowers PAFEF showed five peaks with R_f values in the range of 0.26–0.60 in the optimized solvent system of dichloromethane–methanol (9.5:0.5) (Table 4). The peak number 1 showed higher percentage of area of about 58.42% (Table 4 and Fig. 3a). Ethyl acetate fraction of bark PABEF showed four peaks with R_f values in the range of 0.26–0.62 in the optimized solvent system of dichloromethane–methanol (9.5:0.5) (Table 4). The peak number 3 and 4 showed higher percentage of area of about 49.92 and 50.08% respectively (Table 4 and Fig. 3b).

4. Discussion

In a normal healthy person, the generation of pro-oxidants in the form of ROS and RNS are effectively kept in check by the various levels of antioxidant defense mechanisms. However, when it gets exposed to adverse physicochemical, environmental or pathological agents such as atmospheric pollutants, cigarette smoking, ultraviolet rays, radiation, toxic chemicals, over nutrition and advanced glycation end products (AGEs) in diabetes, this delicately maintained balance is shifted in favor of pro-oxidants resulting in 'oxidative stress'.[20] Due to the increased carcinogenicity effects of synthetic antioxidants, interest has increased toward natural antioxidants as it is having fewer side effects.

The antioxidant activity of the extracts and fractions were analyzed by various *in vitro* antioxidant assay techniques. The free radical scavenging activity using DPPH is based up on the extract's ability to reduce the DPPH, a stable free radical. If a molecule that can donate an electron or hydrogen to DPPH, it can also react with it and thereby bleach the DPPH absorption. DPPH is a purple colour dye having absorption maxima of 517 nm and upon reaction with a hydrogen donor the purple colour fades or disappears due to conversion of it to 2, 2-diphenyl-1-picryl hydrazine resulting in decrease in absorbance.[4] In this study, ethyl acetate fractions of leaves (PALEF), flower (PAFEF) and bark (PABEF) showed the highest DPPH scavenging activity with low IC_{50} values. Hydroxyl radical scavenging activity can be done under two conditions to derive two separate inferences, role on hydroxyl trapping ('non-site-specific assay', where EDTA is added) and role of metal chelation ('site-specific assay', where no EDTA is added).[15] Reducing agents like ascorbic acid can produce OH radical by reducing Fe^{3+} to Fe^{2+} that degrade deoxyribose using Fe^{2+} salts as an important catalytic component and oxygen radicals may attack the sugar, which leads to sugar fragmentation. Addition of transition metal ions such as iron at low concentrations to deoxyribose causes degradation of the sugar into malondialdehyde and other related compounds which form a chromogen with thiobarbituric acid (TBA).[21] Here the ethyl acetate fractions of all the three parts of *P. acerifolium*, PALEF, PAFEF and PABEF showed maximum inhibition in both non-site-specific and site-specific OH radical scavenging methods with minimum IC_{50} values. The total antioxidant activity (phosphomolybdenum method) is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate Mo (V) complex with a maximum absorption at 695 nm.[22] The ethyl acetate fractions reduced molybdenum VI to a green colored phosphomolybdenum V complex significantly.

The inhibition of lipid peroxidation induced by ferrous sulfate in liver homogenate was assayed by measuring the amount of lipid peroxidation product malondialdehyde. Oxidative stress can lead to peroxidation of cellular lipids and can be measured by determining the levels of thiobarbituric acid reactive substances (TBARS). The ethyl acetate fractions of leaves, flowers and bark showed potent lipid peroxidation inhibition by reducing the formation of lipid peroxidation end product malondialdehyde. Hydroxyl radical generated by the Fenton's reaction is also known to cause oxidative induced breaking of DNA strands to yield the open circular or relaxed forms.[15] PALEF and PAFEF showed effective reduction in the formation of nicked DNA and increased super coiled form of DNA. Ethyl acetate fractions of all the examined parts of the plant namely leaf, flower and bark (PALEF, PAFEF, PABEF) showed higher phenolic and flavonoid content as compared to their own fractions and justifies their well-built antioxidant activity. HPTLC is sensible

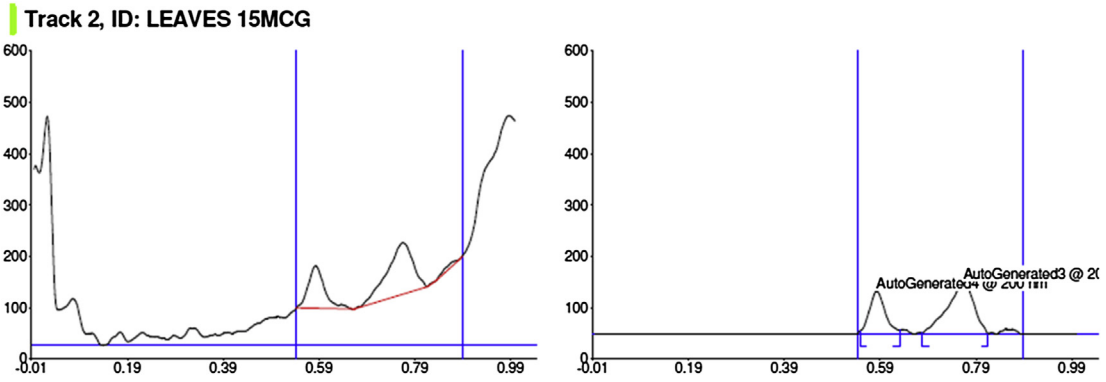

Fig. 2. HPTLC chromatogram of PALEF peak number 3 (AutoGenerated 3) scanned at 200 nm.

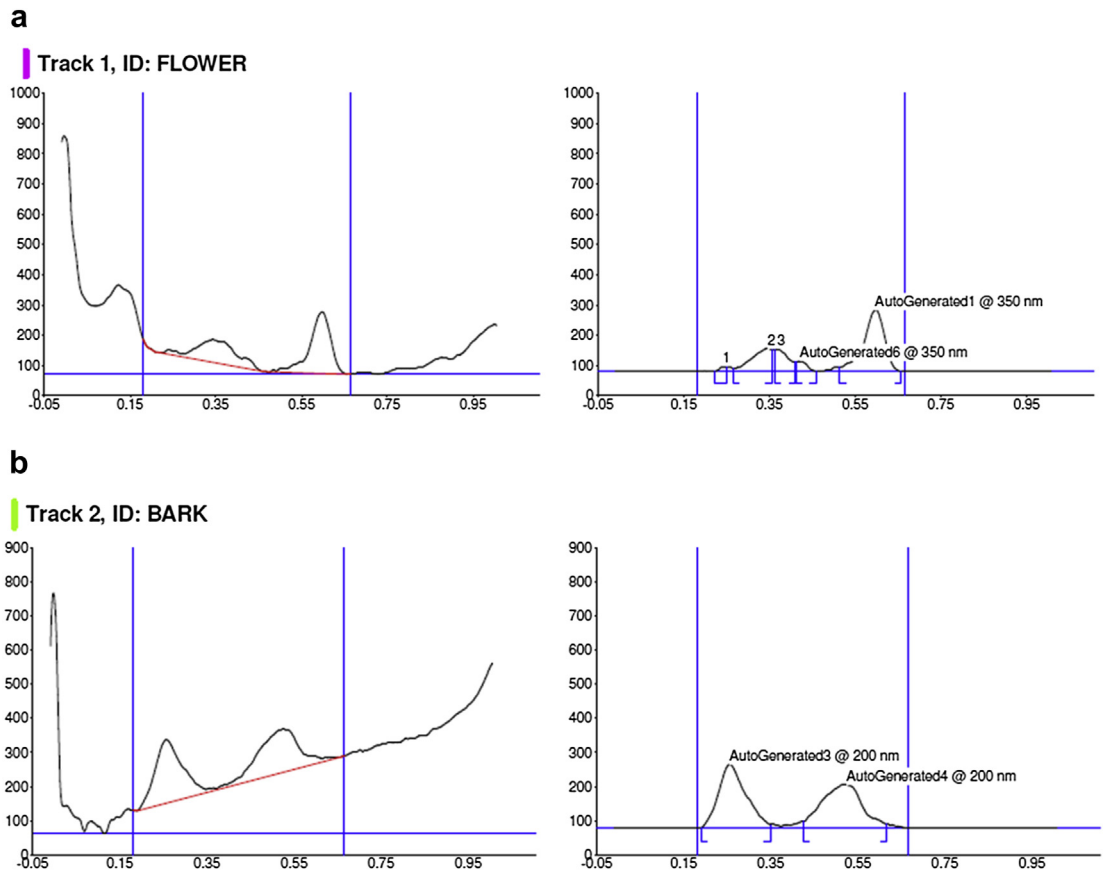

Fig. 3. (a) HPTLC chromatogram of PAFEF peak number 1 (AutoGenerated 1) scanned at 350 nm. (b) HPTLC chromatogram of PABEF peak number 3 & 4 (AutoGenerated 3 & 4) scanned at 200 nm.

method for development of chromatographic fingerprints to determine major active constituents of medicinal plants. In the present study, the HPTLC chromatogram of active ethyl acetate fraction of leaves (PALEF), flowers (PAFEF) and bark (PABEF) of *P. acerifolium* was established. Further works to isolate, characterize and quantitatively estimate with the marker compound of active constituents are in progress.

5. Conclusion

The work demonstrated the *in vitro* antioxidant and DNA nicking potential of different extract and fractions obtained from various parts of *P. acerifolium* and it was found that the ethyl acetate fraction of leaf, bark and stem were the most active fractions. Further work with these active fractions to isolate the active constituents and pin point on its MOA as antidiabetic agent is in progress. This could form the basis for various other pharmacological studies in active fractions.

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Conflicts of interest

All authors have none to declare.

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