

Phytochemical Analysis and Hydroxyl Radical Scavenging Potential of *Murraya exotica* L.

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ABSTRACT

The crude extract/fractions of the leaves of *Murraya exotica* L. have been tested for the qualitative phytochemical analysis, quantitative estimation of total phenolic content and total flavonoid content, hydroxyl radical scavenging activity employing site-specific and non site-specific deoxyribose degradation assay and plasmid nicking assay. The study showed that flavonoids, phenols and tannins were present, but steroids, anthocyanins, proteins, carbohydrates and phlobatanins were absent in the crude extract and its different fractions. It also exhibited the presence of gallic acid, catechin, chlorogenic acid, epicatechin, caffeic acid, umbelliferone, coumaric acid, rutin, tert-Butyl hydroquinone, quercetin and kaempferol in all the fractions viz.; hexane fraction (HF), chloroform fraction (CF), ethyl acetate fraction (EAF), n-Butanol fraction (BF) and water fraction (WF) whereas ellagic acid was detected only in CF. The gallic acid was absent in CF. The investigation involving quantitative estimation of total phenolics showed the highest average of total phenolic content (226.34 µg GAE/mg) in EAF and the highest average of total flavonoids content (304 µg RE/mg) at 1000 µg/ml concentration in CF. HF showed the lowest IC₅₀ value of 1.63 µg/ml in site-specific deoxyribose degradation assay whereas WF showed the lowest IC₅₀ value of 0.00006 µg/ml in non site-specific deoxyribose degradation assay. In plasmid nicking assay, the crude extract and its fractions viz. HF, CF, EAF, BF and WF exhibited hydroxyl radical scavenging activity in a dose dependent manner, with maximum activity at 1000 µg/ml, whereas EAF at 200 µg/ml exhibited the least activity.

Keywords: Antioxidant, deoxyribose, *Murraya exotica*, phytochemicals, scavenging potential

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INTRODUCTION

Plants contain diverse bio-active compounds (phytochemicals) like alkaloids, flavonoids, tannins, terpenoids, phenolic compounds, etc. [1-4]. These compounds act as antioxidants, free radical scavengers, quenchers of singlet and triplet oxygen species and inhibitors of peroxidation [5-7]. Although, a number of plants have been screened for their phytochemistry and antioxidative properties, still majority of plants have not been evaluated for their beneficial uses.

The plant *Murraya exotica* L. belongs to family Rutaceae. Its leaves have astringent properties and are used in diarrhea and dysentery in the Philippines and China. The paste of the leaves is also used to heal cuts

and wounds and to relieve body aches. The leaves and roots are used against rheumatism, cough, and hysteria [8]. Roots are used as antifertility agent in China. They also possess antimicrobial and antipyretic activity. The plant has been reported to contain coumarins, carbazole alkaloids and flavonoids [9-12]. The major coumarins present in the leaves are murrangatin and phebalosin. Murrangatin is reported to possess antithyroid property. Yuehchukene, a bis-indole alkaloid isolated from the roots have antiimplantation activity. Mexolide, a dimeric coumarin from the stem bark is antibacterial. The leaves are reported to possess antifungal and antibacterial activity [13].

In the present study the qualitative and quantitative estimation of phytochemicals in the crude extract and its fractions was evaluated. The crude extract and its fractions were also explored for hydroxyl radical scavenging potential by site-specific and non site-specific deoxyribose degradation assay and plasmid nicking assay. The hydroxyl radical scavenging potential of the leaves was explored as they might be of use against various diseases caused by oxidative stress.

MATERIALS AND METHODS

Plant material

The leaves of *Murraya exotica* L. were collected from the trees growing in the Botanical garden of Guru Nanak Dev University, Amritsar in the month of October.

Sample extraction

The leaves were washed under running tap water and dried at room temperature and were ground to fine powder. The powdered leaves were extracted thrice with 80% methanol and shaken for 24 h on a shaker, at normal temperature. The supernatant was collected and filtered with Whatman No. 1 filter paper. The filtrate was concentrated using rotary evaporator under vacuum, weighed and stored in dry solid form. The separation of crude extract was carried out with solvents in order of increasing polarity i.e. hexane, chloroform, ethylacetate, n-butanol and water.

Phytochemical screening of extract/fractions

The crude extract/ fractions of the leaves of *Murraya exotica* L. were screened for the presence/absence of the various phytochemicals viz. Alkaloids, Flavonoids, Tannins, Proteins, Steroids, Terpenoids, Anthocyanins, Quinones, Phlobatanins, Phenols, Saponins, Carbohydrates and Cardiac glycosides.

Total phenolic content

The total phenolic content of the extract/fractions was determined using Folin-Ciocalteu method [14-15]. To 100 μ l of extract/fractions, 900 μ l of distilled water and 500 μ l of FC (Folin-Ciocalteu) reagent (1:1) was added which was followed by addition of 1.5 ml of 20% sodium carbonate. The volume of the mixture was raised to 10 ml with distilled water. The mixture was then incubated for 2 h at room temperature

and the absorbance of the mixture was taken at 765 nm using UV-VIS spectrophotometer. The phenolic content was calculated as gallic acid (μ g/mg) equivalents on the basis of standard curve of gallic acid.

Total flavonoid content

The total flavonoids content was determined by aluminum chloride colorimetric assay [16]. To 1 ml of extract/fractions, 4 ml of double distilled water was added, followed by addition of 0.3 ml of NaNO_3 and 0.3 ml of AlCl_3 . The mixture was then incubated for 5 minutes at room temperature. After incubation, 2 ml of 1 M NaOH was added and the total volume was raised to 10 ml with double distilled water. The solution was mixed well and the absorbance was measured against blank reagent at 510 nm. The total flavonoid content was expressed as rutin equivalent (RE) in μ g/mg of dry sample.

Liquid Chromatographic analysis

Liquid chromatography was performed on a Shimadzu UHPLC (Nexera). All the standards viz. gallic acid, catechin, chlorogenic acid, epicatechin, caffeic acid, umbelliferone, coumaric acid, rutin, ellagic acid, tert-Butyl hydroquinone, quercetin and kaempferol were purchased from Sigma-Aldrich Bangalore. The HPLC grade methanol and water were purchased from sd fine-chem. Limited (SDFCL) and MERK Mumbai, respectively.

Preparation of standard solutions and samples for UHPLC analysis

10 mg of different fractions viz. hexane fraction (HF), chloroform fraction (CF), ethylacetate fraction (EAF), n-butanol fraction (BF), and water fraction (WF) obtained by the above mentioned separation protocol were dissolved in 1 ml of methanol (HPLC grade) and filtered through 0.2 μ membrane filter. The standard solutions for calibration curve were prepared by diluting the stock solution in methanol at 7 different concentrations (gallic acid, catechin, epicatechin, rutin- 10.416667 ppm to 666.666667 ppm from 8 mg/ml stock solution; chlorogenic acid, caffeic acid, umbelliferone, coumaric acid, ellagic acid, tert-Butyl hydroquinone, quercetin and kaempferol - 5.208334 ppm to 333.333334 ppm from 4 mg/ml stock solution).

Apparatus and chromatographic conditions

Chromatographic separations were done using Enable C-18 G column (150×4.6 mm, 5 µm particle size) at 25°C. The mobile phase for low pressure gradient consisted of A (0.1% acetic acid + water) and B (methanol). The flow rate was set at 1 ml/min and the injection volume was 5 µl. The gradient started with 70% A and 30% B, reaching 45% B at 12 min, 75% B at 13.5-15 min, 50% B at 16.6 min, 25% B at 18-20 min, 30% B at 21 min, and re-equilibrated for 5 min upto 26 min. Detection was made at 280 nm using a PDA detector.

Hydroxyl radical scavenging assays

a) Deoxyribose Degradation assay

The protection against •OH dependent 2-deoxy-D-ribose degradation was estimated using standard deoxyribose degradation assay [17]. The formation of •OH radicals from Fenton reagent were quantified using 2-deoxyribose oxidative degradation.

The deoxyribose degradation assay of the crude extract/fractions was carried out by the experimental procedure as mentioned below [15].

Non-site specific assay: The phosphate buffer (50 mM, pH 7.4) and FeCl₃ (10 mM) were prepared in distilled water. The stock solutions of EDTA (1 mM), ascorbic acid (1 mM), H₂O₂ (10 mM) and deoxyribose (10 mM) were prepared in buffer. The reaction mixture was prepared by adding 100 µl of EDTA, 20 µl of FeCl₃, 100 µl of H₂O₂, 360 µl of deoxyribose, 1 ml of extract/fractions, 320 µl of phosphate buffer and 100 µl of ascorbic acid. The mixture was incubated at 37°C for 1 h. The incubated mixture (1 ml) was mixed with 1 ml of 0.5% TBA in 0.025 M NaOH and heated at 80°C for 1 h to develop pink chromogen, which was measured at 532 nm.

Site-specific assay: The site specific assay was carried out with the same way as described above for non-site specific assay procedure, except that EDTA was replaced by buffer.

b) Plasmid Nicking Assay

The hydroxyl radical scavenging activity of crude extract was estimated using plasmid nicking assay [18].

Supercoiled plasmid DNA (0.5 µg) was added to freshly prepared Fenton's reagent (H₂O₂, FeCl₃, and ascorbic acid), containing extract

and its fractions, and the final volume of the mixture was brought up to 20 µl with water. The mixture was then incubated for 30 minutes at 30°C. DNA strand breaks in supercoiled DNA were analyzed using Gel Doc XR system (Bio-Rad, Hercules, California, USA), after agarose gel electrophoresis. Electrophoresis was accomplished using 1% agarose gel. 0.50 g of agarose was dissolved in 50 ml of 1×TBE buffer and heated, until all of the agarose had dissolved. After cooling it for sometimes, ethidium bromide solution (0.50 µg/ml) was added, followed by casting of gel in tray. The reaction mixture (20 µl) was loaded in the wells and electrophoresis was carried out for 2.5 h at 50 mV.

Statistical analysis

Three different replicates of each concentration were taken and were represented as mean ± S.D. One way ANOVA (Analysis of Variance) was used for analyzing statistical significance of the data and the Tukey's HSD (Honestly Significant Difference) test was used to compare the difference among the means. The regression equation was used to calculate the IC₅₀ value.

RESULTS

I. Phytochemical analysis

The results of the presence/absence of the various phytochemicals in the crude extract/fractions of the leaves of *Murraya exotica* L. are shown in the (Table 1).

II. Total phenolic content

The total phenolic content in the crude extract/ fractions of the leaves of *Murraya exotica* L. was calculated as Gallic acid equivalent from the equation $y = 0.0006x + 0.0002$ (Fig 1) and expressed as µg Gallic Acid Equivalent (GAE) in mg of dry weight of plant extract/fractions as shown in (Table 2). The ethyl acetate fraction (EAF) showed the highest total phenols of 226.34 µg GAE/mg.

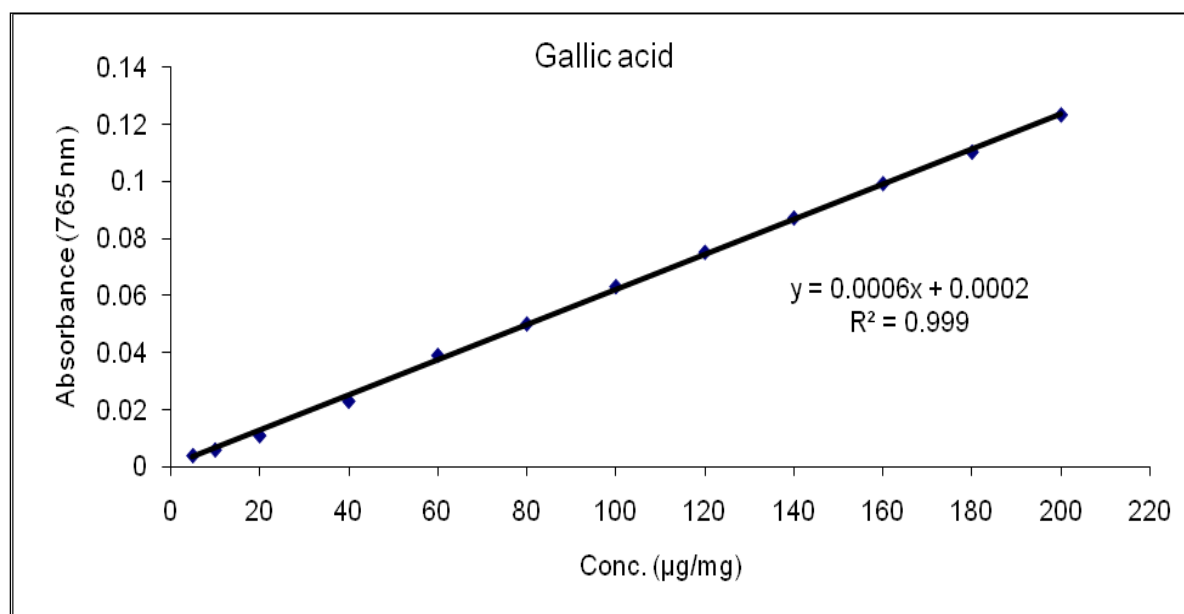
III. Total flavonoid content

The total flavonoid content in the crude extract and fractions of the leaves of *Murraya exotica* L. was calculated as Rutin equivalent from the equation $y = 0.0002x + 0.0062$ (Fig 2) and expressed as µg Rutin Equivalent (RE) in mg of dry weight of plant extract/fractions as shown in (Table 3). The chloroform fraction (CF) showed the highest total flavonoids i.e. 304 µg RE/mg.

Table 1: Qualitative estimation of phytochemicals in the crude extract/fractions of the leaves of *Murraya exotica* L.

S. No.	Phytochemicals	Tests	CE*	HF*	CF*	EF*	BF*	WF*
A.	<u>Alkaloids</u>	Mayer's test	+	+	+	+	+	-
B.	<u>Flavonoids</u>	NaOH test	+	+	+	+	+	+
		H ₂ SO ₄ test	+	+	+	+	+	+
C.	<u>Steroids</u>	Salkowski test	-	-	-	-	-	-
D.	<u>Terpenoids</u>	Salkowski test	+	+	-	+	+	-
E.	<u>Anthocyanins</u>	NaOH test	-	-	-	-	-	-
F.	<u>Proteins</u>	Biuret test	-	-	-	-	-	-
G.	<u>Phenols</u>	FeCl ₃ test	+	+	+	+	+	+
		Libermann's test	+	+	+	+	+	+
H.	<u>Quinones</u>	HCl test	+	+	-	-	-	+
I.	<u>Carbohydrates</u>	Benedict's test	-	-	-	-	-	-
		Fehling's test	-	-	-	-	-	-
J.	<u>Tannins</u>	FeCl ₃ test	+	+	+	+	+	+
K.	<u>Phlobatannins</u>	HCl test	-	-	-	-	-	-
L.	<u>Saponins</u>	Froth test	+	-	+	-	+	+
M.	<u>Cardiac glycosides</u>	Keller-Killani test	+	+	-	+	-	-

*CE: Crude extract, HF: Hexane fraction, CF: Chloroform fraction, EAF: Ethyl acetate fraction, BF: n-Butanol fraction, WF: Water fraction; +: present, -: absent

**Figure 1: Graphical representation of Gallic acid equivalent equation****Table 2: Quantitative estimation of total phenolic content in the crude extract/fractions of the leaves of *Murraya exotica* L.**

S. No.	Extract sample	Total phenolics (µg GAE/mg)
1	Crude extract (CE)	139.67
2	Hexane fraction (HF)	76.34
3	Chloroform fraction (CF)	81.34
4	Ethyl acetate fraction (EAF)	226.34
5	n- Butanol fraction (BF)	83.00
6	Water fraction (WF)	209.67

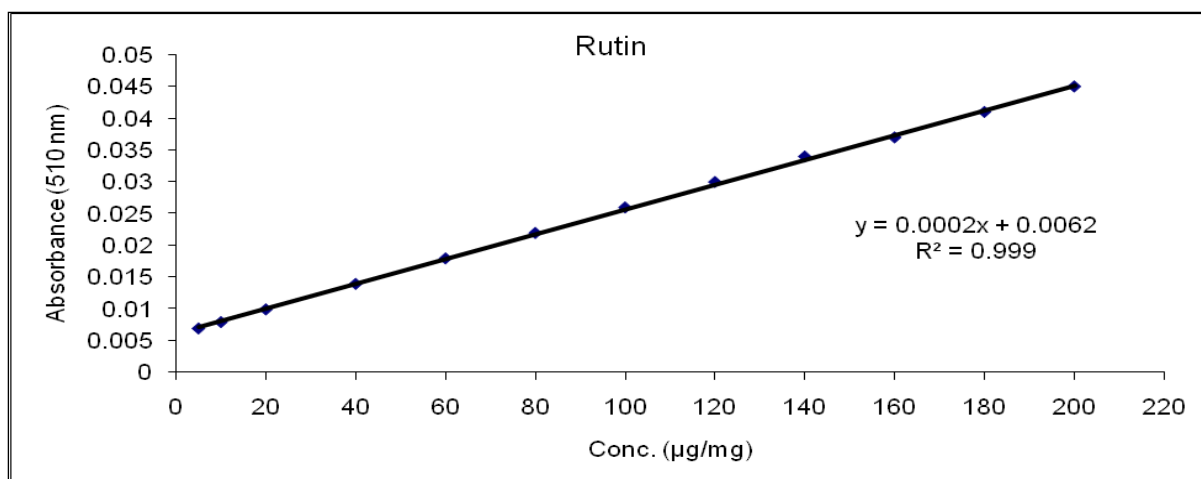


Figure 2: Graphical representation of Rutin equivalent equation

Table 3: Quantitative estimation of total flavonoid content in the crude extract/fractions of leaves of *Murraya exotica* L.

S. No.	Extract sample	Total flavonoids (µg RE/mg)
1	Crude extract (CE)	64
2	Hexane fraction (HF)	109
3	Chloroform fraction (CF)	304
4	Ethyl acetate fraction (EAF)	159
5	n-Butanol fraction (BF)	84
6	Water fraction (WF)	39

Liquid Chromatographic analysis

The mixture of phenol standards (gallic acid, catechin, chlorogenic acid, epicatechin, caffeic acid, umbelliferone, coumaric acid, rutin, ellagic acid, tert-Butyl hydroquinone, quercetin and kaempferol) was analyzed chromatographically (Fig 3), then the fractions viz., HF, CF, EAF, BF and WF were analyzed under the same conditions. (Figures 4-8) represent the chromatographs of the fractions of the plant *Murraya exotica*. After comparison with the standard phenol mixture; it was found that the leaves contain these phenols and some unidentified compounds. It was found that kaempferol is present in higher concentration 226.100, 373.604, 789.390 µg/ml in HF, CF and EAF, respectively; and in BF, tert-Butyl hydroquinone is in the highest concentration of 429.567 µg/ml; whereas in WF, there is presence of epicatechin in the concentration of 39.398 µg/ml, as shown in the (Tables 4-8).

IV. Hydroxyl radical scavenging assays

a) Deoxyribose degradation assay:

The hydroxyl radical scavenging activity of the crude extract and different fractions of the leaves of *Murraya exotica* L. in

deoxyribose degradation assay was observed in both site and non-site specific assay. The gallic acid was used as standard. The extract and different fractions showed pronounced effect in the presence of EDTA which suggested their greater potential to scavenge $\cdot\text{OH}$ present in the solution and thus deoxyribose (detector molecule) is protected from degradation. The extract and different fractions also efficiently chelated Fe (III) in the absence of EDTA making it unavailable to detector molecule and thus impaired the formation of $\cdot\text{OH}$ radicals at a particular site.

i. Site-specific assay:

The effect of different concentrations (% inhibition) of crude leaf extract/ fractions of *Murraya exotica* L. and gallic acid in site-specific deoxyribose degradation assay has been shown in the (Table 9). The observed data revealed that at 200 µg/ml concentration, gallic acid showed maximum hydroxyl radical scavenging activity of 73.60%. Among the extract/fractions, crude extract (CE) showed maximum hydroxyl radical scavenging activity i.e. 68.23%, followed by hexane fraction (HF) which showed 60.52% inhibition, whereas ethyl

acetate fraction (EAF) showed the least hydroxyl radical scavenging activity i.e. 31.43%. Furthermore, the regression curve of % inhibition of gallic acid, crude extract, mAU

different fractions viz. CE, HF, CF, EAF, BF and WF at various concentrations are shown in (Fig 9).

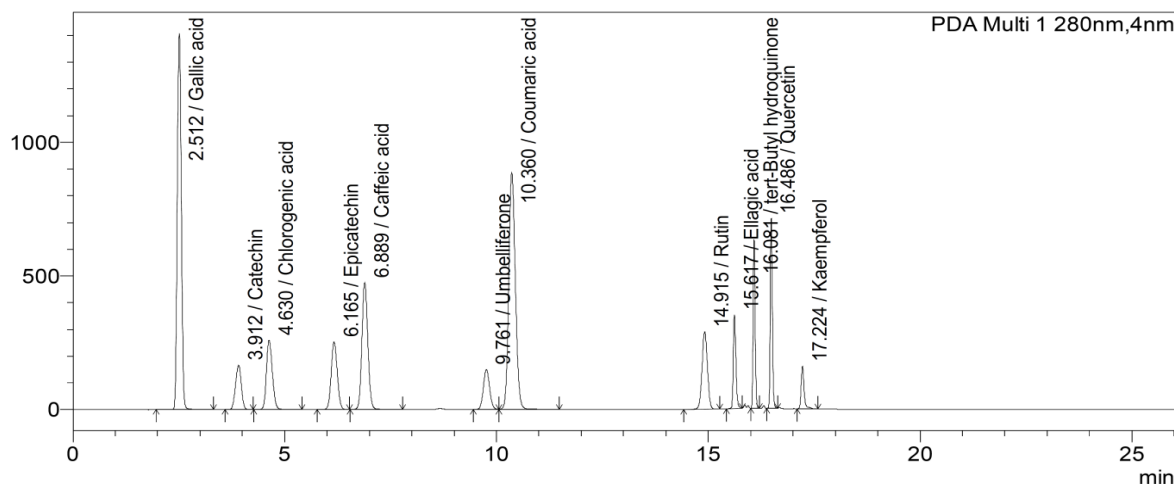


Figure 3: Chromatograms of a mixture of standards (gallic acid, catechin, chlorogenic acid, epicatechin, caffeic acid, umbelliferone, coumaric acid, rutin, ellagic acid, tert-Butyl hydroquinone, quercetin and kaempferol)

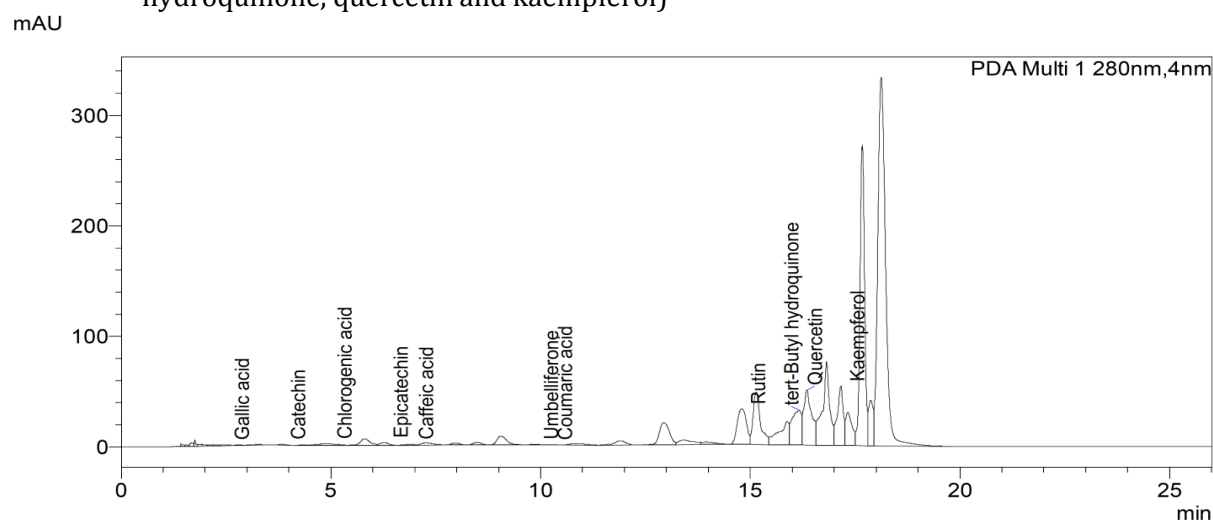


Figure 4: Chromatograms of hexane fraction (HF)

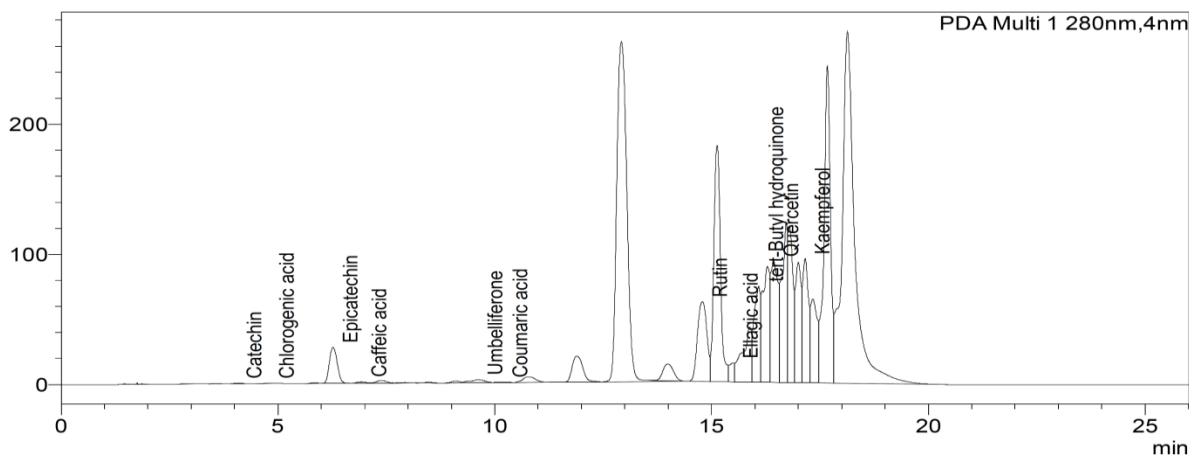


Figure 5: Chromatograms of chloroform fraction (CF)

Table 4: Quantitative estimation of polyphenols in hexane fraction (HF) of the leaves of *Murraya exotica* L.

Name	Ret. Time	Conc. ($\mu\text{g/ml}$)
Gallic acid	2.478	0.701
Catechin	3.829	3.732
Chlorogenic acid	4.933	5.265
Epicatechin	6.263	9.086
Caffeic acid	6.876	0.925
Umbelliferone	9.853	0.671
Coumaric acid	10.194	0.043
Rutin	14.801	127.282
tert-Butyl hydroquinone	16.164	77.666
Quercetin	16.350	93.099
Kaempferol	17.159	226.100

Table 5: Quantitative estimation of polyphenols in chloroform fraction (CF) of the leaves of *Murraya exotica* L.

Name	Ret. Time	Conc. ($\mu\text{g/ml}$)
Catechin	4.051	1.703
Chlorogenic acid	4.808	0.336
Epicatechin	6.270	91.237
Caffeic acid	6.926	1.036
Umbelliferone	9.608	10.957
Coumaric acid	10.182	0.163
Rutin	14.789	239.147
Ellagic acid	15.496	26.399
tert-Butyl hydroquinone	16.086	117.669
Quercetin	16.431	145.655
Kaempferol	17.160	373.604

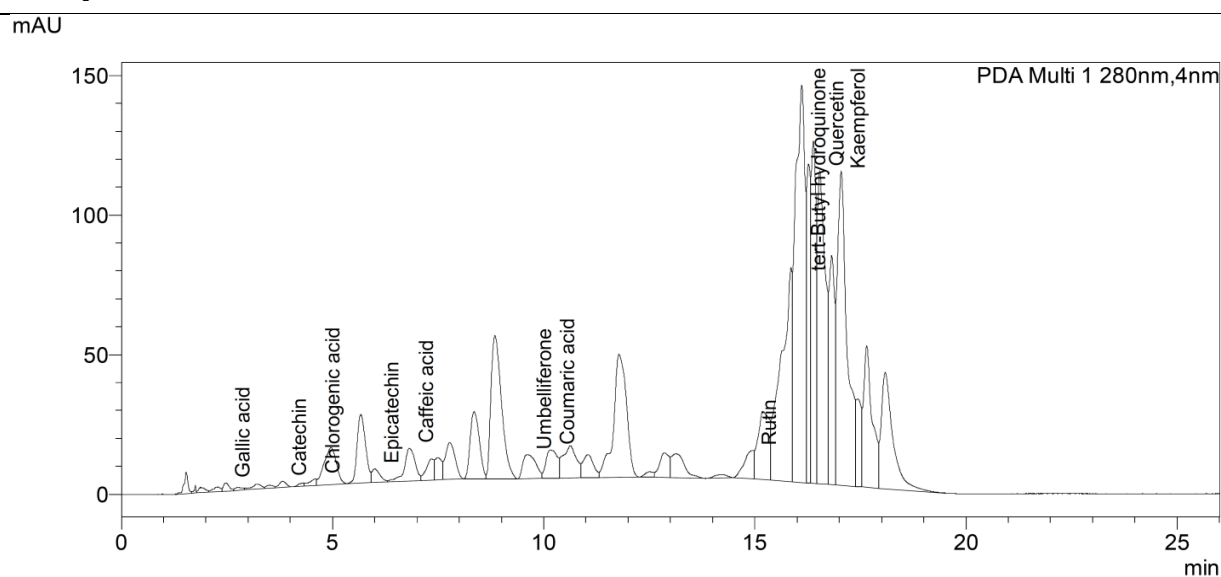
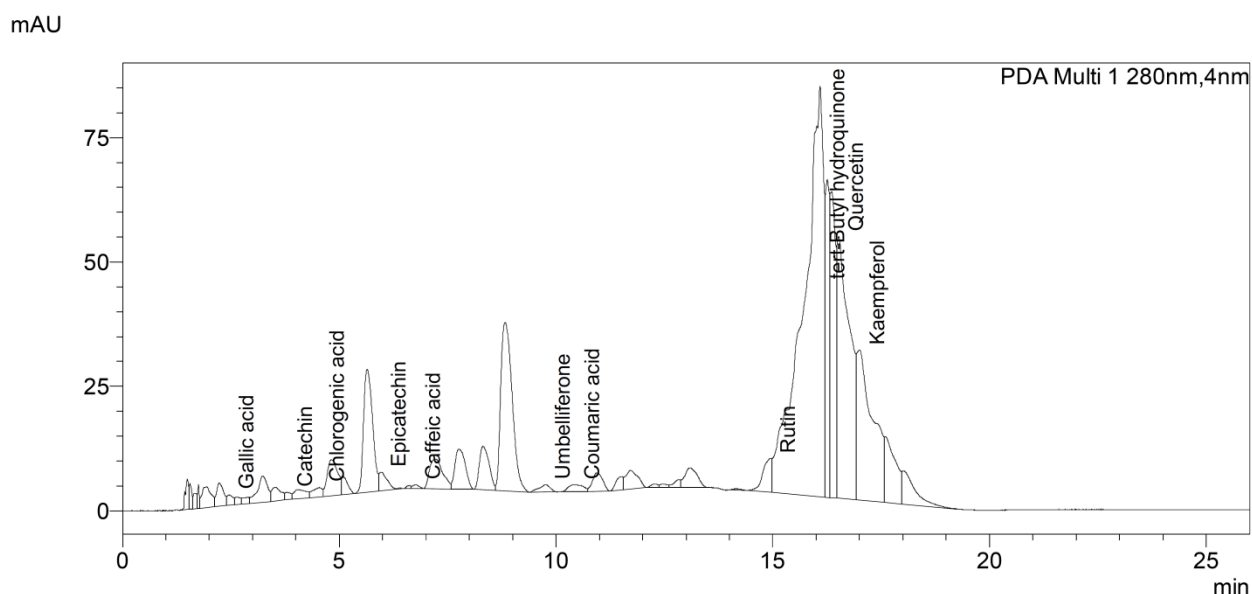
**Figure 6: Chromatograms of ethyl acetate fraction (EAF)**

Table 6: Quantitative estimation of polyphenols in ethyl acetate fraction (EAF) of the leaves of *Murraya exotica* L.

Name	Ret. Time	Conc. ($\mu\text{g/ml}$)
Gallic acid	2.470	1.985
Catechin	3.812	11.304
Chlorogenic acid	4.595	3.547
Epicatechin	6.000	16.653
Caffeic acid	6.818	14.935
Umbelliferone	9.609	39.755
Coumaric acid	10.158	6.431
Rutin	14.944	41.263
tert-Butyl hydroquinone	16.111	361.997
Quercetin	16.539	202.779
Kaempferol	17.045	789.390

**Figure 7: Chromatograms of n-Butanol fraction (BF)****Table 7: Quantitative estimation of polyphenols in n-Butanol fraction (BF) of the leaves of *Murraya exotica* L.**

Name	Ret. Time	Conc. ($\mu\text{g/ml}$)
Gallic acid	2.459	1.477
Catechin	3.801	5.816
Chlorogenic acid	4.536	3.781
Epicatechin	5.971	12.541
Caffeic acid	6.753	0.554
Umbelliferone	9.758	5.579
Coumaric acid	10.431	1.153
Rutin	14.944	24.726
tert-Butyl hydroquinone	16.091	429.567
Quercetin	16.521	149.395
Kaempferol	17.004	330.964

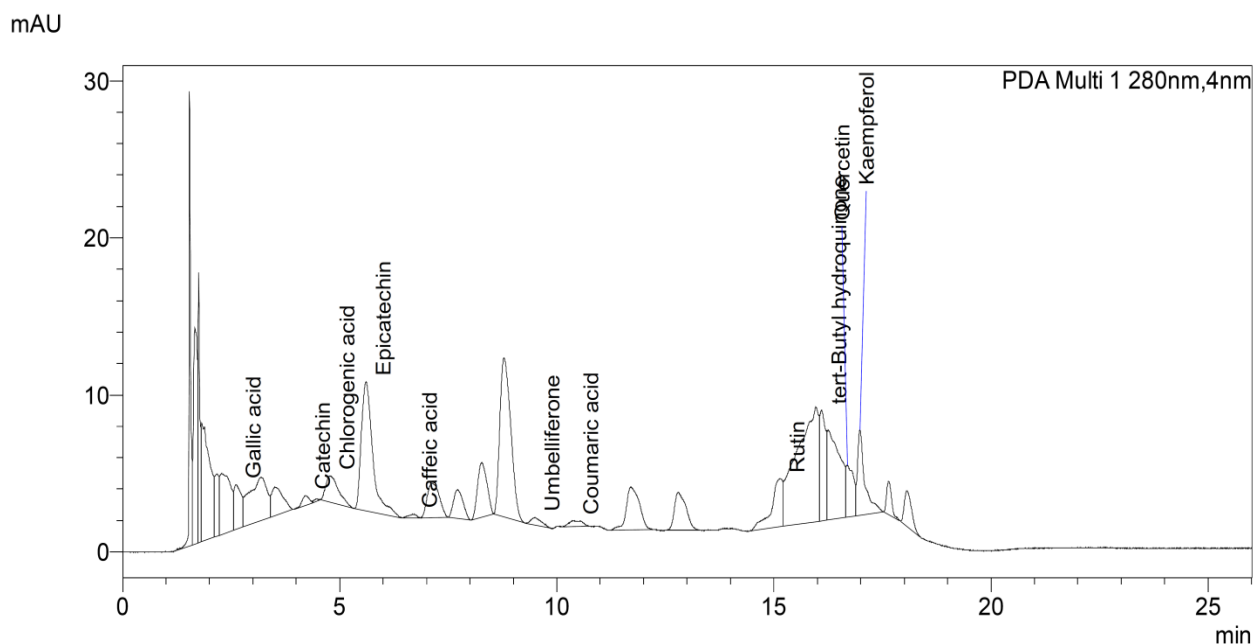


Figure 8: Chromatograms of water fraction (WF)

Table 8: Quantitative estimation of polyphenols in water fraction (WF) of the leaves of *Murraya exotica* L.

Name	Ret. Time	Conc. ($\mu\text{g/ml}$)
Gallic acid	2.612	2.335
Catechin	4.210	2.826
Chlorogenic acid	4.780	4.635
Epicatechin	5.606	39.398
Caffeic acid	6.675	0.232
Umbelliferone	9.490	1.734
Coumaric acid	10.383	0.269
Rutin	15.142	14.585
tert-Butyl hydroquinone	16.099	10.392
Quercetin	16.689	5.400
Kaempferol	16.981	27.271

Table 9: Effect of different concentrations of extract/fractions of the leaves of *Murraya exotica* L. in site specific deoxyribose degradation assay

Conc. ($\mu\text{g/ml}$)	Crude extract (CE)	% Hydroxyl scavenging activity					Water Fraction (WF)	Gallic acid
		Hexane Fraction (HF)	Chloroform Fraction (CF)	Ethyl acetate Fraction (EAF)	n-Butanol Fraction (BF)			
20	30.22±0.09	49.71±0.51	16.47±0.14	17.99±0.48	49.91±1.48	14.07±2.34	41.57±3.59	
40	33.71±0.64	52.22±0.61	21.41±0.65	20.68±1.16	51.49±0.53	16.73±1.93	53.02±3.30	
60	38.60±0.18	53.82±0.65	27.32±0.40	22.26±0.46	52.91±0.74	18.56±2.19	58.92±1.72	
80	45.14±0.74	54.88±0.11	30.89±0.32	23.79±0.02	54.05±0.99	20.63±0.84	61.50±0.50	
100	49.60±0.57	55.92±1.14	34.03±0.64	25.27±0.51	55.12±0.54	22.54±2.20	63.17±0.98	
120	53.41±0.02	56.61±0.21	36.57±1.26	26.65±0.87	55.97±0.52	24.34±1.83	65.72±0.91	
140	59.95±0.58	57.98±0.58	37.95±1.89	27.51±0.37	56.91±0.08	26.35±0.55	67.32±0.43	
160	62.71±0.00	58.59±0.41	39.93±0.75	28.78±0.18	57.78±0.16	29.31±1.57	69.70±0.90	
180	65.13±0.00	59.41±0.91	40.77±0.66	30.05±0.49	58.96±0.43	31.39±1.27	71.60±0.60	
200	68.32±0.00	60.52±0.73	41.98±0.12	31.43±0.32	58.87±0.78	33.44±0.49	73.60±1.16	

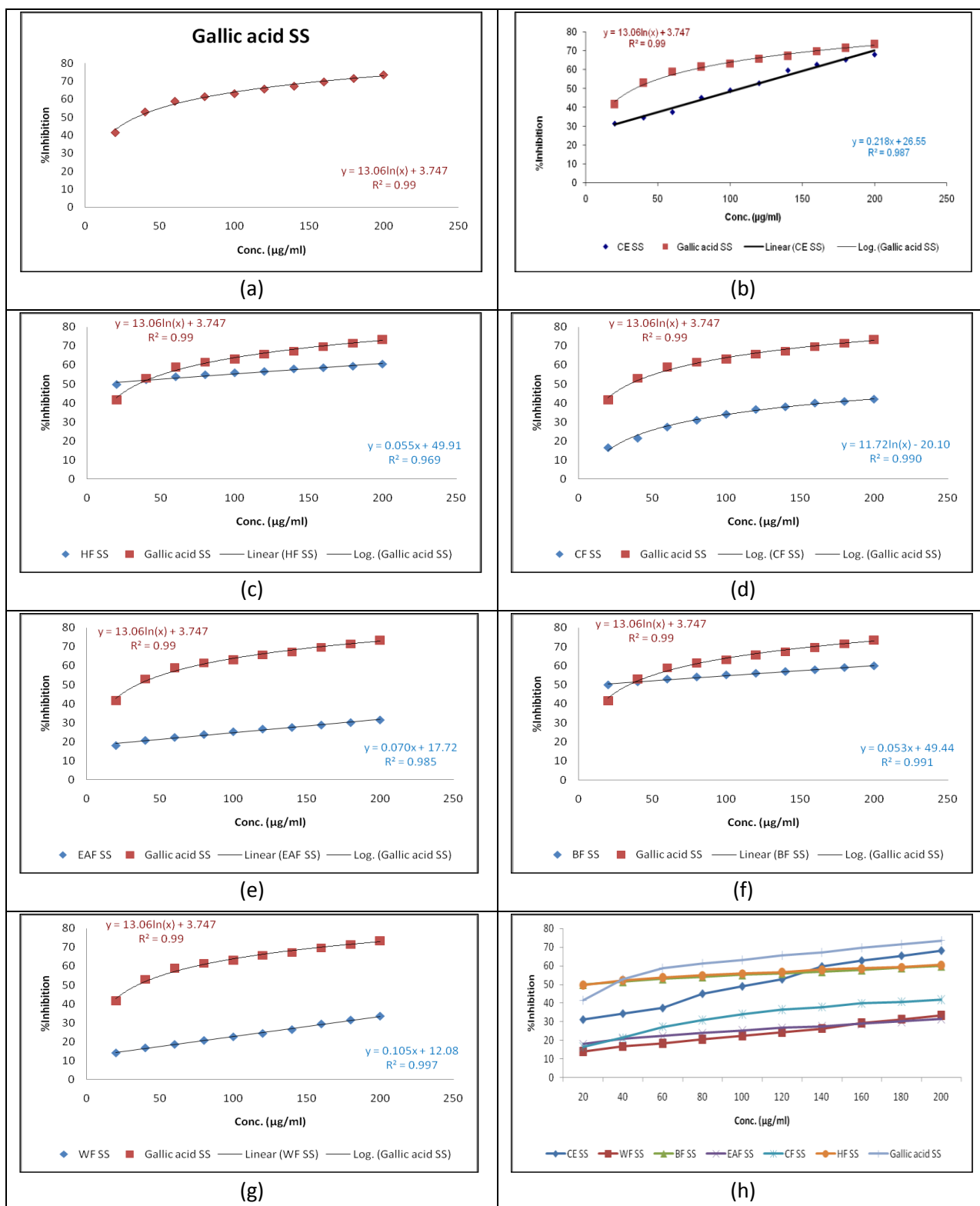


Figure 9: Regression curve of %inhibition in site specific deoxyribose degradation assay: Gallic acid (a); Gallic acid and CE (b); Gallic acid and HF (c); Gallic acid and CF (d); Gallic acid and EAF (e); Gallic acid and BF (f); Gallic acid and WF (g); Comparison of CE, HF, CF, EAF, BF, WF with Gallic acid (h)

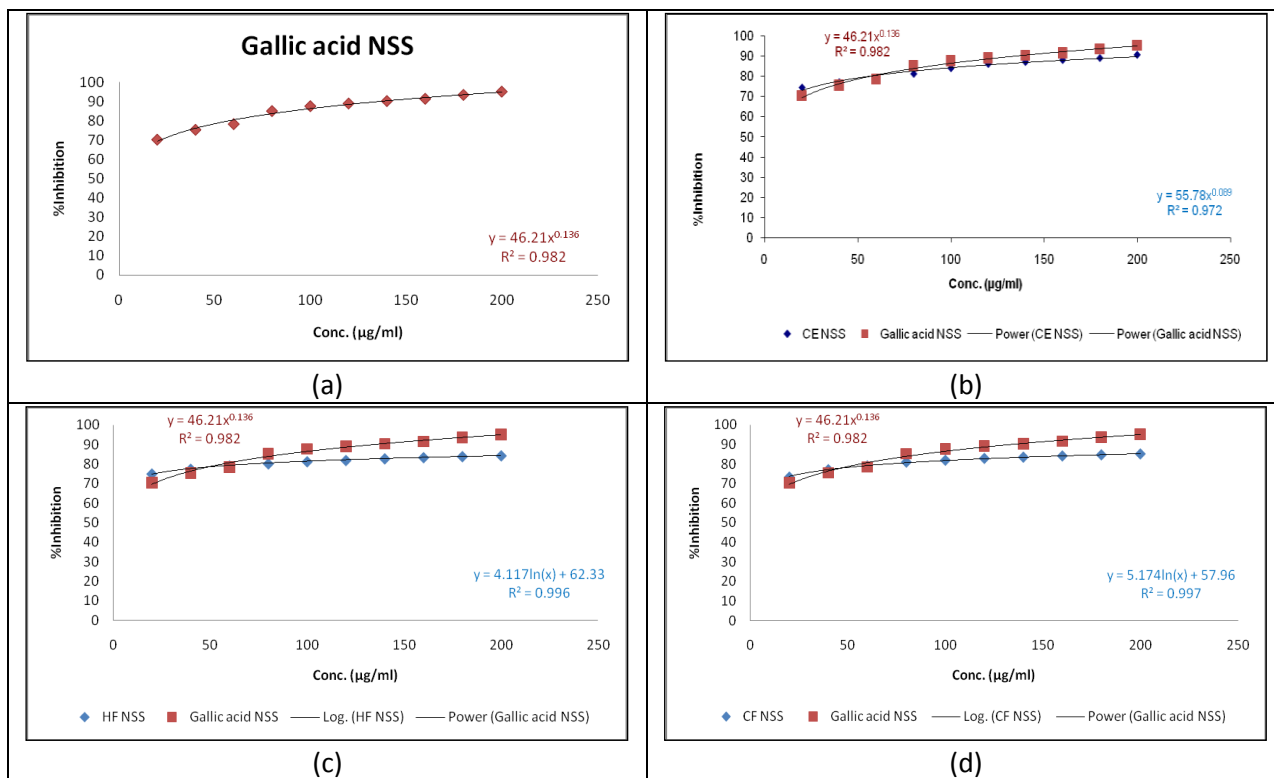
ii. Non-site specific assay:

The effect of different concentrations (%inhibition) of crude leaf extract/fractions of *Murraya exotica* L. and gallic acid in non site-specific deoxyribose degradation assay has been shown in (Table 10). The observed data revealed that at 200 µg/ml of concentration, gallic acid showed hydroxyl radical scavenging activity of 95.23%. Among the

extract/fractions, crude extract (CE) showed maximum hydroxyl radical scavenging activity i.e. 90.59% which was followed by water fraction (WF) with hydroxyl radical scavenging activity of 87.49%. Furthermore, the regression curve of % inhibition of gallic acid, crude extract (CE) and different fractions viz. HF, CF, EAF, BF, and WF at various concentrations are shown in (Fig 10).

Table 10: Effect of different concentrations of extract/fractions of the leaves of *Murraya exotica* L. in non site-specific deoxyribose degradation assay

Conc. (µg/ml)	% Hydroxyl scavenging activity						Gallic acid
	Crude extract (CE)	Hexane Fraction (HF)	Chloroform Fraction (CF)	Ethyl acetate Fraction (EAF)	n-Butanol Fraction (BF)	Water Fraction (WF)	
20	74.32±1.89	74.96±0.10	73.27±0.23	77.98±0.39	75.55±0.39	81.95±0.04	70.32±0.79
40	77.01±0.70	77.36±0.96	77.11±0.32	79.99±0.10	79.14±0.68	83.20±0.04	75.43±1.06
60	79.23±0.98	79.06±0.60	79.06±0.32	81.29±0.41	80.56±1.00	84.13±0.63	78.40±0.68
80	81.16±0.87	80.16±0.15	80.96±0.04	82.47±0.24	81.55±0.40	84.82±0.21	85.16±1.70
100	83.97±0.14	81.23±0.41	81.93±0.52	83.29±0.60	82.12±0.08	85.65±0.52	87.70±0.60
120	86.06±0.38	81.89±0.27	82.90±0.53	83.71±0.18	82.73±0.55	85.93±0.67	89.09±0.62
140	87.01±0.66	82.73±0.10	83.53±1.14	84.13±0.17	83.29±0.22	86.63±0.56	90.34±0.49
160	88.02±0.70	83.35±0.92	84.13±0.72	84.30±0.05	83.74±0.34	87.05±0.56	91.52±0.72
180	89.00±0.57	83.86±0.67	84.72±0.71	84.60±0.21	83.99±0.32	87.25±0.47	93.54±0.98
200	90.59±0.57	84.26±0.70	85.17±0.50	84.92±0.51	84.15±0.05	87.49±0.10	95.23±0.41



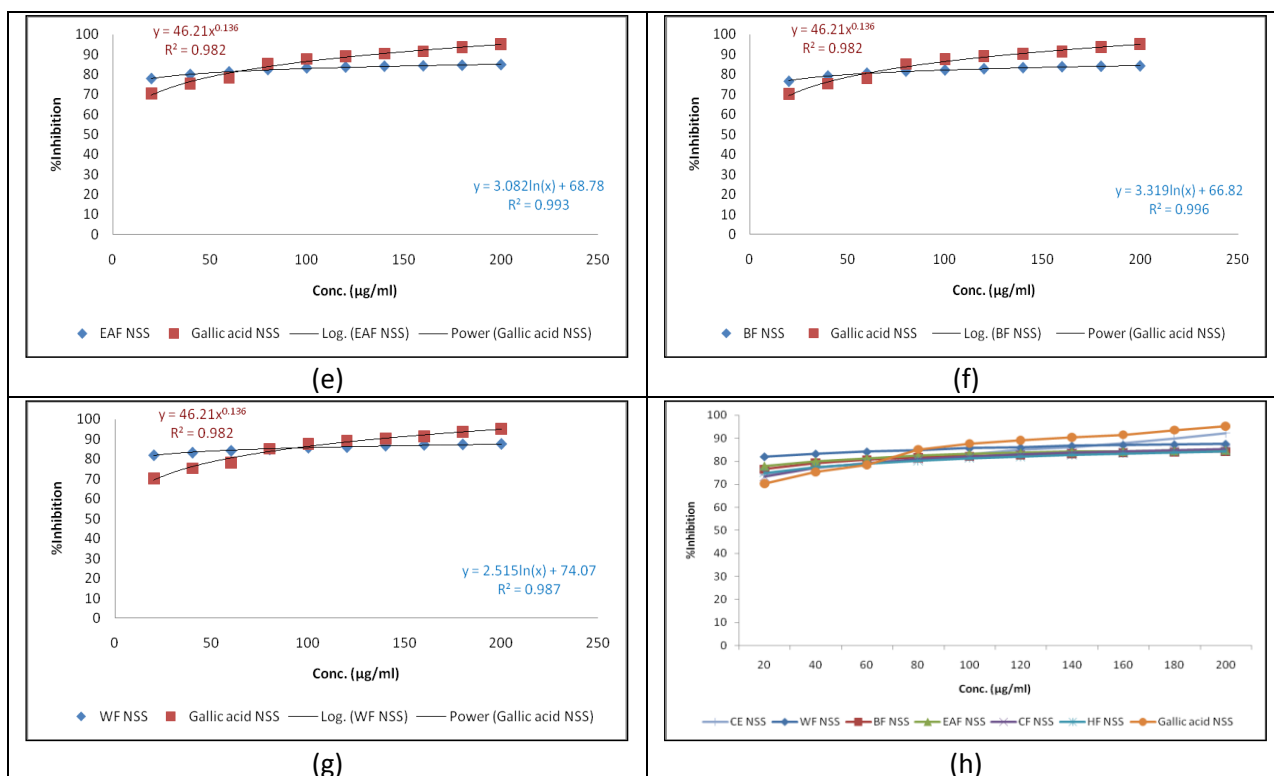


Figure 10: Regression curve of %inhibition in non-site specific deoxyribose degradation assay: Gallic acid (a); Gallic acid and CE (b); Gallic acid and HF (c); Gallic acid and CF (d); Gallic acid and EAF (e); Gallic acid and BF (f); Gallic acid and WF (g); Comparison of CE, HF, CF, EAF, BF, WF with Gallic acid (h)

a) Plasmid Nicking Assay

The hydroxyl radical scavenging activity of crude extract (CE) and its fractions viz. HF, CF, EAF, BF and WF was assessed at different concentrations. It was observed that the crude extract exhibited hydroxyl radical scavenging activity in a dose dependent manner, with maximum activity at 1000 µg/ml as shown in (Fig 11-12) and (Table 11). The observed figure revealed that there was a formation of single- stranded nicked and linear forms of plasmid DNA (forms II and III, respectively) due to hydroxyl radicals generated in Fenton’s reaction mixture.

The extract fractions viz. HF, CF, EAF, BF and WF also exhibited hydroxyl radical scavenging activity in a dose dependent manner, with maximum activity at 1000 µg/ml as shown in (Fig 13-14) and (Table 12). Furthermore, the results also revealed that ethyl acetate fraction at 200 µg/ml exhibited the least activity. The observed results revealed that with the addition of crude extract and its fractions to the reaction mixture, the hydroxyl radical- mediated strand breaking and conversion of supercoiled DNA form I to forms II and III, was reduced.

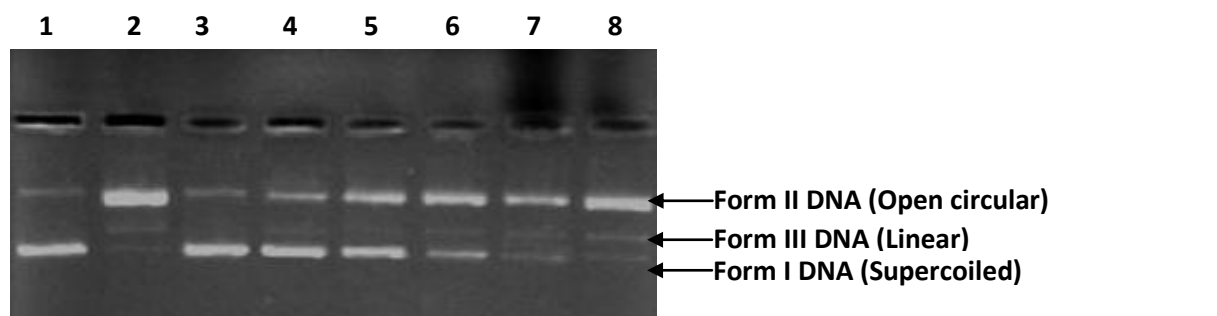
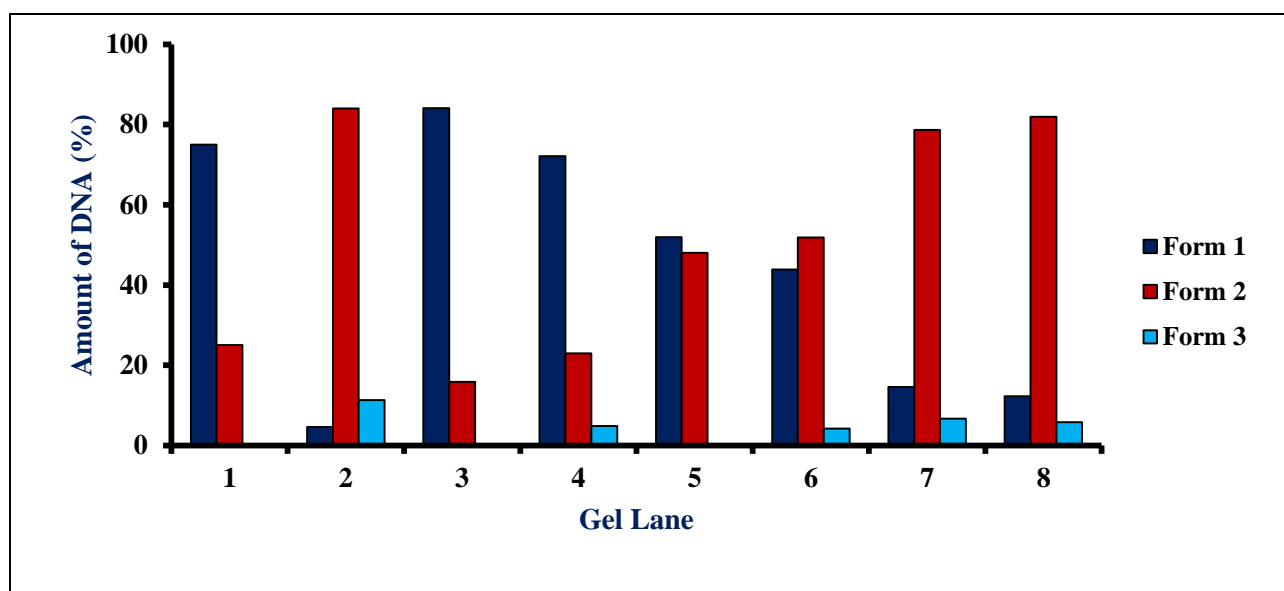


Figure 11: Gel lane showing the effect of different concentrations of crude extract of *Murraya exotica* L. in Plasmid Nicking assay

Lane 1: Negative Control; **Lane 2:** Fenton's Reagent; **Lane 3:** Positive Control (Rutin); **Lane 4-8:** Fenton's Reagent + different concentrations of Crude 80% MeOH extract (1000, 800, 600, 400, 200 µg/ml respectively)

Table 11: Densitometric studies of different forms of DNA after treatment with different concentrations of Crude 80% MeOH extract

Form of DNA	Amount of DNA (%)							
	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8
Form I	74.98	4.61	84.13	72.09	51.93	43.92	14.60	12.25
Form II	25.01	84.05	15.86	23.01	48.06	51.86	78.67	81.96
Form III	0	11.33	0	4.89	0	4.21	6.71	5.78

**Figure 12: Amount of Plasmid DNA (%) after treatment with different concentrations of Crude 80% MeOH extract of *Murraya exotica* L. in Plasmid Nicking assay****CONCLUSION**

The present study revealed the presence of many medicinally active constituents in *Murraya exotica* L. The above biological studies of the leaves extract/fractions of *Murraya exotica* L. suggested that there might be the presence of certain bioactive compounds and antioxidative enzymes in the leaves. The study also showed the presence of gallic acid, catechin, chlorogenic acid, epicatechin, caffeic acid, umbelliferone, coumaric acid, rutin, tert-Butyl hydroquinone, quercetin and kaempferol in all the fractions viz.; hexane fraction (HF), chloroform fraction (CF), ethyl acetate fraction (EAF), n-Butanol fraction (BF) and water fraction (WF); whereas ellagic acid was detected only in the chloroform (CF)

fraction, The gallic acid was found to be absent in the chloroform (CF) fraction. These bio-active compounds could be responsible for the observed strong hydroxyl radical scavenging potential. In the present study the extract/fractions performed better in non-site specific deoxyribose degradation assay, therefore it can be concluded that they are better hydroxyl radical scavengers than iron chelators. The high hydroxyl radical scavenging potential may be attributed due to the presence of alkaloids, flavonoids, phenols and tannins. The strong hydroxyl radical scavenging activity of the leaves revealed that they can be used in the treatment of various diseases caused by oxidative stress.

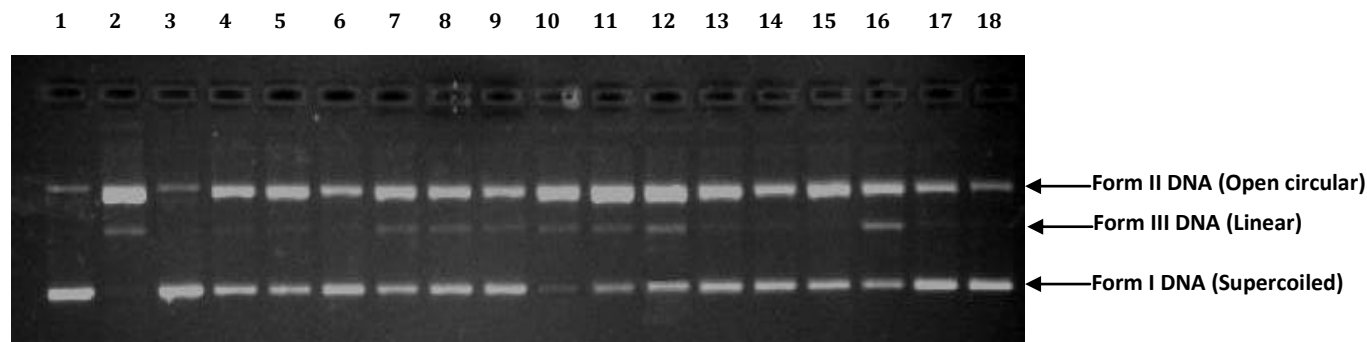


Figure 13: Gel lane showing the effect of different concentrations of fractions of crude extract in Plasmid Nicking assay

Lane 1: Negative Control; **Lane 2:** Fenton’s Reagent; **Lane 3:** Positive Control (Rutin); **Lane 4-6:** Fenton’s Reagent + different concentrations of Hexane fraction (200,600, 1000 µg/ml); **Lane 7-9:** Fenton’s Reagent + different concentrations of Chloroform fraction (200,600, 1000 µg/ml); **Lane 10-12:** Fenton’s Reagent + different concentrations of Ethyl acetate fraction (200,600, 1000 µg/ml); **Lane 13-15:** Fenton’s Reagent + different concentrations of n-Butanol fraction (200,600, 1000 µg/ml); **Lane 16-18:** Fenton’s Reagent + different concentrations of Water fraction (200,600, 1000 µg/ml)

Table 12: Densitometric studies of different forms of DNA after treatment with different fractions of Crude Extract

Form of DNA	Amount of DNA (%)																	
	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8	Lane 9	Lane 10	Lane 11	Lane 12	Lane 13	Lane 14	Lane 15	Lane 16	Lane 17	Lane 18
Form I	65.06	0	64.63	48.44	49.06	53.41	40.57	44.05	47.70	24.21	36.66	44.60	48.63	49.36	48.54	32.31	52.79	60.35
Form II	34.93	67.11	35.36	51.55	50.93	46.58	44.95	45.37	43.75	49.66	52.19	48.27	51.36	50.63	51.45	37.66	47.20	39.64
Form III	0	32.88	0	0	0	0	14.47	10.57	8.54	26.11	11.14	7.11	0	0	0	30.01	0	0

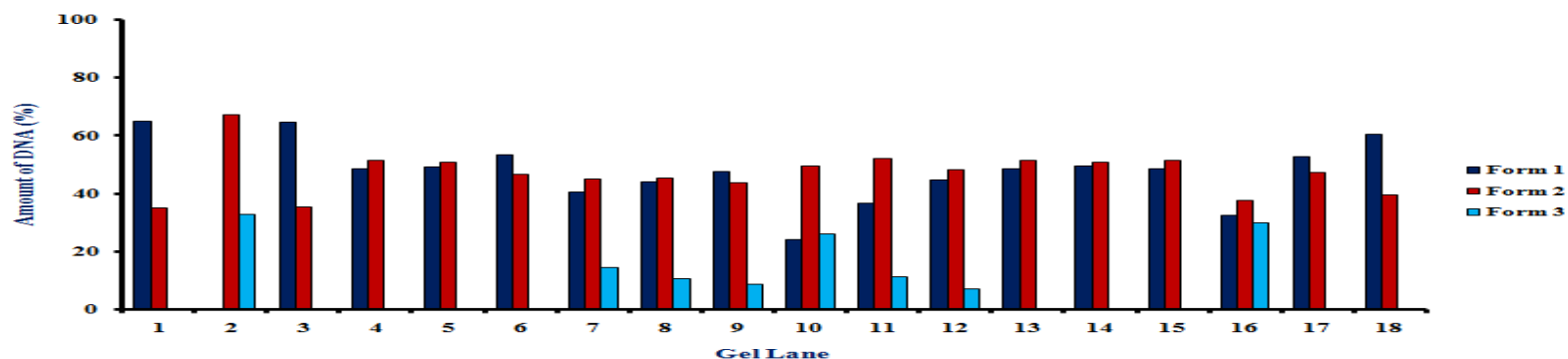


Figure 14: Amount of Plasmid DNA (%) after treatment with different fractions of Crude 80% Methanol extract of *Murraya exotica* L. in Plasmid Nicking assay

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