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Evaluation of Polyphenols, Flavonoids and Antioxidant Activity in different solvent extracts of Sesame (*Sesamum indicum* L.) Genotypes

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Abstract

Sesame (*Sesamum indicum* L) seeds of variety RT-46, RT-54, RT-103, RT-125, RT-127, RT-346 and RT-351 were used to evaluate total phenolics, total flavonoids and antioxidant activity as DPPH radical scavenging IC_{50} value. Water, methanol and hexane extract were used to analyze these activity. The highest total phenolics activity were recorded in water extract of sesame seeds ranging from 164-332 mg GAE $100g^{-1}$ seeds followed by methanol (68-168 mg GAE $100g^{-1}$ seeds) extract and lowest phenols were found in hexane extract (31-52 mg GAE $100g^{-1}$ seeds). Sesame variety RT-125 contained highest phenolics in water extract (332.49 mg GAE $100g^{-1}$). Highest total flavonoid content was recorded in methanol extract followed by hexane and water extract. Sesame variety RT-351 (31.45 QE g^{-1} seed) contained highest total flavonoid content in methanol extract. DPPH radical scavenging IC_{50} value was reported lowest in hexane extract of sesame genotypes showing the potential antioxidant in hexane extract. Variety RT-103 (4.58 mg ml^{-1}) contained lowest IC_{50} value in hexane extract.

Key words : Antioxidants, flavonoids, phenolics, phytochemicals, sesame.

Introduction

Sesame (*Sesamum indicum* L.) is a member of the family *Pedaliaceae.* It is one of the most ancient oilseed crops known to mankind (Akbar *et al.,* 2011). It is grown in tropical and subtropical areas throughout the world (Kun *et al.,* 2014). Sesame plays an important role in human nutrition. Most of the sesame seeds are used for oil extraction and the rest are used for edible purposes (El Khier *et al.,* 2008). Sesame is grown primarily for its oil-rich seeds. Before seeds were appreciated for their ability to add nutty flavour or garnish foods, they were primarily used for oil and wine (Gandhi, 2009). After the extraction of oil, the cake is mostly used for livestock feed or often as manure. Its colour varies from cream-white to charcoal-black but it is mainly white or black. Other colours of some sesame seed varieties include, yellow, red or brown (Shah, 2013).

Secondary metabolites or phytochemicals, naturally occurring in plants are biologically active and play important role in defense system of plants (Lako *et al.,* 2007). These phytochemicals have historically been used as pharmaceuticals, fragrances, flavor compounds, dyes, and agrochemicals (Rathore *et al.,* 2013). *In vitro* studies reported that phytochemicals such as phenolic compounds have potential role against different diseases and used as anti-inflammatory, anti-mutagenic, antiviral and antibacterial, agents (AL Juhaimi *et al.,* 2013; Senevirathne *et al.,* 2006). Sesame seed contains adequate amount of phytochemicals including phenolics, flavonoids alkaloids and tannins (Alege *et al.,* 2014). It is also rich in various bioactive compounds including phytosterols, tocopherols and lignin such as sesamin, sesamolin and sesaminol, which are known to play an important role in providing stability against oxidation of oil and contribute to antioxidative activity (Shahidi *et al.,* 1997; Philip *et al.,* 2010).

Keeping the above facts in view, the present study was undertaken to analyze polyphenols, flavonoids and antioxidant activity in different solvent extracts of sesame genotypes for their suitability in pharmaceutical use.

Materials and methods

Seven sesame genotypes growing in western Rajasthan; RT-46, RT-54, RT-103, RT-125, RT-127, RT-346 and RT-351 were collected from AICRP on Sesame and Niger center of Agricultural Research Station, Mandor for analysis of total phenolics, flavonoid and antioxidant activity in different solvents. The study was conducted at Sanitary and Phytosanitary Laboratory, Agricultural

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Research Station, Mandor, (Agriculture University Jodhpur).

Chemicals and Reagent

The chemicals used in this study were procured from Loba Chemi (India) and Sigma-Aldrich (USA).

Total Phenolics Content (TPC)

The total phenolic content was determined using the Folin-Ciocalteu assay according to procedure describe by Dewanto *et al.,* (2002) with slightly modification and the results were expressed as mg gallic acid $100gm^{-1}$ seed. An aliquot of 1 ml of the crude seed extract was mixed with 1 ml of the Folin-Ciocalteu reagent and 4 ml of a 20 % sodium carbonate solution. Distilled water was added to a final volume of 25 ml. Following incubation for 30 min, the absorbance of the reaction mixture was measured at 765 nm using Lab India make spectrophotometer against a blank. Gallic acid was used as the standard. The amount of total phenolic was calculated by using the standard curve of gallic acid drawn within a concentration range of $8.0x10^{-4}$ to $4.0x10^{-3}$ mg ml^{-1} having R^2 value 0.994 and was expressed as mg Gallic acid equivalents $100g^{-1}$ (mg GAE $100g^{-1}$) seed.

Total Flavonoid Content (TFC)

The total flavonoid content in methanol extract was determined using aluminium trichloride ($AlCl_3$), protocol described by Chang *et al*., (2002) with slight modification. Briefly, 2 ml of 2 % aluminium trichloride ($AlCl_3$) solution in methanol was mixed with 2 ml of a diluted stock solution (0.01 or 0.02 mg ml^{-1}). Absorption readings were taken at 415 nm (Lab India spectrophotometer) after 10 min against a methanol blank, Quercetin was used as the standard. The total flavonoid content was determined using a standard curve of Quercetin drawn within a concentration range of 4.0×10^{-3} to 2.0×10^{-2} mg ml^{-1} having R^2 value 0.996 and was expressed as mg Quercetin equivalents g^{-1} seed (mg QE g^{-1} seed).

Antioxidant activity DPPH Assay

There are several methods commonly used to determine the antioxidant activity of natural products, however 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical reagent was chosen in the present study as it is an easy, precise, and accurate method. 2, 2- diphenyl-1-picryl-hydrazyl DPPH is a free radical, and produces a violet solution in alcohol. It is reduced in the presence of an antioxidant molecule. Antioxidant activity of the methanol extract of sesame seed and standard were assessed on the basis of the radical scavenging effect of the stable 2, 2-diphenyl-1-picrylhydrazyl hydrate radical (DPPH). The diluted working solutions of the test samples were prepared in methanol. Gallic acid was used as the standard in solutions ranging from 5×10^{-4} to 4×10^{-3} mg ml^{-1}. 0.135 mM DPPH solution was prepared in methanol. Then 2 ml of this DPPH solution was mixed with 2 ml of sample solutions (ranging from 1 mg ml^{-1} to 10 mg ml^{-1}) and the standard solution was tested separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Lab India make spectrophotometer against methanol. 2 ml of methanol with 2 ml of DPPH solution was used as control. (Khan *et al*., 2017; Saxena *et al*., 2015; Shimada 1992) The optical density (O.D.) was recorded and percentage of inhibition (Figure 3-5) was calculated using the formula given below:

% of inhibition of DPPH activity =

$$\frac{\text{O.D. of the control Ɗ O.D. of the sample}}{\text{O.D. of the control}} \times 100$$

The IC_{50} values were calculated using linear regression of plots where the abscissa represented the concentration of the test solution and the ordinate was the percent of antioxidant activity.

Results and discussion

Total Phenolics Content (TPC)

The total phenolic content in water extract ranged from 164-332 mg GAE $100g^{-1}$ seeds. Highest phenolics were recorded in RT-125 (332.49 mg GAE) followed by RT- 127 (317.16 mg GAE) while lowest total phenolics was found in RT-54 (164.98 mg GAE) (Table-1).

In methanol extract of sesame genotypes total phenolics content ranged from 68-168 mg GAE $100g^{-1}$ seeds. Highest phenolics were recorded in RT-125 (168.40mg GAE) while variety RT-54 (68.06 mg GAE) found lowest phenols content in methanol extract. Hexane extract was found low in phenolics content ranging from 31-52 mg GAE $100g^{-1}$ seeds (Table-1). The comparative analysis of total phenolics in different extracts is presented in fig.-1.

Bopitiya and Madhujith (2013) reported 26 mg GAE g^{-1} total phenolic content in sesame oil methanolic extracts and conclude that sesame oil extract contained higher phenolics compared to other commonly available vegetable oils. Zhou *et al.,* (2016) reported total phenolic contents in black sesame varieties that ranged from 4.54 to 7.32mg/GAE/kg. Nadeem *et al.,* (2004) reported the total phenolics extracted from sesame cake were 1.72mg/GAE/kg. Shahidi *et al.,* (2006) reported that the total phenolic contents of two sesame seed cultivars (black and white) were 29.9 ± 0.6 and 10.6 ± 1.6mg catechin equivalents/kg crude ethanolic extract. Lin *et al.,* (2017) analyzed total phenolics content in six varieties of white sesame from china and reported 370.5-786.8 mg

Table 1. Total Phenolics Content in sesame genotypes expressed as mg GAE 100 g^{-1} seed

S. No.	Variety	Total Phenolics Content mg GAE 100g^{-1} seed		
		Water Extract	Methanol Extract	Hexane Extract
1.	RT-46	214.66 ± 0.28	140.61 ± 0.26	42.98 ±0.09
2.	RT-54	164.98 ± 0.31	68.06 ± 0.15	44.50 ±0.07
3.	RT-103	304.76 ± 0.24	165.56 ± 0.43	47.43 ±0.07
4.	RT-125	332.49 ± 0.46	168.40 ± 0.42	31.13 ±0.04
5.	RT-127	317.16 ± 0.11	109.50 ± 0.20	46.39 ±0.23
6.	RT-346	230.56 ± 0.39	129.89 ± 0.33	52.46 ±0.07
7.	RT-351	301.91 ± 0.32	68.37 ± 0.11	63.82 ±0.11

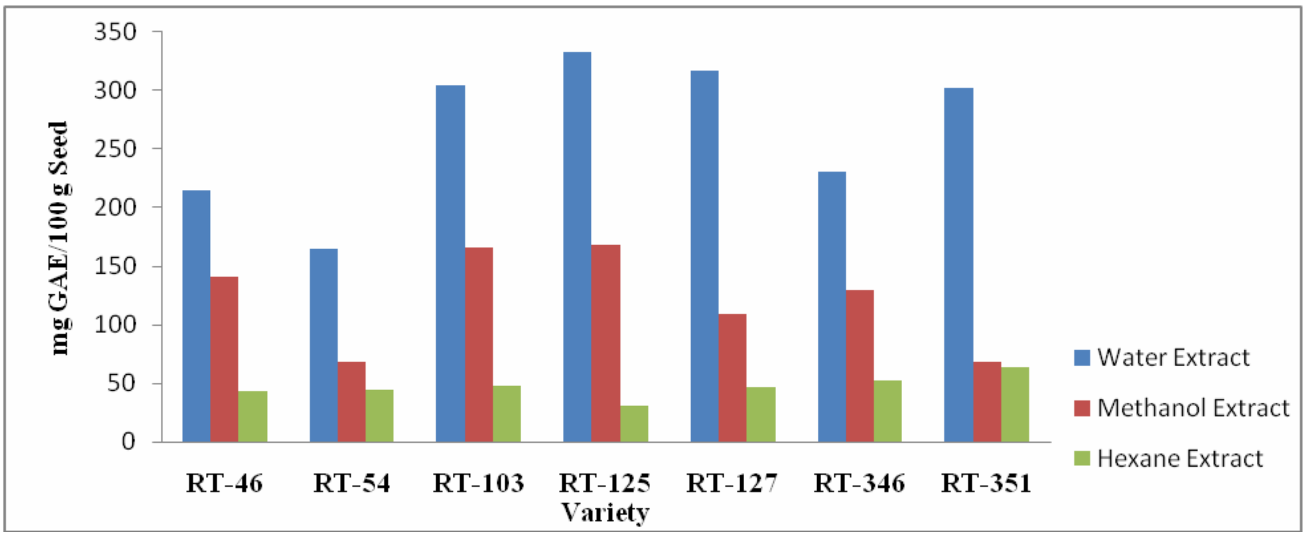

Fig. 1. Total Phenolics Content in different solvent extracts of sesame genotypes

GAE 100g^{-1} phenolics. Nigam *et al*., (2015) reported 19.48 mg/g total phenolics in methanolic extract of sesame.

Total Flavonoid Content (TFC)

The total flavonoid content in water extract ranged from 0.84-6.28 mg QE g^{-1} seed. Highest Flavonoid was found in RT-127 (6.28 mg QE g^{-1}) followed by RT-346 (6.22 mg QE g^{-1}), while RT-54 contained lowest flavonoid (0.84 mg QE g^{-1}) in water extract (Table-2). In methanolic extract of sesame genotypes total flavonoid content ranged from 22-31 mg QE/g seed. Sesame variety RT-351 (31.45 mg QE g^{-1}) contained highest flavonoid while variety RT-127 (22.60 mg QE g^{-1}) contained lowest flavonoid among seven genotypes methanolic extract. Hexane extract of seven sesame genotypes were also rich in flavonoid content ranging from 22-28 mg QE g^{-1} seed. Sesame variety RT-351 (28.43 mg QE g^{-1}) contained highest flavonoid followed by RT-103 (27.15 mg QE g^{-1}), RT-127 (25.65 mg QE g^{-1}), RT-346 (24.47 mg QE g^{-1}), RT-125 (23.83 mg QE g^{-1}), RT-46 (23.48 mg QE g^{-1}) and lowest flavonoid was reported in RT-54 (22.28 mg QE g^{-1}). The comparative analysis of total flavonoids in different extracts is presented in fig.-2.

Zhou *et al.,* (2016) reported total flavonoid contents that ranged from 5.80 to 8.04 g CE/kg among six varieties of sesame from China. No significant differences of the flavonoid contents were observed between black and white sesame varieties. Lin *et al.,* (2017) reported 714.0-1354.7 mg CE 100g^{-1} total flavonoid contents of in six varieties of white sesame from China.

Antioxidant activity (DPPH Assay)

The determination of the antioxidant activity of sesame seed extracts was based on the DPPH radical scavenging activity through the IC_{50} parameter, which represents the concentration of the material necessary to inhibit 50% of free radicals. Thus, a lower IC_{50} value shows a superior ability to neutralize free radicals and potential antioxidant content.

Table 2. Flavonoid Content in sesame genotypes expressed as mg QE g^{-1} seed

S. No.	Variety	Total Flavonoid Content mg QE g^{-1} seed		
		Water Extract	Methanol Extract	Hexane Extract
1.	RT-46	3.25 ± 0.66	29.50 ± 0.65	23.48 ± 0.96
2.	RT-54	0.84 ± 0.12	27.86 ± 0.63	22.28 ± 1.39
3.	RT-103	1.96 ± 0.24	29.58 ± 1.36	27.15 ± 0.81
4.	RT-125	2.54 ± 0.21	26.81 ± 0.68	23.83 ± 1.71
5.	RT-127	6.28 ± 0.44	22.60 ± 0.51	25.65 ± 0.68
6.	RT-346	6.22± 0.30	27.53 ± 0.95	24.47 ± 1.11
7.	RT-351	4.95 ± 0.45	31.45 ± 1.37	28.43 ± 1.14

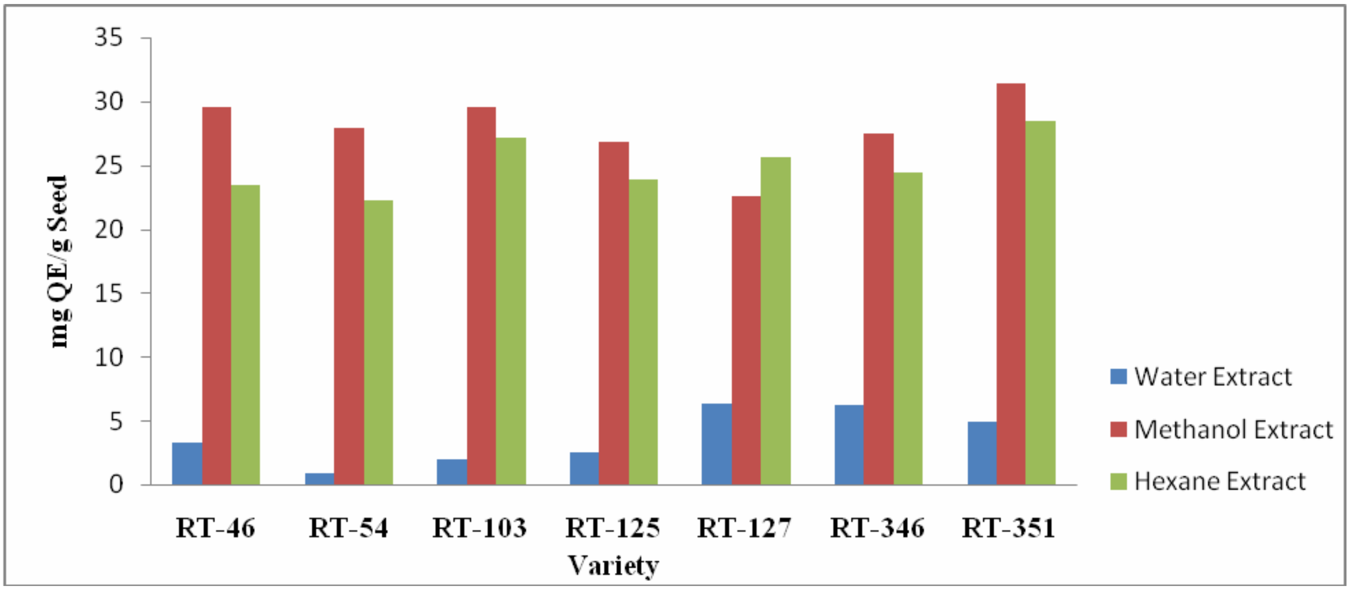

Fig. 2. Total Flavonoid Content in different solvent extracts of sesame genotypes

The DPPH scavenging percent ability of water, methanol and hexane extract of sesame is presented in (fig. 3, 4 & 5) Table-3 and IC_{50} of DPPH radical are shown in Table-4. IC_{50} values of water extract ranged from 4.88-8.81 mg ml^{-1}. RT-127 having lowest IC_{50} value among seven genotypes of sesame in water extracts. In methanolic extract variety RT-103 (5.53 mg ml^{-1}) recorded lowest IC_{50} value among other genotypes (Table-4). In hexane extract RT-103 (4.58 mg ml^{-1}) also recorded lowest IC_{50} value.

Study results reveal that lowest IC_{50} value in sesame genotypes were recorded in hexane extract followed by methanol extract, while water extract showed higher IC_{50} value among other extract. In sesame oil Bopitiya and Madhujith (2013) reported 0.026 mg ml^{-1} DPPH radical scavenging activity. Aleksander *et al.,* (2008) reported that the IC_{50} value of selected vegetable oil extracts, namely soy, sunflower, rapeseed, corn ranged from 29.7 to 34.0 µg ml^{-1}. Current study result reveals that western Rajasthan growing sesame genotypes contains adequate amount of antioxidants.

Conclusion

Current study reveals that sesame genotypes contains adequate amount of secondary metabolites. Highest total phenolics were obtained in aqueous extract while higher total flavonoid was recorded in methanolic extract. Hexane extracts were potential antioxidant showing lower IC_{50} value among three extract. Western Rajasthan growing genotypes of sesame contained rich amount of polyphenols, flavonoids and antioxidants and an immense genetic variability among seven genotypes in all solvent extracts were observed. The information provided by this research will help breeders in future breeding programmes to develop improved varieties of sesame.

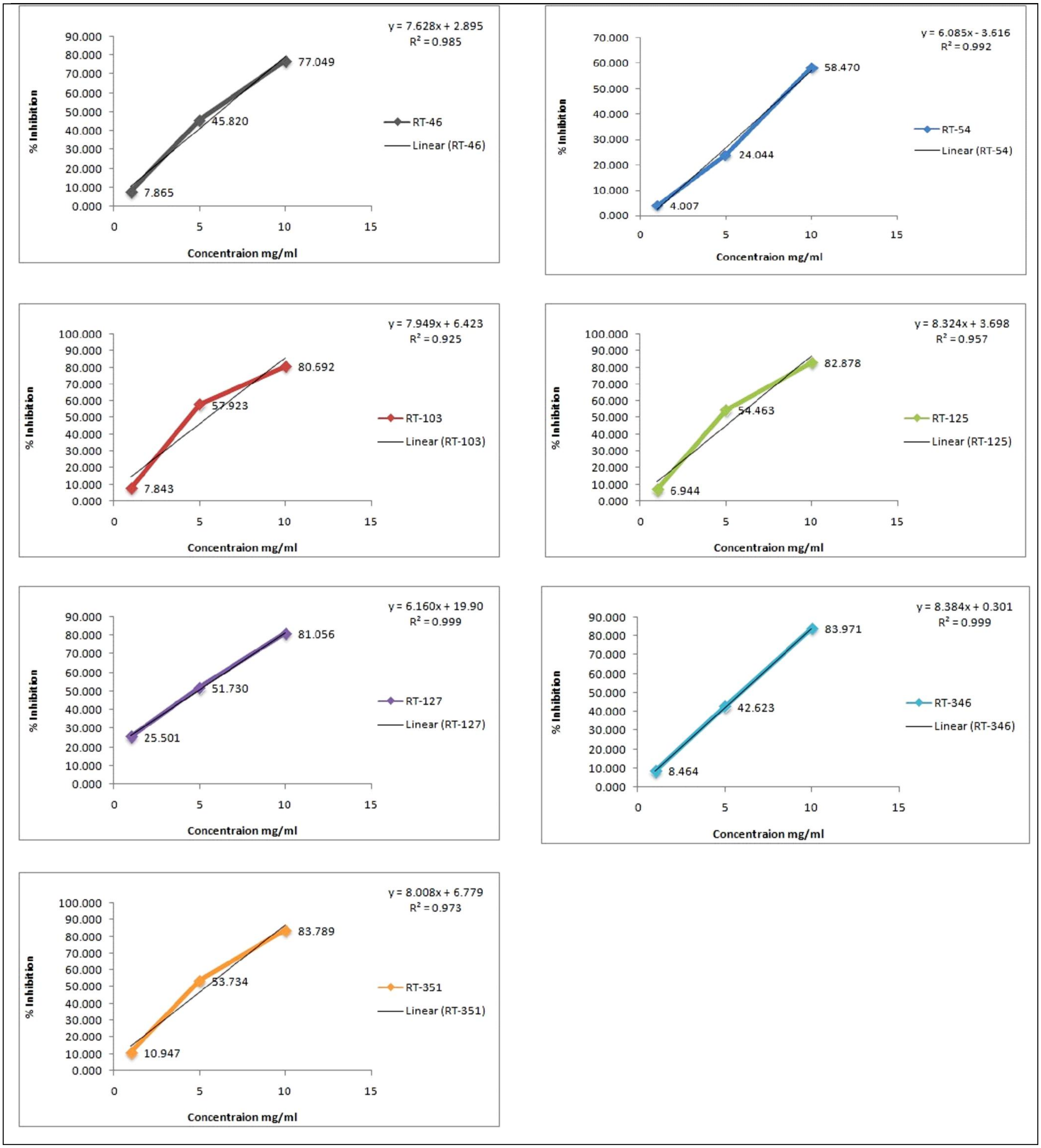

Fig. 3. DPPH radical scavenging activity in water extract of sesame genotypes

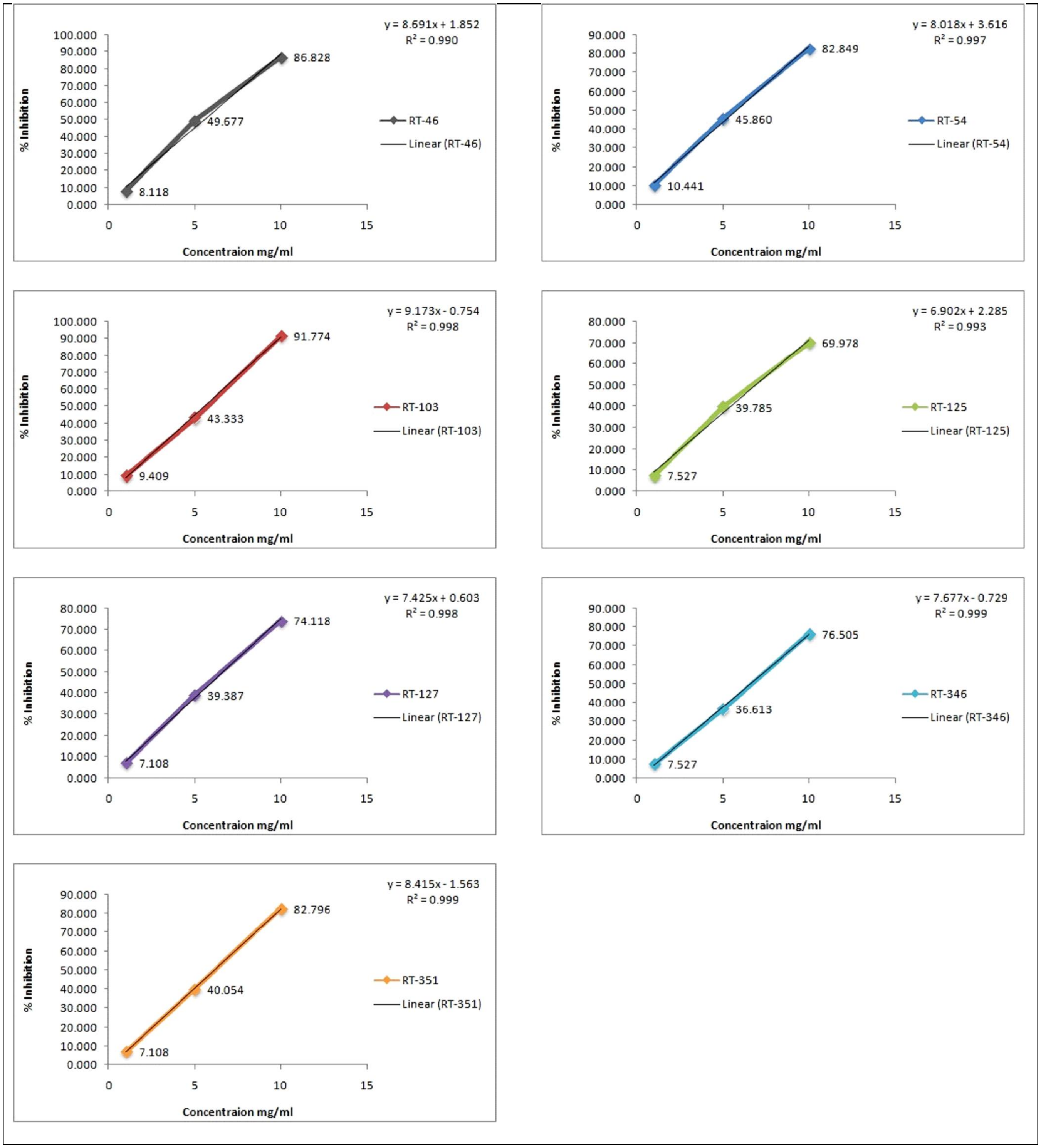

Fig. 4. DPPH radical scavenging activity in Methanol extract of sesame genotypes

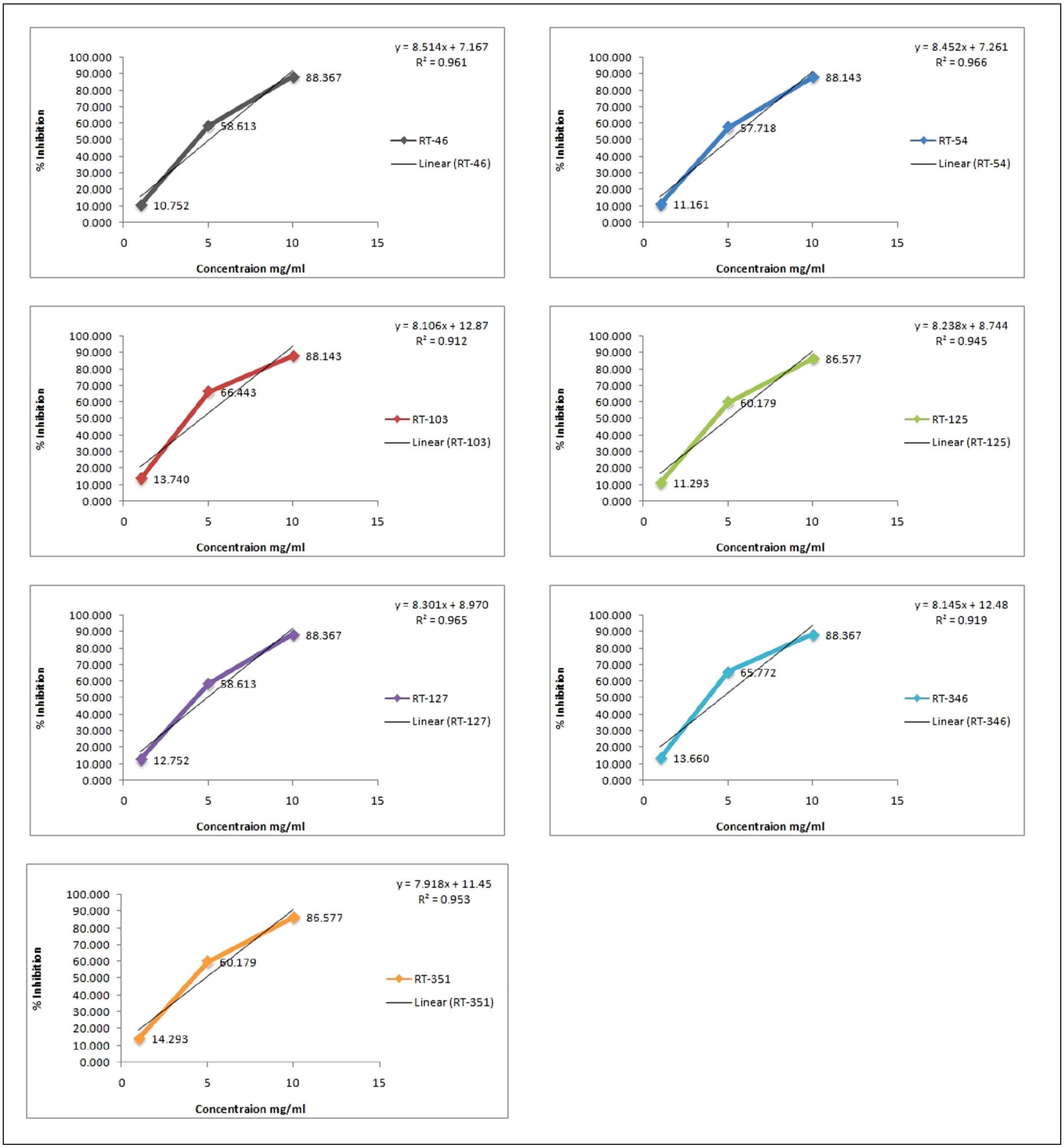

Fig. 5. DPPH radical scavenging activity in Hexane extract of sesame genotypes.

Table 3. DPPH radical scavenging % in different extract of sesame genotypes

S. No.	Variety	DPPH radical scavenging %								
		Water Extract (mg ml^{-1})			Methanol Extract (mg ml^{-1})			Hexane Extract (mg ml^{-1})		
		1.0	5.0	10.0	1.0	5.0	10.0	1.0	5.0	10.0
1.	RT-46	7.87	45.82	77.05	8.12	49.68	86.83	10.75	58.61	88.37
2.	RT-54	4.01	24.04	58.47	10.44	45.86	82.85	11.16	57.72	88.14
3.	RT-103	7.84	57.92	80.69	9.41	43.33	91.77	13.74	66.44	88.14
4.	RT-125	6.94	54.46	82.88	7.53	39.79	69.98	11.29	60.18	86.58
5.	RT-127	25.50	51.73	81.06	7.11	39.39	74.12	12.75	58.61	88.37
6.	RT-346	8.46	42.62	83.97	7.53	36.61	76.51	13.66	65.77	88.37
7.	RT-351	10.95	53.734	83.79	7.108	40.05	82.79	14.29	60.18	86.58

Table 4. Antioxidant Activity IC_{50} Value of sesame genotypes

S. No.	Variety	Antioxidant Activity IC_{50} Value mg ml^{-1}		
		Water Extract	Methanol Extract	Hexane Extract
1.	RT-46	6.18	5.54	5.03
2.	RT-54	8.81	5.78	5.06
3.	RT-103	5.48	5.53	4.58
4.	RT-125	5.56	6.91	5.01
5.	RT-127	4.88	6.65	4.92
6.	RT-346	5.93	6.61	4.61
7.	RT-351	5.39	6.13	4.87

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