

Evaluation of *in Vitro* Antioxidant Potential of *Carissa Carandas* Genotype found in NE India

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ABSTRACT

The present work was designed to study the antioxidant potential of various parts of *Carrisa carandas* using different assay systems. *C. carandas* is a semi-wild shrubby plant species found in Assam (India). The species is being used in various traditional medicinal practices in Assam and other parts of NE India. The methanol extract of the fruits of *C. carandas* showed highest radical scavenging activity against DPPH (1, 1-diphenyl-2-picryl hydrazyl). Ethanol extract of the fruits showed highest radical scavenging activity against H₂O₂. The study revealed that the fruits, leaves and seeds extract of *C. carandas* possesses potent antioxidant activity. The antioxidant potential was determined to be in the following order: Fruit > Leaf > Seed extracts.

Keywords: *Carissa Carandas*, Antioxidant, Radical Scavenging, DPPH, Reducing Power.

I. INTRODUCTION

Plants are well known source of biologically active natural products with potent therapeutic value. Phytoconstituents such as carotenoids, tocopherols and polyphenols possess considerable antioxidant capacity. It is known that reactive oxygen species (ROS) play a crucial role in the development of various ailments such as arthritis, asthma, dementia, carcinoma and several others. Moreover, the excess production of active oxygen species like O₂, H₂O₂ and OH⁻ may lead to tissue injury, DNA damage, thiol oxidation and lipid peroxidation, cardiac disorder, chronic gut inflammation, cancer and AIDS in humans [1]. However, free radicals play a positive role in phagocytosis, energy production and regulation of cell growth. But free radicals produced in the body react with various biological molecules namely lipids, proteins and deoxyribonucleic acids resulting in oxidative stress [1], [2], [3], [4]. Oxidative stress is commonly defined as a disturbance in the prooxidant and antioxidant balance. Although human body is

protected by natural antioxidant defense, there is always a demand for antioxidants from plant sources. Therefore, identification of antioxidant rich plant resources and evaluation of their antioxidant potential is considered as a high priority area of research.

C. carandas L. is a 3-5 m tall evergreen shrub or climbing tree with strong thorns in pairs (Figure 1). The species has been found in semi-wild situation in home gardens in several places of Assam and other parts of North East India [5]. "Semi-wild" applies to those plants that are indigenous and naturalized to the region while nurtured also through maintenance or acceptance by people in their crop fields and home gardens [6]. Stem is fresh green, cylindrical in shape, smooth on surface, 4-5 cm internodes. The branches are set with sharp simple or forked thorns in pairs in the axils of the leaves, 2-4 cm long and are usually alternate, numerous and spreading, forming dense masses; branches are usually without spines. Bark is smooth light grey or yellowish brown [7]. Leaves are simple, evergreen, opposite [8]. Flowers are white,

often tinged with pale-pink coloured, found in groups, scarcely odorous. Fruits have fairly thin but tough peel. Peel and pulp are usually green and pinkish white when immature but turns purplish-red to dark-purple and shiny when ripened depending on the genotype. Ripe fruits are sweet and edible. Unripe ones are sour and pickled. Various parts of *C. carandas* have been used in many ethno-medicines as stated in several published work [9], [10], [11], [12], [13]. Roots are used as stomachic and anthelmintic in traditional medicinal practices of Assam. Decoction of leaves is given in the treatment of remittent fever [5]. However scientific validation of the medicinal information for the *C. carandas* genotype found in Assam is obscure. Considering the medicinal importance, the objectives of this study was designed to evaluate the antioxidant potential of various parts viz., fruit, leaf and seed of *Carissa carandas* using DPPH (1,1-diphenyl-2-picrylhydrazyl) and hydrogen peroxide (H₂O₂) radical scavenging methods.

II. METHODS AND MATERIAL

Plant samples were collected from a semi-wild population of *C. carandas* found in a local Garden in Nagaon district of Assam, India. The fresh leaves, fruits and seeds were gathered during the month of June to August, 2018. The specimen was identified in the Department of Botany, Gauhati University, Assam, India. All the chemicals and solvents were of analytical grade obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai. A UV-visible spectrophotometer (Thermo Fischer scientific) was used for recording the spectra.

The collected samples were cleaned and washed thoroughly using tap water followed by distilled water. The ripened fruits were sliced into small pieces to increase the surface area; seeds were removed and then dried under shade for 7 days. Dried samples were then ground to produce fine homogenous powder using a grinder. The powdered samples were

preserved in clean plastic containers away from light, heat and moisture until use. For preparation of cold extracts, 10g of each powdered plant sample (leaf, fruit and seed) soaked in 100mL of selected solvents viz., Ethanol, Acetone, Methanol, Ethyl Acetate, Diethyl ether, petroleum ether, Chloroform and Distilled water at room temperature. Each sample was filtered using Whatman no.1 filter paper and the filtered solutions were evaporated to dryness using water bath at 40°C for overnight. The plant extracts were then stored at 4°C for further analysis. Chemical analysis was done to estimate antioxidant activity of the samples.

The antioxidant activity of 8 different extracts (methanol, ethanol, acetone, chloroform, petroleum ether, distilled water, ethyl acetate and diethyl ether) of *C. carandas* plant material was assayed. The extracts were tested for their radical scavenging ability against DPPH as per method described by Blois [14]. The scavenging activity of the extracts on hydroxyl radical was measured according to a method earlier described by Ruch [15] and Yu *et al.* [16]. Reducing Power Assay was carried out as per the method described by Yildrin [17].



Figure 1: A fruiting twig of *Carissa carandas* found in Assam (India)

DPPH Scavenging Assay

Stock solutions of all the extracts of different plant parts with the concentration of 1000µg/ml were prepared by dissolving 20mg of the extract separately in eight number of different solvents (Ethanol, Acetone, Methanol, Ethyl Acetate, Diethyl ether, petroleum ether, Chloroform and Distilled water) and the final volume was made up to 20ml. The stock was diluted to various concentrations like 10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml and 160µg/ml solutions with each solvent earlier used for extraction. The total volume was made 1ml in all the test tubes and to this 2ml of solvent solution of DPPH was added thereby making the final volume to 3ml. The test tubes were incubated at 37°C for 30minutes. Optical densities of these samples were measured for degree of discoloration from purple to yellow at 517 nm along with blank. Each of the above mentioned eight solvents with respective plant extracts served as blank. Ascorbic acid was used as a standard. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

The DPPH radical scavenging activity of the plant extracts were calculated by the following equation:

$$\% \text{ inhibition} = [A_0 - A_s] / A_0 \times 100$$

Where,

A_0 = Absorbance of DPPH (unreduced)

A_s = Absorbance of the sample and standard

The percentage scavenging activity of each extract was compared with L-Ascorbic acid, the positive control. IC50 is defined as the concentration of antioxidant that causes 50% loss of the initial DPPH free radical concentration (color). IC50 of reference antioxidant compound i.e. ascorbic acid was used for comparison to IC50 of the extracts. The least value of IC50 represents the better antioxidant and high value for antioxidant activity. IC50 value of each extract was determined from the plotted graph of percentage DPPH neutralization versus concentration of extract.

Statistical Analysis

The data were subjected to statistical analysis by Origin ver. 6.1. All the assays were recorded in triplicates and the values were expressed as mean \pm S.D. Each measurements were significantly different at $p < 0.05$.

Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM pH 7.4). Different concentration of the plant extracts (10 µg/ml to 160 µg/ml) was added to 600 µl of H₂O₂ (0.6 ml, 40 mM) and the final volume was made 3ml with distilled water. The concentration of hydrogen peroxide was determined by absorption at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was taken as standard. The percentage of hydrogen peroxide scavenging of both the extracts and standard compounds was calculated as in the case of DPPH.

$$\% \text{ inhibition} = [A_0 - A_s] / A_0 \times 100$$

Where,

A_0 = Absorbance of H₂O₂ (unreduced)

A_s = Absorbance of the sample and standard

Hydrogen peroxide produces hydroxyl radicals in cells. Scavenging of these radicals by the test extract is used as a test for antioxidant activity. The reduction of these radicals is seen by the decreased absorbance at 230nm with increasing concentration of the test extract. The mean percentage H₂O₂ inhibition values were plotted against concentration.

Reducing Power Assay

Substances having the reducing potential react with potassium ferricyanide (Fe³⁺) to potassium ferrocyanide (Fe²⁺) which then further react with ferric chloride to form a Ferric –Ferrous complex which has an absorbance maximum at 700nm. Phosphate buffer was prepared by mixing Dibasic Sodium Phosphate (37.50ml of 0.2M) with 62.5ml of monobasic sodium

phosphate and diluted to 100ml with distilled water. Different concentration of the plant extracts (10µg/ml-160µg/ml) were mixed with 2.5ml of phosphate buffer (pH6.6) and 2.5ml of 10g/l (1%) potassium ferricyanide. The mixture was incubated at 50°C for 30 minutes and an aliquot (2.5 ml) of 10% of trichloroacetic acid was added to the mixture which was then centrifuged at 3000 rpm for 10minutes. Finally 2.5ml of the upper layer was mixed with 2.5ml of distilled water and 0.5 ml of 1g/l (0.1%) ferric chloride. The absorbance of resulting solution was measured at 700nm by using a UV-visible spectrophotometer. Water was used as blank while L-Ascorbic acid was used as standard. Increasing absorbance of the reaction mixture indicates increase in reducing power assay. Reducing power of each extract was estimated from the plotted graph of percentage reducing power versus concentration of extract.

III.RESULTS AND DISCUSSION

DPPH Radical scavenging assay

The fruit extracts exhibited a potent free radical scavenging activity. The inhibition percentage of methanol extract of fruit showed highest radical scavenging activity gradually increasing from 28.37% to 91.22% at 10µg/ml -160µg/ml. Ethyl acetate extract showed less activity compared to ethanol, chloroform, acetone, water, diethyl ether and petroleum ether extracts respectively (Table1; Figure 2). Methanol and ethanol extracts of fruit showed 50% and above inhibition at 40µg/ml. fruits extracts in chloroform, water, acetone and petroleum ether showed at 80µg/ml while diethyl ether at 160µg/ml and ethyl acetate showed above 160µg/ml. The extracts showed significant influence when compared to ascorbic acid. The leaf extracts exhibited radical scavenging activity against DPPH. The inhibition percentage of leaf methanol extract showed 28.37% to 80.79% at 10µg/ml -160µg/ml while petroleum ether showed less scavenging activity compared to ethanol, chloroform,

acetone, water, diethyl ether and ethyl acetate respectively (Table 2; Figure 3). Methanol, ethanol, chloroform and acetone extracts of leaf showed 50% and above inhibition at 40µg/ml and water showed at 80µg/ml while diethyl ether and petroleum ether at 160µg/ml and ethyl acetate showed above 160µg/ml. The leaf extracts showed less significant scavenging activity compared to fruit extract. The inhibition % of seed extracts increased with increasing the concentration. The inhibition percentage of seed methanol extract showed 28.94% to 78.05% at 10-160µg/ml while petroleum ether showed less scavenging activity compared to chloroform, ethanol, acetone, water, ethyl acetate and diethyl ether respectively (Table 3; Figure 4). The seed methanol extract showed 50% and above inhibition at 20µg/ml while chloroform and ethanol extract showed at 40µg/ml. Acetone showed at 80µg/ml while water, ethyl acetate and diethyl ether at 160µg/ml and petroleum ether showed above 160µg/ml.

H₂O₂ Radical scavenging assay

The inhibition % of Fruit extracts against H₂O₂ exhibited a strong free radical scavenging activity. The inhibition percentage of fruit ethanol extract showed 55.80% to 88.73 % at 10µg/ml-160µg/ml while petroleum ether showed less scavenging activity compared to the extracts in chloroform, methanol, acetone, water, ethyl acetate and diethyl ether respectively (Table 4; Figure 5). The fruit ethanol extract showed 50% and above inhibition at 20µg/ml while chloroform at 40µg/ml. Methanol extract showed at 10µg/ml, acetone and water at 80µg/ml, ethyl acetate at 160µg/ml and, diethyl ether and petroleum ether showed above 160µg/ml concentration. The inhibition % of leaf extracts increased with increase in the concentration. The inhibition percentage of leaf ethanol extract showed 45.95% to 96.02 % at 10µg/ml-160µg/ml while ethyl acetate showed less scavenging activity compared to chloroform, water, methanol, acetone, petroleum ether and diethyl ether respectively (Table 5, Figure 6).

Ethanol and chloroform extract of leaf showed 50% and above inhibition at 20µg/ml. Water extract of leaf showed activity at 40µg/ml, methanol and acetone extracts showed at 80µg/ml while petroleum ether and diethyl ether showed activity at 160µg/ml. The leaf extracts showed significant influence when compared to ascorbic acid. The seed extracts exhibit radical scavenging activity against H₂O₂. The inhibition percentage of seed ethanol extract showed 45.75% to 92.11 % at 10µg/ml-160µg/ml while diethyl ether showed less scavenging activity compared to methanol, chloroform, water, acetone, petroleum ether and ethyl acetate respectively (Table 6, Figure 7). The seed ethanol extract showed 50% and above inhibition at 20µg/ml, methanol and chloroform showed at 40µg/ml while water, acetone, petroleum ether showed at 160µg/ml and diethyl ether and ethyl acetate at above 160µg/ml. The seed extracts showed less significant scavenging activity compared to fruit and leaf extracts.

Reducing power assay

The fruit extracts showed an increase in reducing power with increase in the concentration. The fruit methanol extract showed 0.188 to 1.915 at 10µg/ml-160µg/ml while petroleum ether showed less reducing power compared to ethanol, chloroform, acetone, water, diethyl ether and ethyl acetate (Table 7, Figure 8). The leaf methanol extract showed 0.167 to 1.574 at 10µg/ml-160µg/ml while petroleum ether showed less reducing power compared to chloroform, ethanol, acetone, water, ethyl acetate and diethyl ether (Table 8, Figure 9). The seed ethanol extract showed increasing reducing power gradually from 0.141 to 1.265 at 10µg/ml -160µg/ml while ethyl acetate showed less reducing power compared to methanol, acetone, chloroform, water, diethyl ether and petroleum ether (Table 9, Figure 10).

Table 1: %inhibition concentration for DPPH radical scavenging activity of Fruit extracts of *Carissa carandas* L.

Sl. No.	Conc. (µg/ml)	%inhibition								
		ascorbic acid	methanol	ethanol	chloroform	acetone	water	diethyl ether	petroleum ether	ethyl acetate
1	10	19.91±0.11	28.37±0.29	27.42±0.11	21.25±0.11	16.60±0.19	14.31±0.19	12.79±0.19	10.31±0.33	5.53±0.19
2	20	25.05±0.89	38.04±0.11	32.25±0.19	37.79±0.19	20.68±0.11	38.17±0.19	23.35±0.11	21.57±0.19	11.13±0.29
3	40	28.90±0.76	76.97±0.11	54.01±0.19	48.15±0.11	40.49±0.19	40.49±0.19	28.18±0.29	28.37±0.29	20.99±0.19
4	80	32.87±0.74	80.85±0.11	75.19±0.19	77.67±0.19	51.85±0.22	67.94±0.19	49.55±0.11	53.50±0.11	25.83±0.11
5	160	42.07±0.91	91.22±0.29	90.52±0.11	85.31±0.19	79.96±0.19	75.76±0.19	64.38±0.11	56.11±0.42	49.68±0.11

*Data are mean of triplicate measurements ± standard deviation. Each measurement were significantly different at p<0.05.

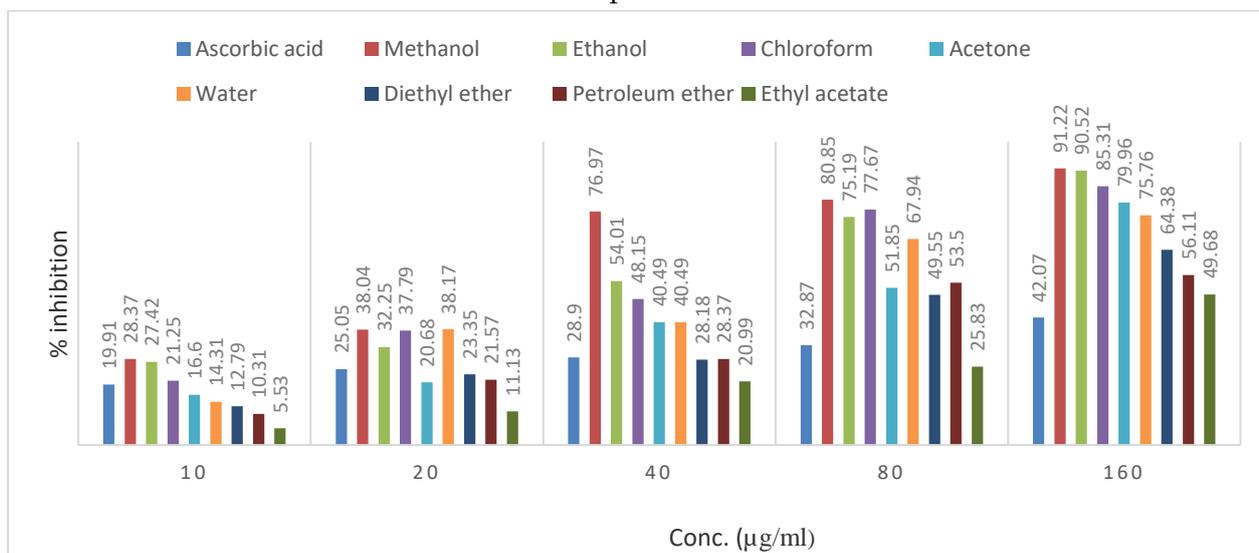


Figure 2: DPPH scavenging activity of Fruit extracts of *Carissa carandas* L.

Table 2: % inhibition concentration for DPPH radical scavenging activity of Leaf extracts of *Carissa carandas*

Sl. No	Conc. (µg/ml)	%inhibition									
		ascorbic acid	methanol	ethanol	chloroform	acetone	water	diethyl ether	petroleum ether	ethyl acetate	
1	10	19.91±0.11	28.37±0.29	25.83±0.11	20.48±0.11	16.60±0.19	14.76±0.61	11.13±0.29	10.43±0.11	8.52±0.11	
2	20	25.05±0.89	43.38±0.58	49.55±0.11	38.17±0.19	37.47±0.11	29.14±0.19	28.44±0.19	27.42±0.11	18.70±0.19	
3	40	28.90±0.76	51.21±0.29	56.30±0.19	52.16±0.11	59.22±0.11	43.38±0.58	33.97±0.19	30.60±0.11	30.47±0.11	
4	80	32.87±0.74	71.18±0.19	69.21±0.11	69.66±0.19	49.68±0.11	58.91±0.22	43.70±0.19	44.34±0.29	38.42±0.29	
5	160	42.07±0.91	80.79±0.11	79.58±0.19	78.82±0.19	76.91±0.19	63.10±0.11	59.73±0.19	56.81±0.11	48.16±0.11	

*Data are mean of triplicate measurements ± standard deviation. Each measurement were significantly different at p<0.05.

Table 3: % inhibition concentration for DPPH radical scavenging activity of Seed extracts of *Carissa carandas*

Sl. No	Conc. (µg/ml)	%inhibition									
		ascorbic acid	methanol	ethanol	chloroform	acetone	water	diethyl ether	petroleum ether	ethyl acetate	
1	10	19.91±0.11	28.94±0.29	21.37±0.19	23.41±0.11	19.66±0.19	18.70±0.19	8.46±0.11	6.87±0.19	10.37±0.11	
2	20	25.05±0.89	58.84±0.29	48.60±0.72	49.11±0.72	38.23±0.22	38.17±0.19	21.37±0.19	12.85±0.11	21.12±0.29	
3	40	28.90±0.76	63.10±0.58	55.53±0.19	58.27±1.23	44.95±0.19	44.53±0.22	37.85±0.22	38.23±0.22	37.98±0.38	
4	80	32.87±0.74	71.76±0.38	68.34±0.94	64.70±0.19	65.39±0.11	49.62±0.19	43.64±0.22	38.97±0.19	48.16±0.11	
5	160	42.07±0.91	78.05±0.19	71.76±1.16	76.15±0.19	68.64±0.29	63.04±0.11	56.49±0.19	44.21±0.11	59.73±0.19	

*Data are mean of triplicate measurements ± standard deviation. Each measurement were significantly different at p<0.05.

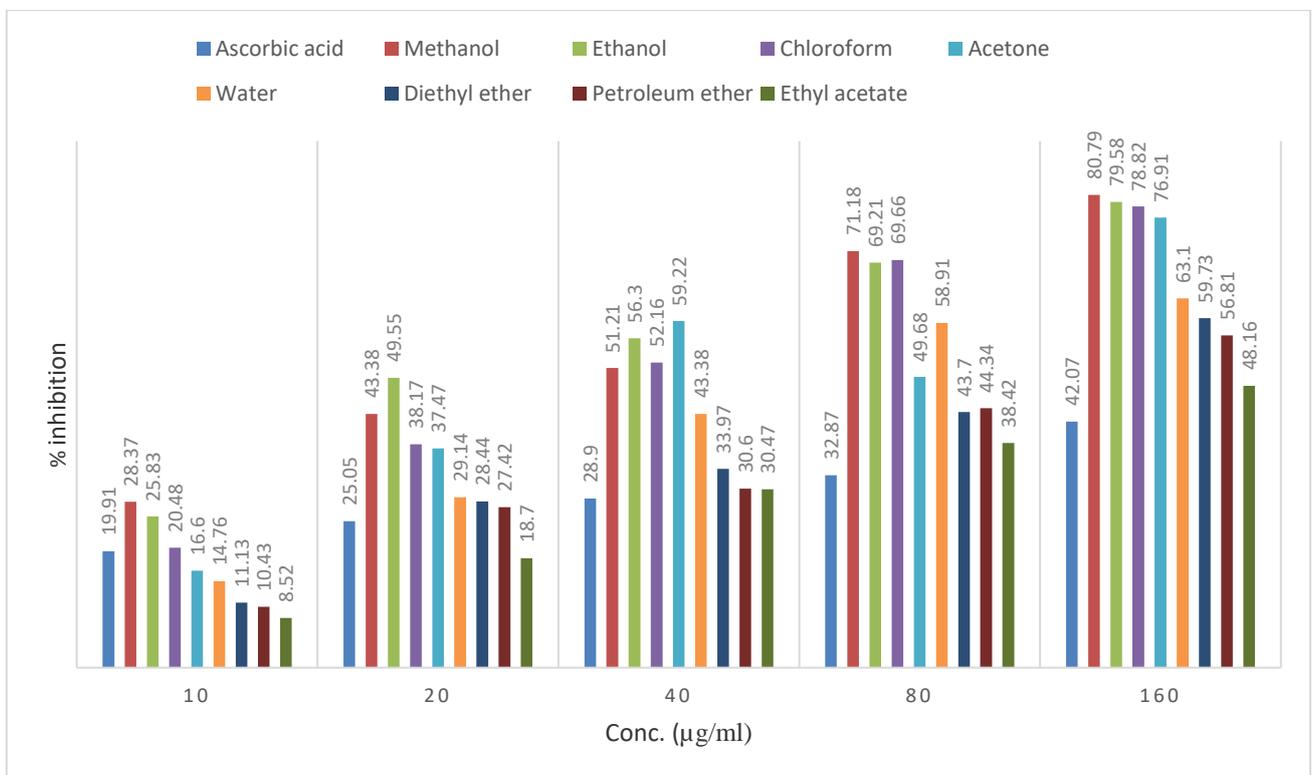


Figure 3: DPPH scavenging activity of Leaf extracts of *Carissa carandas* L.

Table 4: % inhibition concentration for H₂O₂ radical scavenging activity of Fruit extracts of *Carissa carandas*

Sl. No	Conc. (µg/ml)	%inhibition								
		ascorbic acid	methanol	ethanol	chloroform	acetone	water	diethyl ether	petroleum ether	ethyl acetate
1	10	10.79±0.20	55.80±0.12	48.38±0.20	39.88±0.20	20.85±0.20	16.80±0.20	11.07±0.12	5.40±0.23	12.96±0.20
2	20	27.47±0.20	60.53±0.20	63.56±0.41	49.80±0.20	30.77±0.20	28.14±0.20	21.26±0.20	8.64±0.23	26.92±0.20
3	40	36.47±0.47	68.22±0.20	74.97±0.12	52.43±0.20	49.06±0.31	47.17±0.20	33.60±0.20	9.72±0.20	31.16±0.02
4	80	53.36±0.59	80.36±0.20	91.36±0.31	79.76±0.20	67.41±0.20	55.67±0.20	42.11±0.20	25.37±0.12	45.95±0.20
5	160	54.5±0.45	88.73±0.42	92.31±0.20	87.65±0.20	74.29±0.20	93.52±0.12	46.09±0.31	35.83±0.20	80.84±0.20

*Data are mean of triplicate measurements ± standard deviation. Each measurement were significantly different at p<0.05.

Table 5: % inhibition concentration for H₂O₂ radical scavenging activity of Leaf extracts of *Carissa carandas* L.

Sl. No	Conc. (µg/ml)	%inhibition								
		ascorbic acid	methanol	ethanol	chloroform	acetone	water	diethyl ether	petroleum ether	ethyl acetate
1	10	10.79±0.20	20.99±0.12	45.95±0.20	39.68±0.20	20.65±0.20	32.19±0.20	5.47±0.20	14.17±0.20	8.77±0.23
2	20	27.47±0.20	21.05±0.20	62.82±0.31	58.30±0.20	21.12±0.12	46.15±0.20	8.70±0.20	28.07±0.23	11.10±0.12
3	40	36.47±0.47	42.71±0.20	85.83±0.41	80.77±0.20	41.70±0.20	68.15±0.12	17.21±0.20	46.49±0.42	27.80±0.62
4	80	53.36±0.59	60.12±0.20	92.11±0.20	91.50±0.20	62.75±0.20	87.65±0.20	31.98±0.20	48.58±0.20	33.60±0.20
5	160	54.5±0.45	89.27±0.20	96.02±0.31	92.11±0.20	89.34±0.42	90.49±0.20	57.29±0.20	70.38±0.31	45.69±0.31

*Data are mean of triplicate measurements ± standard deviation. Each measurement were significantly different at p<0.05.

Table 6: % inhibition concentration for H₂O₂ radical scavenging activity of Seed extracts of *Carissa carandas*

Sl. No	Conc. (µg/ml)	%inhibition								
		ascorbic acid	methanol	ethanol	chloroform	acetone	water	diethyl ether	petroleum ether	ethyl acetate
1	10	10.79±0.20	24.70±0.20	45.75±0.20	28.14±0.20	10.53±0.20	8.70±0.20	12.82±0.12	5.87±0.20	7.49±0.20
2	20	27.47±0.20	37.25±0.20	60.39±0.12	45.55±0.20	18.62±0.20	18.42±0.20	28.34±0.20	20.65±0.20	10.73±0.20
3	40	36.47±0.47	55.06±0.20	70.11±0.31	63.16±0.20	30.57±0.20	21.86±0.41	33.20±0.20	31.98±0.21	25.44±0.12
4	80	53.36±0.59	63.09±0.31	91.10±0.20	83.20±0.20	46.22±0.31	40.49±0.20	46.15±0.70	48.18±0.20	38.80±0.31
5	160	54.5±0.45	85.43±0.20	92.11±0.20	91.30±0.20	74.49±0.21	63.36±0.20	47.71±0.31	86.44±0.20	46.97±0.20

*Data are mean of triplicate measurements ± standard deviation. Each measurement were significantly different at p<0.05.

Table 7: Reducing power of Fruit extracts of *Carissa carandas* L.

Sl. No	Conc. (µg/ml)	Ascorbic acid	methanol	ethanol	chloroform	acetone	water	diethyl ether	petroleum ether	ethyl acetate
1	10	0.454±0.002	0.188±0.001	0.181±0.002	0.175±0.001	0.163±0.002	0.141±0.001	0.130±0.001	0.107±0.002	0.128±0.001
2	20	0.646±0.001	0.294±0.001	0.382±0.002	0.288±0.001	0.412±0.001	0.504±0.001	0.350±0.001	0.437±0.002	0.267±0.001
3	40	0.734±0.002	0.425±0.001	0.518±0.001	0.508±0.001	0.616±0.001	0.713±0.001	0.466±0.001	0.556±0.002	0.520±0.002
4	80	0.816±0.001	0.624±0.001	0.740±0.001	0.830±0.001	0.902±0.001	0.818±0.001	0.769 ±0.001	0.889±0.002	0.908±0.002
5	160	1.098±0.001	1.915±0.002	1.806±0.001	1.624±0.001	1.514±0.001	1.332±0.001	1.315±0.002	1.258±0.001	1.303±0.002

*Data are mean of triplicate measurements ± standard deviation. Each measurement were significantly different at p<0.05.

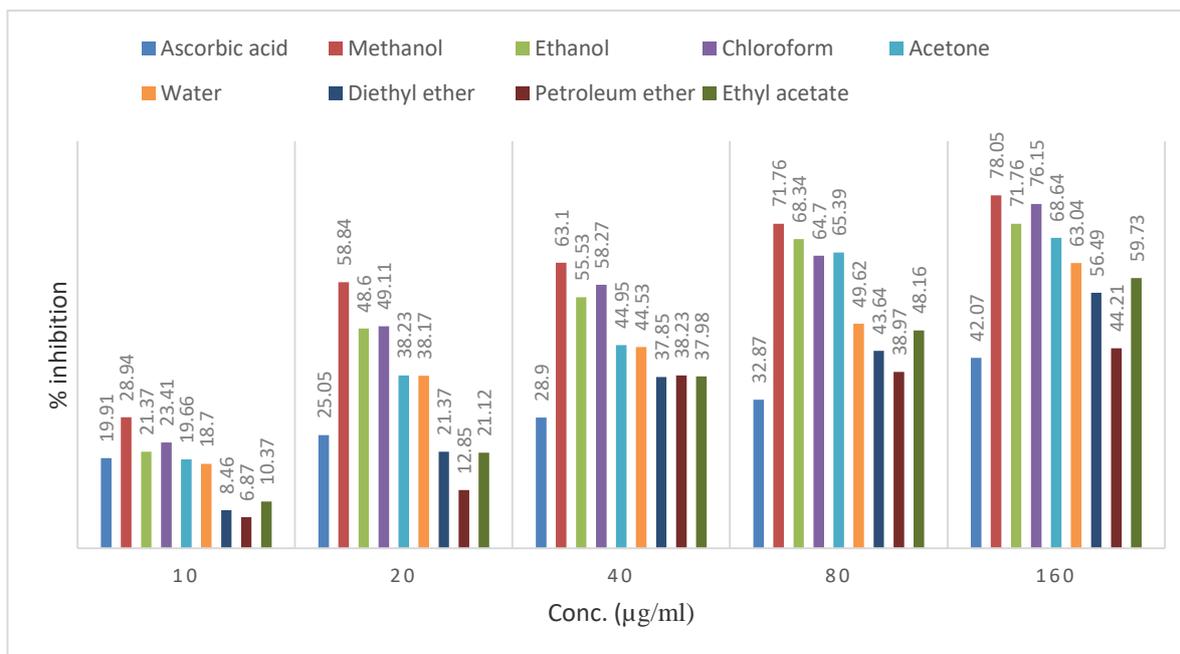


Figure 4: DPPH scavenging activity of Seed extracts of *Carissa carandas*

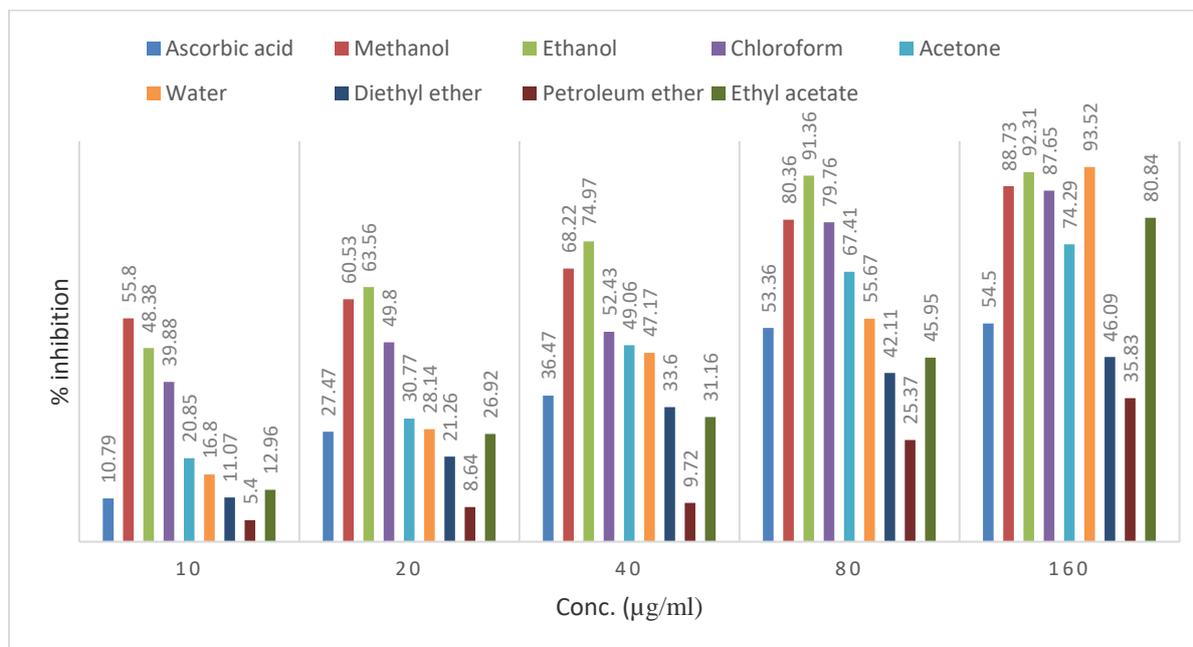


Figure 5: H₂O₂ scavenging activity of Fruit extracts of *Carissa carandas*

Table 8: Reducing power of Leaf extracts of *Carissa carandas*

Sl. No	Conc. (µg/ml)	Ascorbic acid	methanol	ethanol	chloroform	acetone	water	diethyl ether	petroleum ether	ethyl acetate
1	10	0.454±0.002	0.167±0.002	0.128±0.002	0.162±0.001	0.105±0.001	0.096±0.002	0.086±0.002	0.085±0.001	0.092±0.001
2	20	0.646±0.001	0.252±0.001	0.426±0.003	0.313±0.001	0.214±0.001	0.357±0.002	0.244±0.001	0.396±0.002	0.108±0.002
3	40	0.734±0.002	0.394±0.001	0.552±0.001	0.405±0.001	0.315±0.002	0.473±0.002	0.350±0.002	0.567±0.001	0.568±0.001
4	80	0.816±0.001	0.820±0.001	0.915±0.002	0.620±0.001	0.728±0.001	0.916±0.002	0.619±0.002	0.915±0.004	0.796±0.001
5	160	1.098±0.001	1.574±0.001	1.497±0.001	1.572±0.001	1.402±0.002	1.357±0.001	1.241±0.001	1.213±0.002	1.341±0.001

*Data are mean of triplicate measurements ± standard deviation. Each measurement were significantly different at p<0.05.

Table 9: Reducing power of Seed extracts of *Carissa carandas*

Sl. No	Conc. (µg/ml)	Ascorbic acid	methanol	ethanol	chloroform	acetone	water	diethyl ether	petroleum ether	ethyl acetate
1	10	0.454±0.002	0.135±0.001	0.141±0.001	0.110±0.001	0.113±0.001	0.095±0.001	0.090±0.001	0.085±0.001	0.073±0.002
2	20	0.646±0.001	0.245±0.001	0.244±0.001	0.423±0.002	0.324±0.001	0.313±0.001	0.293±0.001	0.364±0.001	0.414±0.002
3	40	0.734±0.002	0.667±0.008	0.548±0.001	0.809±0.001	0.720±0.002	0.708±0.001	0.585±0.001	0.627±0.001	0.704±0.002
4	80	0.816±0.001	1.207±0.002	1.194±0.001	0.913±0.001	1.092±0.001	0.812±0.002	0.943±0.002	0.887±0.002	0.716±0.002
5	160	1.098±0.001	1.218±0.002	1.265±0.001	1.197±0.001	1.205±0.002	1.171±0.001	1.152±0.001	1.125±0.002	1.113±0.002

*Data are mean of triplicate measurements ± standard deviation. Each measurement were significantly different at p<0.05.

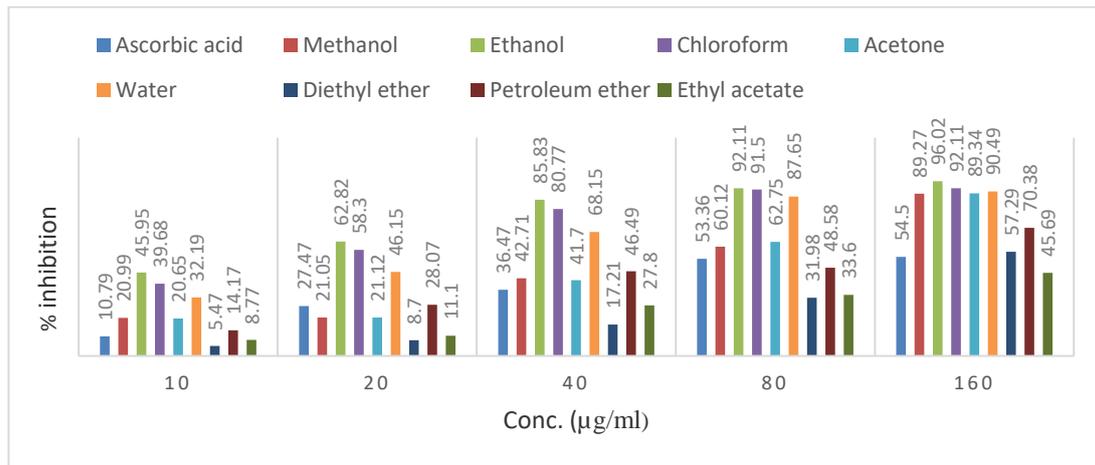


Figure 6: H₂O₂ scavenging activity of Leaf extracts of *Carissa carandas*

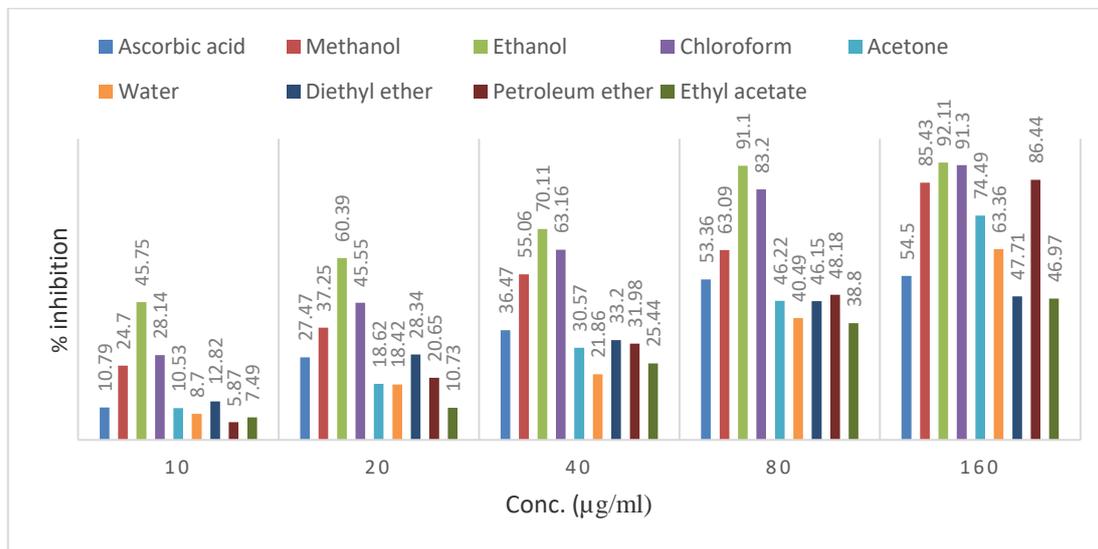


Figure 7: H₂O₂ scavenging activity of Seed extracts of *Carissa carandas*

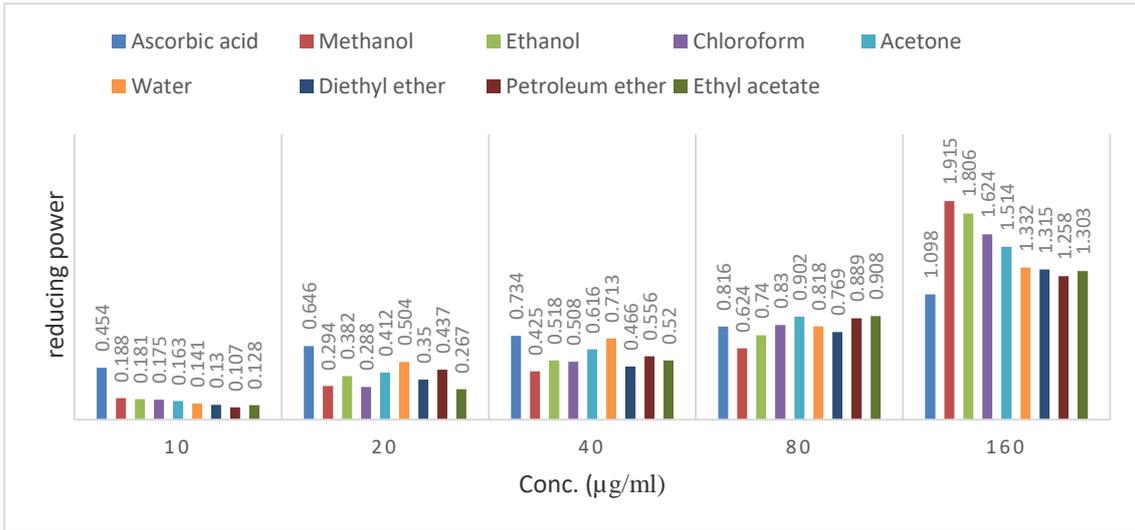


Figure 8: Reducing power of Fruit extracts of *Carissa carandas*

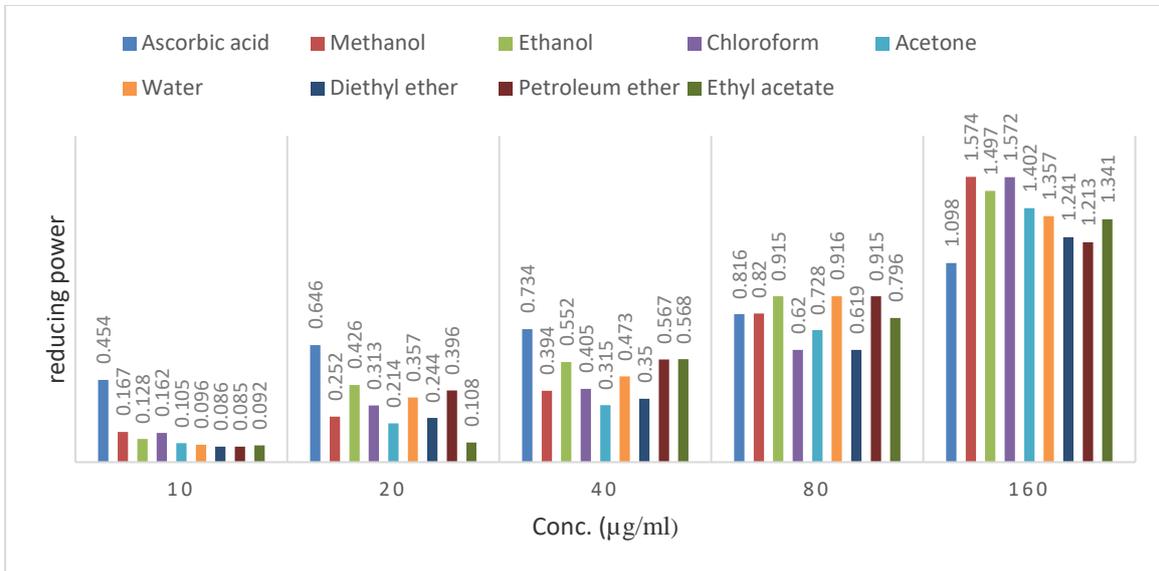


Figure 9: Reducing power of Leaf extracts of *Carissa carandas*

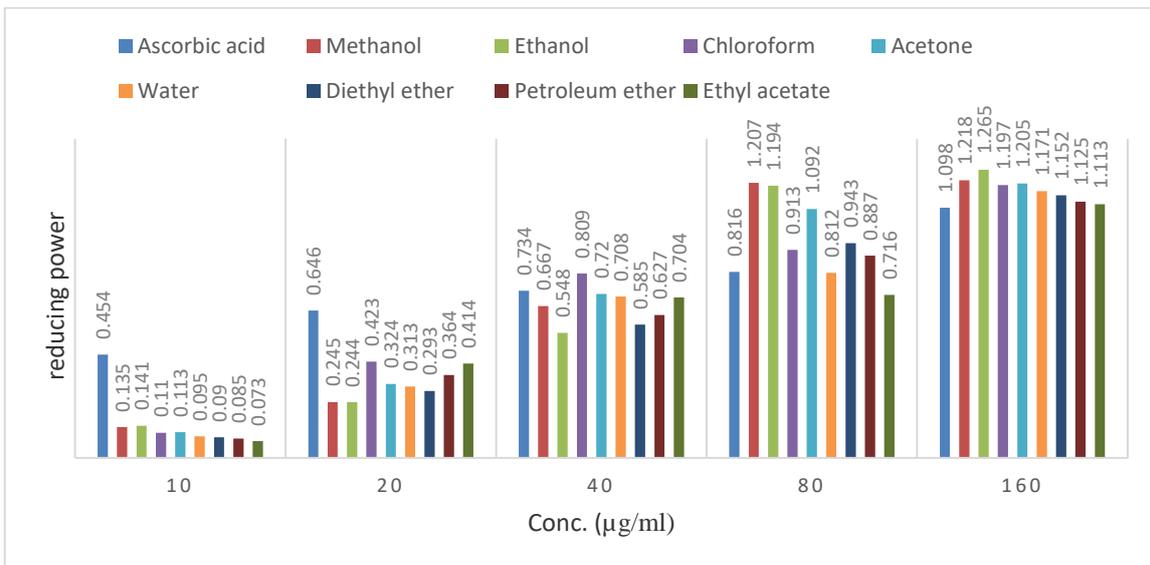


Figure 10: Reducing power of Seed extracts of *Carissa carandas*

The results revealed that DPPH and H₂O₂ radical scavenging activity was recorded in terms of % inhibition as shown in the tables and figures. It was observed that extract with the highest effective DPPH radical scavenging activity and reducing power was the methanol extract of *C. carandas* fruit while ethanol extract of fruit showed the highest effective H₂O₂ radical scavenging activity. Petroleum ether, ethyl acetate and diethyl ether showed lowest radical scavenging activity and reducing power assay. The results were compared to the standard (L-Ascorbic acid). Higher % inhibition represented better antioxidant potential of the extract. The extracts were investigated for their reducing capabilities by using potassium ferricyanide reduction method. The reducing ability may serve as a significant indicator of potential antioxidant activity [18]. The fruit, leaf and seed extracts increased in reducing powers with increasing concentration. The fruit and leaf extracts exhibited highest reducing ability than the seed extracts. The methanol extracts of fruit and leaf showed the highest activity and petroleum ether showed lowest while ethanol extract of seed showed the highest and ethyl acetate showed lowest reducing activity. Table 7-9 depicted the reducing power of the extracts.

The fruit and leaf extracts had significantly higher antioxidant properties than seed. This result was correlated with reducing power assay where fruit and leaf extracts were also found more significant than seed extracts. The antioxidant potential was determined to be in the following order: Fruit>Leaf>Seed extracts. The studies suggest that the fruits, leaves and seeds extracts of *C. carandas* possesses potent *in vitro* antioxidant activity and act as an effective free radical scavenger. Methanol extracts of *C. carandas* show exceeding good results to extract TPC and TFC and its antioxidant activity. Further study on the same can be of immense value as therapeutic drugs and thus protecting our body

from various diseases. Earlier work on various other plant species was also in conformity with the present findings [1], [2], [18], [19], [20], [21], [23]. In the present study, the fruit and leaf extracts of *C. carandas* showed significantly higher antioxidant properties than seed. The result was correlated with reducing power assay where fruit and leaf extracts were found more significant than seed extracts. The studies suggested that the fruits, leaves and seeds extracts of *C. carandas* possesses antioxidant ability and act as an effective free radical scavenger.

IV. CONCLUSION

From the present study, it can be concluded that solvent extracts of various parts of *Carissa carandas* exhibited potent free radical scavenging properties. The fruit extracts of *C. carandas* exhibited a higher free radical scavenging ability. The inhibition percentage of fruit methanol extract showed highest radical scavenging activity gradually increasing from 28.37% to 91.22% at 10µg/ml to 160µg/ml concentration of the extracts. Methanol and ethanol extract of fruits showed 50% and above inhibition at 40µg/ml concentration. The leaf extracts exhibited radical scavenging activity from 28.37% to 80.79% at 10µg/ml -160µg/ml.

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