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In Vitro Antioxidant Property of Laticiferous Plant Species from Western Ghats Tamilnadu, India

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Abstract

Purpose: To determine the antioxidant and free radical scavenging properties of the ethanol and aqueous extracts from roots of *Carissa carandas* and *Pergularia daemia* in various *in vitro* systems.
Methods: Reducing power, DPPH radical, superoxide radical, nitric oxide radical and hydrogen peroxide radical scavenging assays were carried out to evaluate the antioxidant potential of the ethanol and aqueous extracts from roots of *C. carandas* and *P. daemia*. Total phenolic content and total flavonoids were also evaluated.
Results*:* In the DPPH radical scavenging assay the IC_{50} values were 178.84 and 169.39 µg/ml for ethanol extract of *C. carandas* and *P. daemia* respectively. In the nitric oxide radical scavenging assay the ethanol extract of *P. daemia* showed maximum percentage inhibition (65.14±0.0115), while the aqueous extract of *P. daemia* exhibited least percentage inhibition (62.69±0.0461) on superoxide radical scavenging, when compared with other extracts. The aqueous extract of *C. carandas* and *P. daemia* exhibited 50% scavenging activity at 208.07 and 243.09 µg/ml on H_2O_2 radical. The reducing power of the extracts increased dose dependently. All the extracts exhibited significant antioxidant ($p < 0.01$) activity.
Conclusion: The extracts of the roots of *C. carandas* and *P. daemia* possess antioxidant properties and could serve as free radical inhibitors or scavengers.

Keywords: *Carissa carandas; Pergularia daemia*; Free radicals; Antioxidant; Phenolic compounds.

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Introduction

Antioxidants are vital substances which possess the ability to protect the body from damages caused by free radical induced oxidative stress. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism [1]. The most common reactive oxygen species (ROS) include superoxide ($O_2^{\cdot}$) anion, hydrogen peroxide (H_2O_2), peroxyl ($ROO^{\cdot}$) radicals and reactive hydroxyl ($OH^{\cdot}$) radicals. The nitrogen derived free radicals are nitric oxide ($NO^{\cdot}$) and peroxynitrite anion ($ONOO^{\cdot}$). Free radicals contribute to more than one hundreds of diseases or disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, aging, liver diseases, cancer and AIDS [2]. Both exogenous and endogenous antioxidants (whether synthetic or natural) can be effective in the prevention of the free radical formation by scavenging or promoting the decomposition and suppression of such disorders [3]. Therefore, the search for natural antioxidant has greatly increased in the recent years.

Ethnopharmacological surveys conducted among herbal practitioners of Western Ghats Tamilnadu, India have revealed a large numbers of laticiferous plant species are used as sources of herbal therapies. Accordingly, we selected two medicinal plants including *Carissa carandas* and *Pergularia daemia* used to treat liver disease and jaundice, condition in which oxidative stress is prominent for this study. The plant *Pergularia daemia* (Asclepiadaceae) is known as "Veliparuthi" in Tamil, "Uttaravaruni" in Sanskrit and "Utranajutuka" in Hindi while the plant, *Carissa carandas,* belonging to the family Apocynaceae, is commonly known as Christ's thorn or Bengal Currant 'Kalakke' in Tamil [4]. Traditionally the plant *P.daemia* is used as anthelmintic, laxative, antipyretic and expectorant, and is also used to treat infantile diarrhoea and malarial intermittent fevers [5-7]. *C. carandas* was traditionally used as stomachic, antidiarrheal and anthelmintic. The stem bark was used as strengthen tendons and fruits have been used in skin infections, while the leaves have been used as remedy for fevers, earache and syphilitic pain [4-7]. In the present study, the ethanol and aqueous extracts of roots of *C. carandas* and *P. daemia* were screened for antioxidant properties using *in vitro* standard procedures so as to assess the medicinal potential of these two plants and thus justify their folklore uses.

Materials and Methods

Chemicals

Butylated hydroxytoluene (BHT), L- ascorbic acid, gallic acid, catechin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium nitropruside, nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), nicotinamide adenine dinucleotide(NADH), trichloroacetic acid (TCA) and ferric chloride were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Plant Material

The plant materials were collected from Maruthamalai Hills, Coimbatore, India in the month of November 2006. The both plants were taxonomically identified by Dr P. Jayaraman, Plant Anatomy Research Centre, Chennai, Tamil Nadu, India. The voucher specimen of *P. daemia* (PARC/2007/52) and *C. carandas* (PARC/2007/53) has been preserved in our laboratory for further study and reference.

Preparation of extracts

The roots of *P. daemia* and *C. carandas* were dried under shade, powdered with a mechanical grinder and passed through sieve no 40. The sieved powder was stored in airtight container and kept in room temperature until further study. The dried

powdered material (500 g) was extracted with 95% ethanol using soxhlet apparatus for about 48 h. The aqueous extract was prepared by cold maceration (72 hr) and the solvents were removed from the extracts under reduced pressure using rotary vacuum evaporator.

Preliminary phytochemical analysis

The ethanol and aqueous extracts of roots of *P. daemia* (PDEE & PDAE, respectively) and *C. carandas* (CCEE & CCAE, respectively) were subjected to phytochemical tests [8] in order to identify the nature of chemical constituents present in the plant material and show the presence of various phyto-constituents including alkaloids, glycosides, tannins, flavonoids, steroids and terpenoids.

Determination of total phenol

Total phenolic contents in the extracts were determined by the using the Folin-Ciocalteu method [9]. A dilute solution of each plant extract (0.5 ml of 1:10 g ml^{-1}) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous sodium carbonate (4 ml, 1M). The mixtures were allowed to stand for 15 min and the total phenols were determined by UV-VIS spectrometer (Pharmaspec 1700, Shimadzu) at 765 nm and the content expressed in terms of gallic acid equivalent using the following equation based on the calibration curve: $y = 0.045\ x$, $r^2 = 0.960$, where x was the absorbance and y was gallic acid equivalent (mg/g).

Determination of total flavonoid

Aluminum chloride colorimetric method was used for flavonoids determination [10]. Each plant extracts (0.5 ml of 1:10 g ml^{-1}) in methanol was separately mixed with1.5 ml of 1M potassium acetate and 2.8 ml of distilled water. It was maintained at room temperature for 30 min and then the absorbance of the mixture was measured at 415 nm with UV-VIS spectrometer (Pharmaspec 1700, Shimadzu). The total flavonoids content was expressed in terms of catechin equivalent using the following equation based on the calibration curve: $y = 0.05\ x$, $r^2 = 0.992$, where x was the absorbance and y was catechin equivalent (mg/g).

Determination of DPPH radical scavenging activity

The free radical scavenging activity of CCEE, CCAE, PDEE, PDAE and butylated hydroxyl toluene (BHT) was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH [11]. DPPH solution (0.1 mM) in ethanol was prepared and 1 ml of this solution was added to 3 ml of extract solution in water at different concentrations (10-300 µg/ml). After 35 min, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH \cdot scavenged \cdot (\%) = \frac{A_{cont} - A_{test}}{A_{cont}} \times 100$$

where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts.

Determination of NO radical scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent [12]. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Sodium nitroprusside (5 mM) in phosphate-buffered saline (PBS) was mixed with 3 ml of different concentrations (10 – 300 µg/ml) of the CCEE, CCAE, PDEE,

PDAE and BHT dissolved in the suitable solvent systems and incubated at 25 ℃ for 150 min. The samples from the above were reacted with Greiss reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm. The nitric oxide radicals scavenging activity was calculated as in the case of DPPH.

Determination of superoxide anion radical scavenging activity

Measurement of super oxide anion scavenging activity of the CCEE, CCAE, PDEE and PDAE based on the method described by Liu et al., with slight modification [13]. Super oxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of nitro blue tetrazolium (NBT). In this experiment, the super oxide radicals were generated in 3 ml of Tris-HCl buffer (16 mM, pH 8) containing1.0ml of NBT (50 µM) solution, 1 ml NADH (78 µM) solution and sample solution of the extracts (10-300 µg/ml) in water. The reaction started by adding 1.0ml of phenazine methosulphate(PMS) solution (10 µM) to the mixture. The reaction mixture was incubated at 250 ℃ for 5 min, and the absorbance at 560 nm was measured against blank samples. L-Ascorbic acid was used as a reference compound. Decreased absorbance of the reaction mixture was indicated an increased superoxide anion scavenging activity. The % inhibition of super oxide anion generation was calculated as in the case of DPPH.

Determination of H_2O_2 radical scavenging activity

The ability of extracts to scavenge $H_2O_2$was determined according to the method of Ruch et al [14]. A solution of H_2O_2 was prepared in phosphate buffer (pH 7.4).Extracts (10-300 µg/ml) in distilled water were added to a H_2O_2 solution (0.6 ml, 40 mM).Absorbance of H_2O_2 at 230 nm was determined10 min later against a blank solution containing the phosphate buffer without H_2O_2. The % of H_2O_2 scavenging of both the extracts and standard compounds was calculated as in the case of DPPH.

Determination of reducing power

The reducing power of the extracts was determined according to the method of Oyaizu [15]. Various concentrations of the CCEE, CCAE, PDEE, PDAE and L-Ascorbic acid (10-300 µg/ml) in 1.0 ml of de ionized water were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and 1% potassium ferricyanide (2.5ml). The mixture was incubated at 50 ^oC for 20min. Aliquots of trichloroacetic acid (2.5 ml,10%) were added to the mixture, which was then centrifuged at 1036 x g for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared $FeCl_3$solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm. Increased absorbance of their action mixture indicated increased reducing power.

Statistical analysis

The experimental results were expressed as mean ± standard error of mean (SEM) of three replicates. Where applicable, the data were subjected to one way analysis of variance (ANOVA) and followed by Dunnet's test for multiple comparisons. P values less than 0.05 were regarded as significant and p values <0.01 as very significant. The amount of extracts needed to inhibit free radicals concentration by 50% (IC_{50}) was graphically estimated using linear regression lines.

Results

Preliminary phytochemical tests of CCEE, CCAE, PDEE and PDAE indicated the presence of compounds that include alkaloids, glycosides, steroids, flavonoids,

Table 1: Total phenolic and flavonoid content of *Carissa carandas* and *Pergularia daemia*

Material	Total phenolic content [a]	Total flavonoid content [b]
Ethanol extract of *C. carandas* (CCEE)	18.33 ± 0.1011	2.76 ± 0.0611
Aqueous extract of *C. carandas* (CCAE)	15.82 ± 0.1039	1.69 ± 0.0862
Ethanol extract of *P. daemia* (PDEE)	17.69 ± 0.1039	2.57 ± 0.0520
Aqueous extract of *P. daemia*(PDAE)	12.62 ± 0.1375	1.27 ± 0.0352

Values represent mean ± SEM (n=3)
[a] Expressed as mg of gallic acid equivalents / g of dry plant extract
[b] Expressed as mg of catechin equivalents / g of dry plant extract

Table 2: DPPH free radical scavenging activity of *Carissa carandas* and *Pergularia daemia* root extracts at different concentration

Concentration (µg / ml)	Percentage of inhibition				
	Reference compound (BHT)	CCEE	CCAE	PDEE	PDAE
10	36.19 ± 0.0115	07.66 ± 0.0346*	03.06 ± 0.0202*	08.2 8± 0.0260*	01.84 ±0.0173*
50	50.12 ± 0.0233	27.91 ± 0.0202*	19.03 ± 0.0057*	28.00 ± 0.0525*	25.76 ± 0.0230*
100	69.01 ± 0.0145	36.80 ± 0.0173*	26.07 ± 0.0115*	38.95 ± 0.0230*	38.03 ± 0.0173*
150	82.52 ± 0.0523	43.24 ± 0.0176*	38.03 ± 0.0173*	46.61 ± 0.0664*	42.33 ± 0.0115*
200	96.63 ± 0.0260	54.91 ± 0.0208*	50.30 ± 0.0260*	56.38 ± 0.0600*	52.14 ± 0.0288*
250	-	68.71 ± 0.0173*	58.89 ± 0.0120*	69.93 ± 0.0115*	61.33 ± 0.0655*
300	-	70.55 ± 0.0600*	61.96 ± 0.0400*	74.43 ± 0.0300*	64.40 ± 0.0152*
IC_{50} (µg / ml)	48.99	178.84	216.18	169.39	197.82

Values are mean± S.E.M., n=3; * P<0.01 vs. Reference compound; CCEE, Ethanol extract of *C. carandas;* CCAE, Aqueous extract of *C. carandas ;* PDEE, Ethanol extract of *P. daemia ;* PDAE, Aqueous extract of *P. daemia*

saponin, terpenoids, carbohydrates, gums, mucilage, tannin and phenolic compounds. The content of total phenolics (determined using Folin-Ciocalteu assay) and flavonoids in CCEE, CCAE, PDEE and PDAE were significant (Table 1). CCEE and PDEE had higher amounts of total flavonoid and phenol content than CCAE and PDAE.

The antioxidant properties of CCEE, CCAE, PDEE, PDAE and BHT led to decrease in the concentration of DPPH radical (Table 2). A 200 µg/ml of CCEE, PDEE and BHT exhibited percentage inhibition of 54.91± 0.0208, 52.14±0.0288 and 96.63±0.0260%, respectively. The IC_{50} values were found to be 178.84, 216.18, 169.39, 197.82 and 48.99 µg/ml for CCEE, CCAE, PDEE, PDAE and BHT, respectively. CCEE, CCAE, PDEE, PDAE and BHT effectively reduced nitric oxide formation at 201.52, 240.74, 174.52, 226.46 and 63.39 µg/ml, respectively (Table 3). The extracts and BHT compete with oxygen to react with NO and thus inhibit the peroxynitrite formation. The superoxide scavenging activity of CCEE, CCAE, PDEE, PDAE and ascorbic acid exhibited 50% scavenging activity at 129.62, 163.96, 149.81, 192.53 and 33.10 µg/ml, respectively on superoxide radical are presented in Table 4. With H_2O_2 radical, the extracts and ascorbic acid exhibited 50% scavenging activity at 164.58, 208.07, 184.55, 243.09 and 72.64 µg/ml, respectively (Table 5). The reducing capacity of the extracts was less than that of ascorbic acid. However, the reducing power of CCEE, CCAE, PDEE and PDAE increased with increasing dosage (Table 6).

Discussion

Polyphenols are the most wide spread secondary metabolite in the plant kingdom. These diverse groups of compounds have received much attention as potential natural antioxidant in terms of their ability to act as efficient radical scavengers and it is believed to be mainly due to their redox properties [16]. The high phenolic and flavonoid content

Table 3: Nitric oxide radical scavenging activity of *Carissa carandas* and *Pergularia daemia* root extracts at different concentration

Concentration (µg / ml)	Percentage of inhibition				
	Reference compound (BHT)	CCEE	CCAE	PDEE	PDAE
10	34.85 ± 0.0173	26.05 ± 0.0288*	23.94 ± 0.0155*	28.82 ± 0.0115*	25.00 ± 0.0173*
50	46.47 ± 0.0115	30.97 ± 0.0145*	30.28 ± 0.0404*	33.45 ± 0.0115*	32.40 ± 0.0240*
100	57.04 ± 0.0173	37.31 ± 0.0693*	35.91 ± 0.0173*	40.49 ± 0.0461*	35.21 ± 0.0088*
150	70.42 ± 0.0115	41.54 ± 0.0404*	39.28 ± 0.0288*	45.75 ± 0.0115*	39.78 ± 0.0288*
200	88.73 ± 0.0288	47.88 ± 0.0155*	46.12 ± 0.0230*	52.11 ± 0.0145*	46.47 ± 0.0115*
250	-	59.85 ± 0.0173*	51.05 ± 0.0288*	63.02 ± 0.0230*	53.66 ± 0.0176*
300	-	61.61 ±0 .0230*	55.98 ± 0.0057*	65.14 ± 0.0115*	58.45 ± 0.0230*
IC_{50} (µg / ml)	63.39	201.52	240.74	174.52	226.46

*Values are mean± S.E.M., n=3; * P<0.01 vs. Reference compound; CCEE, Ethanol extract of C. carandas; CCAE, Aqueous extract of C. carandas ; PDEE, Ethanol extract of P. daemia ; PDAE, Aqueous extract of P. daemia*

Table 4: Superoxide radical scavenging activity of *Carissa carandas* and *Pergularia daemia* root extracts at different concentration

Concentration (µg/ml)	Percentage of inhibition				
	Reference compound (Ascorbic acid)	CCEE	CCAE	PDEE	PDAE
10	41.66 ± 0.0173	25.04 ± 0.0514*	23.80 ± 0.0173*	24.60 ± 0.0229*	21.03 ±0.0664*
50	59.14 ± 0.0620	38.06 ± 0.0230*	35.69 ± 0.0229*	36.45 ± 0.0173*	34.12 ± 0.0173*
100	65.07 ± 0.05169	43.65 ± 0.0173*	40.87 ± 0.0173*	41.66 ± 0.0346*	40.47 ± 0.0230*
150	82.93 ± 0.0230	51.98 ± 0.0115*	49.60 ± 0.0519*	50.39 ± 0.0145*	47.61 ± 0.0230*
200	96.02 ± 0.0260	63.09 ± 0.02308	57.93 ± 0.0173*	60.71 ± 0.0230*	51.19 ± 0.0288*
250	-	74.20 ± 0.0519*	63.49 ± 0.0346*	63.87 ± 0.0318*	55.55 ± 0.0230*
300	-	83.73 ± 0.0346*	65.85 ± 0.0260*	74.20 ± 0.0461*	62.69 ± 0.0461*
IC_{50} (µg / ml)	33.10	129.62	163.96	149.81	192.53

Values are mean± S.E.M., n=3; * P<0.01 vs. Reference compound; CCEE, *e*thanol extract of *C. carandas;* CCAE, *a*queous extract of *C. carandas ;* PDEE, ethanol extract of *P. daemia;* PDAE, aqueous extract of *P. daemia*

Table 5: Hydrogen peroxide radical scavenging activity of *Carissa carandas* and *Pergularia daemia* root extracts at different concentration

Concentration (µg / ml)	Percentage of inhibition				
	Reference compound (Ascorbic acid)	CCEE	CCAE	PDEE	PDAE
10	29.13 ± 0.0173	25.59 ± 0.0519*	22.083 ± 0.0288*	24.80 ± 0.0404*	20.86 ± 0.0288*
50	44.09 ± 0.0461	32.48 ± 0.0623*	30.90 ± 0.0218*	31.88 ± 0.0288*	25.97 ± 0.0260*
100	61.81 ± 0.0230	41.76 ± 0.0609*	33.080 ± 0.0288*	38.50 ± 0.0461*	30.70 ± 0.0115*
150	74.01 ± 0.0173	49.21 ± 0.0346*	40.15 ± 0.0115*	46.85 ± 0.0155*	36.61 ± 0.0173*
200	83.07 ± 0.0115	56.29 ± 0.0230*	46.81 ± 0.0230*	50.39 ± 0.0288*	45.27 ± 0.0230*
250	95.66 ± 0.0404	63.38 ± 0.0288*	58.66 ± 0.0173*	61.47 ± 0.0155*	35.14 ± 0.0173*
300	-	67.71 ± 0.0173*	62.57 ± 0.0710*	64.18 ± 0.0288*	55.90 ± 0.0288*
IC_{50} (µg / ml)	72.64	164.58	208.07	184.55	243.09

Values are mean± S.E.M., n=3; * P<0.01 vs. Reference compound; CCEE, *e*thanol extract of *C. carandas;* CCAE, *a*queous extract of *C. carandas;* PDEE, ethanol extract of *P. daemia;* PDAE, aqueous extract of *P. daemia*

Table 6: Reducing power capacity of *Carissa carandas* and *Pergularia daemia* root extracts at different concentration

Concentration (µg / ml)	Absorbance				
	Reference compound (Ascorbic acid)	CCEE	CCAE	PDEE	PDAE
10	0.32 ± 0.0155	0.25 ± 0.0176*	0.21 ± 0.0155*	0.25 ± 0.0033*	0.18 ± 0.0100*
50	0.38 ± 0.0155	0.32 ± 0.0208*	0.28 ± 0.0155*	0.33 ± 0.0666*	0.26 ± 0.0881*
100	0.49 ± 0.0155	0.38 ± 0.0057*	0.35 ± 0.0033*	0.37 ± 0.0033*	0.32 ± 0.0057*
150	0.56 ± 0.0057	0.51 ± 0.0033*	0.45 ± 0.0057*	0.48 ± 0.0066*	0.40 ± 0.0066*
200	0.70 ± 0.0066	0.62 ± 0.0057*	0.52 ± 0.0033*	0.57 ± 0.0033*	0.49 ± 0.0033*
250	0.81 ± 0.0066	0.68 ± 0.0088*	0.63 ± 0.0057*	0.66 ± 0.0057*	0.56 ± 0.0033*
300	0.88 ± 0.0033	0.73 ± 0.0088*	0.65 ± 0.0088*	0.69 ± 0.0033*	0.61 ± 0.0066*

Values are mean± S.E.M., n=3; * P<0.01 vs. Reference compound; CCEE, *e*thanol extract of *C. carandas;* CCAE, *a*queous extract of *C. carandas;* PDEE, ethanol extract of *P. daemia;* PDAE, aqueous extract of *P. daemia*

in the CCEE, CCAE, PDEE and PDAE may be responsible for its free radical scavenging activity.

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [17]. The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl groups. We can infer that, the antioxidant activities of these extracts may be probably due to the presence of compounds with hydroxyl group.

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and is involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases. Oxygen reacts with the excess of NO to generate nitrite and peroxynitrite anions, which act as free radicals [12]. Our results suggest that the phenolic components present in the extract might be responsible for NO scavenging effect.

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, is very harmful to the cellular components in a biological system [18]. The CCEE, CCAE, PDEE and PDAE may have decreased the mean rate of absorption by inhibiting NBT reduction by the superoxide anion radicals.

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly, and once inside the cell, H_2O_2 probably reacts with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical which may be the origin of many of its toxic effects [19]. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. The CCEE, CCAE, PDEE and PDAE scavenged H_2O_2 and this may be attributed to the presence of phenolics, which could donate electrons thereby neutralizing it into water.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Antioxidant activity has been attributed to various chain mechanisms, among which are the prevention of chain initiation, the binding of transition metal ion catalysts, decomposition of peroxides, the prevention of continued hydrogen abstraction, the reductive capacity and radical scavenging [20]. The CCEE, CCAE, PDEE and PDAE had reductive ability which increased with increasing concentrations of these extracts.

Conclusion

The results obtained in the present study indicates that the extracts from the roots of *C.carandas* and *P. daemia* exhibit antioxidant activities which may be attributed to the presence of polyphenolics and other phytochemicals constituents. The roots of *C.carandas* and *P.daemia* could be potential sources of natural antioxidant that could have great importance as therapeutic agents in preventing various liver disorders and oxidative stress related degenerative diseases.

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