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Research Article

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Potential health benefits of *Carissa carandas* dried fruit methanol extract

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ABSTRACT

Carissa carandas is used in traditional medicinal system for its various diseases curing property. Extraction of dried fruits of Carissa carandas were carried out with petroleum ether and methanol. The methanol extract and petroleum ether extract were selected for performing antioxidant activity. Here we have performed various in-vitro antioxidant assays including DPPH, metal chelating, H_2O_2, super oxide, anti- lipid peroxidation of petroleum ether and methanol extracts from the selected fruit. The methanol extract have showed strong antioxidant activities when compared with petroleum ether extract, which were correlated with its high level of phenolic and flavonoid. The extract possesses more phenolic and flavonoid content that causes the antioxidant activity. Generally antioxidant capacity of plant extracts was attributed to secondary metabolites present in it. Therefore evaluation of % inhibition by a single method may not provide proper result.

Keywords: Antioxidant activities, *Carissa carandas* fruits, Methanol extract, Petroleum ether extract.

INTRODUCTION

Antioxidant compounds from plants plays an important role in health protecting factor in human biological system. Primary source for antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes and phenolic acids are potentially useful for the reducing risk of several chronic diseases [1]. During the metabolism of oxygen in the system, an entire group of highly reactive molecules has been generated which is known as reactive oxygen species (ROS). These are formed for specific metabolic requirements and the main characteristic of an antioxidant is its ability to trap free radicals and oxygen species which are present in biological systems from a variety of sources. When the generation of these species exceeds the level of antioxidant mechanism it leads to an extensive damage to the cells, tissues and cause degenerative diseases such as cardio vascular diseases, ageing, neuron degenerative diseases like Alzheimer's disease, mutation, cancer etc.Also the free radicals may oxidize nucleic acids, proteins, lipids or DNA and this can initiate degenerative diseases[2].The reactive oxygen species (ROS) which includes hydroxyl (OH^-), superoxide (O_2^-), nitric oxide (NO), peroxyl (RO_2), lipid peroxyl (LOO^-). The antioxidant compounds like flavones, phenolic acids, polyphenols and flavonoid may scavenge these free radicals such as peroxide, hydro peroxide produced through the metabolism. Thus it inhibits the oxidative mechanism that leads to degenerative diseases.

Carissa carandas L. (Apocynaceae), commonly known as karaunda, is widely used as medicinal plant. The fruits, leaves, barks and roots of *C. carandas* have been used for ethno-medicine in the treatment of human diseases, such as, diarrhea, stomachic, anorexia, intermittent fever, mouth ulcer, sore throat, syphilitic pain, burning sensation, scabies, and epilepsy[3]. The chemical constituents include steroids, terpenes, tannins, flavonoid, benzenoides, phenylpropanoid, lignans, sesquiterpenes and coumarins[4]. Here both petroleum ether extract and methanol extract of *Carissa carandas* dried fruits were evaluated for antioxidant studies. Potent antioxidant activity manifested in scavenging DPPH, superoxide's, hydrogen peroxide, lipid peroxidation, reducing activity and iron chelating activity[5]. Even organic chemist focused their research towards the medicinal compounds [6-11]. Literature review says that the extract of *Carissa carandas* root bark can relatively safe for usage at doses between 250 and 1000

mg/kg[12]. The dried fruits of *Carissa carandas* could be used as a source of potential antioxidant or functional food material. To our knowledge, there is no report on antioxidant studies[13]. The methanol extract showed significant antioxidant activity compared to petroleum ether due to the presence of phenolic compounds and flavonoid in the extract.

EXPERIMENTAL SECTION

Collection and Authentication of Plant material

Carissa carandas fruits were collected from in and around of Vellore District, Tamil Nadu, India during the month of October, 2012. The sample was authenticated by Dr. M. Palanisamy and a voucher specimen was deposited at the Botanical Survey of India, Coimbatore (BSI/SRC/5/23/2013-14/Tech.1119). The fresh fruits of *Carissa carandas* were dried under shade and pulverized to a coarse powder. This powder was used to carry out the extraction process to obtain the petroleum ether and methanol extract.

Preliminary Phytochemical Studies

The preliminary phytochemical studies were performed by using petroleum ether and methanol extracts of dried fruits of *Carissa carandas* to screen the presence of various secondary metabolites[14].

Preparation of the Extract

The powdered sample material (500 g) was defatted with petroleum ether (1000 mL) and then subjected for distillation to get a thick dark green mass called petroleum ether extract (PTE). Then the residue was extracted with methanol (1000 mL) at room temperature for effective extraction. After filtering through folded paper the methanol extract was concentrated in a rotary evaporator to yield a dark brown mass (10 g) called methanol extract (MTE) as illustrated in **Fig. 1**. The petroleum ether extract and methanol extract were subjected for different methods of antioxidant studies (**Fig. 2**)[15].The preliminary phytochemical analysis was carried out as per the procedure given in Madhumitha et al [16].

Antioxidant Studies by Using Dried Fruit Extract

DPPH Method

The scavenging of 1, 1-diphenyl-2-picrylhydrazyl radical by the extract is based on the method by Madhumitha [17]. To 1 mL of the methanol extract of concentrations (100-500 µg/mL) was mixed with 3 mL of 0.1mM solution of DPPH. The mixture was kept in dark for 30 min. The absorbance was measured after incubation at 517 nm, against a blank of Absolute ethanol without DPPH. The control solution is a mixture of 1 mL ethanol and DPPH. The standard used for the DPPH assay was Ascorbic acid. The results were expressed as percentage of inhibition of the DPPH radical, which was calculated according to the following equation **(1)**

$$\% \text{ Inhibition} = [AC - AS/AC] \times 100 \% \quad \textbf{(1)}$$

Where,
AC - Absorbance of Control
AS - Absorbance of Sample

Reducing Power Assay

The crude petroleum ether and methanol extracts of concentrations (100-500 µg/mL) were mixed with 0.5 mL of 0.2 M phosphate buffer (pH 6.6) and 0.5 mL of 1 % potassium ferricyanide to check the reducing power of the extracts. The mixture was then incubated at 50^{o}C in a water bath for 20 min. The samples were centrifuged at 1500 rpm for 10 min followed by the addition of 0.5 mL of 10 % trichloroacetic acid to the supernatant. The solution was mixed with 1mL distilled water and 0.2 mL of 0.1 % $FeCl_3.6H_2O$ and it was then allowed to stand at room temperature for 10 min. The color change was monitored at 700 nm for control, extracts and standard in UV- Vis spectrophotometer. The higher the absorbance, the better the reducing power of the sample[18].The percentage of inhibition was calculated by equation **(1)**.

Hydrogen Peroxide Scavenging Activity

The H_2O_2scavenging assay was carried out as per the procedure described by Sumaira [19]. The principle of this method is that there is a decrease in absorbance of H_2O_2 upon oxidation of H_2O_2. A solution of 43 mM H_2O_2 was prepared in 0.1M phosphate buffer whose pH is 7.4. The concentration of methanol extracts ranges from 100-500 µg/mL were mixed in 3.4 mL phosphate buffer which was added to 0.6 mL of H_2O_2 solution (43 mM) and absorbance of the reaction mixture was recorded at 230 nm. The sodium phosphate buffer without H_2O_2 was taken to be blank solution. The absorbance of the control, standard ascorbic acid and the extracts were noted. Further the inhibition was calculated by equation **(1)**

Metal Chelating Assay

The chelating of ferrous ions by extracts was estimated by following the procedure depicted in Mohammad[20]. The PTE and MTE (100-500 µg/mL) was added to a solution of 0.05 mL 2 mM$FeCl_2$. The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine. The mixture was shaken vigorously and allowed to stand for 10 min at room temperature. The absorbance of the solution was measured by UV-Vis spectroscopy at 562 nm for extracts and the standard ascorbic acid. The percentage inhibition of ferrozine- Fe^{2+} complex formation was calculated by using the equation **(1)**.

Lipid Peroxidation Inhibition Assay

The lipid peroxidation inhibition assay was carried out using the spectrophotometer studies. The method was done by using both using chick liver and goat liver procured from the market. The liver was washed thoroughly in cold phosphate buffered saline of pH 7.4. The liver was then homogenized in a homogenizer to give a 10 % homogenate. The homogenate was then filtered using muslin cloth to remove the unwanted debris. The filtrate was centrifuged at 10,000 rpm for 10 min under refrigerated conditions. The supernatant was then used to carry out the assay. To 0.5 mL of the 10 % homogenate, 0.5 mL of the PTE and MTE of concentrations (100-500 µg/mL) was added. To this, 0.05 mL of 0.07 M ferrous sulphate was added. The solution was incubated at room temperature for 30 min. To the incubated solution, 1.5 mL of 20 % acetic acid (pH 3.5), 1.5 mL of 0.8 % TBA (in 1.1 % SDS) and 0.05 mL of 20 % TCA were added and mixed well. The tubes were then incubated at 100 ^{0}C for 1 h and cooled to room temperature. About 3 mL of butanol was added to each tube. The solution was mixed well and then centrifuged at 3000 rpm for 10 min. The upper layer was used to read the absorbance at 532 nm. The percentage inhibition was then calculated using the equation **(1).**

Super Oxide Anion Radical Scavenging Activity

The assay for superoxide anion radical scavenging activity was done with slight modification in the procedure. The reaction mixture contained 1 mL of NBT solution (312 µM prepared in phosphate buffer, pH- 7.4) and diluted different extracts were added. Finally, the reaction were accelerated by adding 100µL PMS solution (120µM prepared in phosphate buffer, pH-7.4) to the mixture. The reaction mixture is incubated at 25 °C for 5 min and absorbance at 560nm was measured against control. The standard used here was Ascorbic acid. The percentage inhibition of superoxide anion generation was calculated using the equation **(1)**

RESULTS AND DISCUSSION

Phytochemical Screening

The extracts obtained from solvent extraction with petroleum ether and methanol was then subjected to various qualitative preliminary tests for the identification of secondary metabolites. The results showed the presence of alkaloids, glycosides, flavonoid, terpenes, steroids and tannins in the methanol extract. Whereas the petroleum ether extract showed only carbohydrates and steroids. The results were recorded in **Table 1**.

Antioxidant Activity

Plant phenolic content was correlated to several biological activities like antioxidant, anti-cancer etc. The higher phenolic and flavonoid content inhibits the free radical formation. Generally antioxidant capacity of plant extracts was attributed to secondary metabolites present in it. Therefore evaluation of % inhibition by a single method may not provide proper result. Hence we have performed various in-vitro antioxidant assays including DPPH, metal chelating, H_2O_2, super oxide, anti- lipid peroxidation of petroleum ether and methanol extracts from the selected fruit[21]. The free radicals are produced under certain environmental condition and during normal cell function in the body. These molecules are very reactive because missing of an electron which gives them an electric charge. To neutralize this electric charge free radicals try to capture an electron from or donate an electron to a neighbouring molecule. Antioxidants work against the molecules that form free radicals, by destroying it before they can begin the neutralizing effect that leads to oxidative damage in biological system[22].

The diphenylpicrylhydroxyl is a stable free radical having absorption maximum at 517 nm in absolute methanol. The extract decreases their absorption which indicates their neutralizing nature and the colour of the DPPH turns from purple to yellow. The extent of decrease in absorption is a measure of their radical scavenging ability. Thus their percentage of inhibition increases. **Fig. 2(A)**represents the % of inhibition by plotting both petroleum ether and methanol extracts. From this we could be concluding that petroleum ether possesses moderate DPPH radical scavenging activity whereas methanol extract shows effective radical scavenging activity of 90.1 %[23]. The mechanism illustrated in **Fig. 2(B)**.

In **Fig. 3(B)**the substances which have reduction potential reacted with potassium ferricyanide (Fe^{3+})to form potassium ferricyanide (Fe^{2+}), which then reacted with ferric chloride to form ferric-ferrous complex that has an

absorption maximum at 700 nm. The observed result showed as in **Fig. 3(A)**, methanol extract has more reducing power than petroleum ether extract which is due to the presence of potent secondary metabolites present in it[24].

The Hydrogen peroxide scavenging capacity of an extract was directly related to its antioxidant capacity. Both PTE and MTE extracts showed antioxidant activity in a dose dependent manner at concentrations of 100-500 µg/mL. The percentage of inhibition of methanol extract (93.78%) is greater than of petroleum ether (92.79%). The ability of extracts to capture hydroxyl radicals relates directly the prevention of propagation of the process of lipid peroxidation as given in **Fig. 4(A)**[25].

In metal chelating method, the maximum inhibition was showed by methanol extract and petroleum ether extract shows moderate activity which diagrammatically represented in **Fig. 4(B)**. Here the decrease of absorbance value at 560 nm indicates the usage of superoxide ion in the reaction mixture.

The free radicals are produced under certain environmental condition and during normal cell function in the body. It causes damage to lipid membranes thereby causing various disease conditions in humans. Here we have used Goat liver and chicken liver for illustrating the index of antioxidant potential in terms of lipid peroxidation inhibition. The MTE extract of dried fruits of *Carissa carandas* showed a good degree of lipid peroxidation inhibition in goat liver (92.65 %) than chick liver (85.51 %) as represented in **Fig. 5(A) & (B)**

From the **Fig 6**, it can be inferred that at the least concentration of 100µg/mL, the percentage of radical scavenging activity of petroleum ether was found to be 17% and for methanol extract it showed 68.62 %which is significant. At higher concentration 500 µ/mL, the radical scavenging activity was 60% for petroleum ether extract and 83.23% for methanol extract.

The % inhibition of all the free radicals was given in the **Table 2.** From the preliminary phytochemical studies, the methanol extract of *Carissa carandas* was found to contain flavonoid, glycosides, terpenes, carbohydrates and steroids. Therefore these components were responsible for the antioxidant activity of the extract furthermore it drives to search new compounds from the respective fruit.

Table 1: Qualitative Phytochemical Screening of Dried Fruits of *Carissa carandas*

Chemical constituent	PET	MET
Carbohydrates	+	+
Alkaloids	_	+
Flavonoid	_	+
Tannins and phenolic	_	+
Steroids	+	+
Terpenoids	_	+

(- = negative, + = Positive)

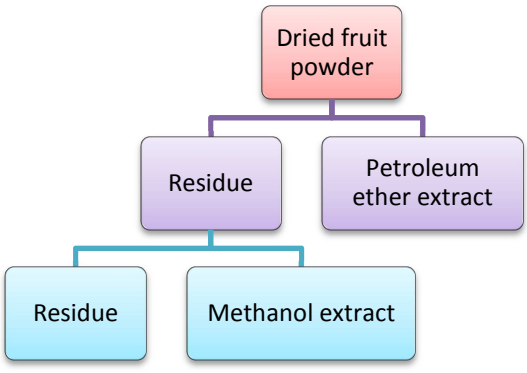

Fig.1 Preparation of extracts from dried fruits of *Carissa carandas*

Table 2: The Experimental Results of Various Antioxidant Activities of Dried Fruits of *Carissa carandas*

Extract	DPPH		Reducing assay		Metal chelating		Hydrogen peroxide		Lipid peroxidation (Chick liver)		Lipid peroxidation (Goat liver)		Total antioxidant		SOD	
	Conc.	% of inhibition	Conc.	% of inhibition	Conc.	% of inhibition	Conc.	% of inhibition	Conc.	% of inhibition	Conc.	% of inhibition	Conc.	% of inhibition	Conc.	% of inhibition
	10	35.1	100	59.15	100	12.65	100	41.36	100	71.34	100	44.83	100	16.7	100	17
	50	41.75	200	61.22	200	19	200	55.73	200	76.96	200	46.09	200	25.6	200	25.9
PTE	100	51.63	300	65.89	300	22.85	300	76.63	300	76.69	300	66.93	300	31.3	300	31.5
	250	63.26	400	66.79	400	35.77	400	81.06	400	79.38	400	71.44	400	48.7	400	55.46
	500	63.45	500	74.49	500	77.3	500	92.79	500	82.03	500	72.87	500	67.7	500	60
	10	51.73	100	69.15	100	89.35	100	81.35	100	76.29	100	80.79	100	22.3	100	68.62
	50	69.96	200	69.23	200	91.59	200	84.68	200	78.71	200	82.16	200	45.1	200	72.71
MTE	100	73.38	300	82.18	300	94.37	300	89.59	300	79.24	300	86.04	300	68.8	300	77.36
	250	88.53	400	92.38	400	96.04	400	90.12	400	81.27	400	90.36	400	74.2	400	82.10
	500	90.14	500	94.88	500	97.64	500	93.98	500	85.51	500	92.65	500	74.9	500	83.23
	10	31.21	100	54.69	100	91.08	100	95.6	100	63.69	100	67.99	100	19.6	100	70.85
	50	65.25	200	67.59	200	91.68	200	95.9	200	78.5	200	69.64	200	23.9	200	71.78
Std	100	83.79	300	65.74	300	91.73	300	95.97	300	83.21	300	75.98	300	34.4	300	74.45
	250	88.59	400	87.63	400	95.85	400	96.3	400	85.57	400	82.05	400	59.2	400	76.59
	500	90.1	500	94.83	500	98.55	500	97.08	500	86.76	500	84.96	500	85.5	500	86.15

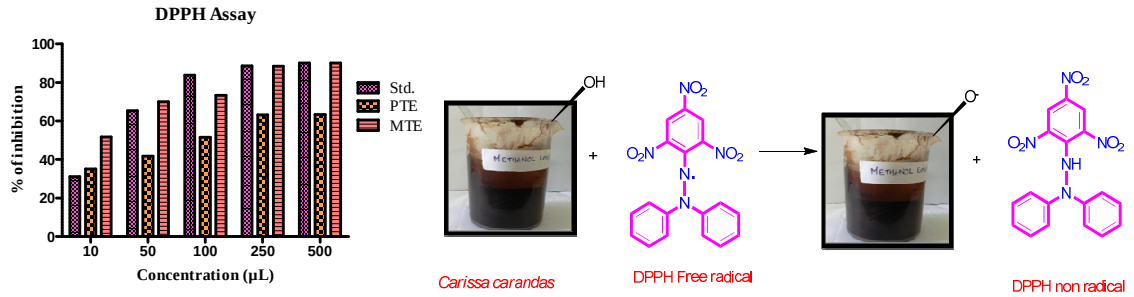

Fig. 2 Graph representing inhibition percentage of extracts by DPPH method& mechanism

Fig. 3 Graph representing inhibition percentage of extracts by reducing power assay& mechanism

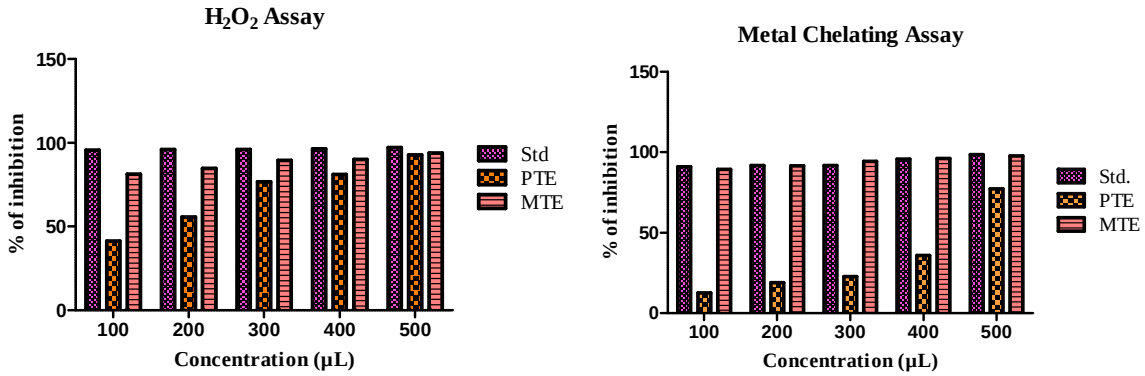

Fig. 4 (A, B) Graph representing Hydrogen peroxide assay & Metal chelating assay

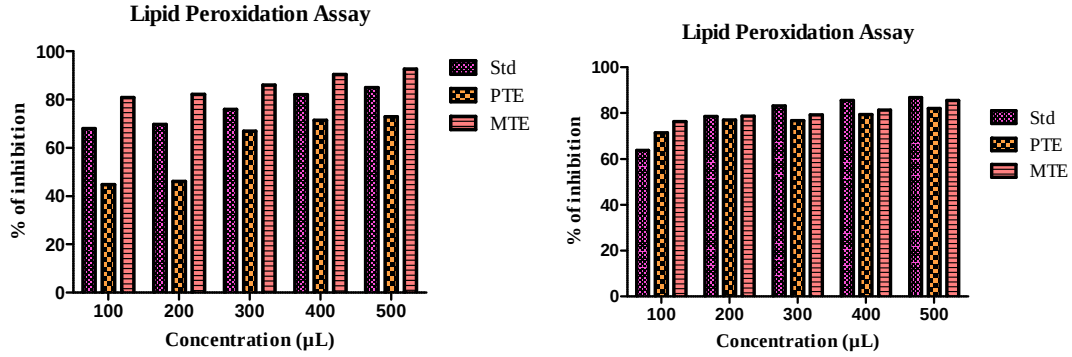

Fig. 5 Graph representing Lipid peroxidation assay of goat liver and chick liver

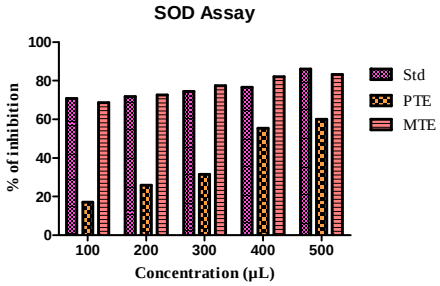

Fig. 6 Graph representing SOD assay

CONCLUSION

Here we analyzed the potential of edible fruits for antioxidant capacity by different antioxidant methods. The synergistic effect of metabolites present in the methanol extract of *Carissa carandas* showed significant scavenging activity. The dried fruits of *Carissa carandas* have been more often used as a food for its medicinal purpose in local areas, by reporting the antioxidant activity of the fruit. The methanol extract of dried fruit of *Carissa carandas* could be used as food supplement.

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