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Antioxidant Activity of *Carissa spinarum*

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ABSTRACT

Objective: The objective of this study was to evaluate the antioxidant activity of ethyl acetate and methanolic extract of leaves of *Carissa spinarum*. **Methods:** The leaf powder of *Carissa spinarum* was subjectd to successive solvent extraction by using soxhlet apparatus with various solvents like petroleum ether (60ºC-80ºC), chloroform, acetone, ethyl acetate and methanol. The antioxidant activity of the extracts were examined by biochemical assays namely diphenylpicrylhydrazyl (DPPH) and hydrogen peroxide radical scavenging activity. **Results:** The phytochemical screening of various extracts showed the presence of various phytoconstituents like alkaloids, glycosides, tannins, proteins, phenols and flavonoids. The Total Phenolic and total Flavonoid Contents by quantitative method in ethyl acetate and methanolic extract of *Carissa spinarum* was found to be 30.634, 36.120 and 28.36, 26.60 respectively. The assays revealed that the ethyl acetate and methanolic extracts exhibited stronger antioxidant activity. DPPH radical scavenging ability of ethyl acetate, methanolic extracts of *Carissa spinarum* and Ascorbic acid was found to be 69.994±0.587, 56.314±0.639% and 93.968±0.682 % inhibition at 200 µg/ml respectively. The IC50 value DPPH radical scavenging model of ethyl acetate and methanolic extracts were found to be 114.400±0.281, 119.221±0.342 and 48.120 ±0.231µg/ml respectively. Hydrogen peroxide radical scavenging activity with a significant decrease in the concentration of free radicals due to the scavenging ability of the extracts of *Carissa spinarum* and Ascorbic acid was found to be as 73.464±0.602, 62.871±0.428 and 77.624±1.706% inhibition at 200 µg/ml respectively. The IC50 value hydrogen peroxide radical scavenging model of ethyl acetate and methanolic extracts and ascorbic acid were found to be 124.530±0.942, 164.560±0.980 and 122.654±0.610µg/ml respectively. **Conclusion:** A positive, significant linear relationship between antioxidant activity and compounds in the plant. The present study revealed the significant antioxidant activity of the plant *Carissa spinarum*.

INTRODUCTION

Many terrestrial plants have been subjected to chemical, analytical and pharmacological screening methods, in order to evaluate their potency as drugs in medicine. Natural products are important sources for new pharmaceutical compounds, the ethnomedicinal approach represents an important method for identifying biologically active plant-based natural products as well as a means of documenting and preserving[1]. Natural antioxidant agents have attracted much interest because of their ability to scavenge free radicals [2]. The burden of chronic diseases is rapidly increasing worldwide. It has been calculated that, in 2001, chronic diseases contributed approximately 60% of the 56.5 million people, the total reported deaths[3]. in the world are approximately 46% of the global burden of disease. It has been projected that, by 2020, chronic diseases will account for almost three-quarters of all deaths worldwide and that 71% of deaths due to Ischaemic Heart Disease (IHD), 75% of deaths due to stroke, and 70% of deaths due to diabetes will occur in developing countries[4]. The number of people in the developing world with diabetes will increase by more than 2.5-fold, from 84 million in 1995 to 228 million in 2025 [5].

Plants containing natural products have been used worldwide in traditional medicine since antiquity [6] and are potential sources of drugs [7]. The plants with higher potency are rich source for new drugs, the research on the plants is still largely unexplored, and among the estimated 250,000- 500,000 plant species, only a few compounds have been submitted to biological or pharmacological studies. Diet has been known for many years to play a key role and as a risk factor for many chronic diseases. Several studies have demonstrated the potential role of fruits and vegetable in promoting health benefits and preventing diseases. Herbs have been used as food and for medicinal purposes for centuries. During the past two decades there has been a tremendous resurgence in the interest and use of medicinal plant products and an intense interest in “nutraceuticals” or “functional foods” in which phytochemicals can have long-term health promoting or medicinal properties. In different herbs, a wide variety of active phytochemicals, including flavonoids, terpenoids, alkaloids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins, and phthalides have been identified. The oxidative environment presents a range of free radicals including superoxide, hydroxyl radical, nitric oxide and peroxynitrite, for living organisms to deal with. There are a numerous of evidences about the role of free radicals in the development of various diseases including Cancer, neurodegeneration and some inflammatory diseases [8-10].

Oxidants are ubiquitous in biological systems and can cause significant damage to membranes, proteins, and nucleic acids. Plants with antioxidant activity lower the risk for ROS-mediated chronic diseases, such as cancer, ulcer, diabetes and cardiovascular disease [11-16]. Antioxidants have therefore gained importance for their capacity to neutralize free radicals.

Free radicals are responsible for causing a large number of diseases including cancer[17], cardiovascular disease [18] neural disorders[19] Alzheimer's disease[20] mild cognitive impairment[21] Parkinson's disease[22] alcohol induced liver disease ulcerative colitis[23] aging[24] and atherosclerosis[25].Protection against free radicals can be enhanced by ample intake of dietary antioxidants substantialiy evidences indicates that the foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in disease prevention.

Free radicals are atoms which have one or more unpaired electrons in the outermost shell[26]. These unpaired electrons are very unstable and can attack adjacent molecules such as lipids, proteins and carbohydrates and induce cellular damage[27]. Free radicals involving with oxygen are termed reactive oxygen species (ROS) such as peroxide radicals, hydroxyl radical and hydrogen peroxide[28,29]. The oxidative damage caused by excess RO may lead to development of many diseases such as heart diseases, congestive heart failure, hypertension, cerebrovascular accidents and diabetic complications[30]. Antioxidant are molecules which prevent effects of oxidation in tissues and can protect cell damaging from free radicals[31]. Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery.

Carissa spinarum is known as conkerberry or bush plum, it is a large shrub belonging to the family Apocynaceae, and is widely distributed in tropical regions of Africa, Southern Asia, Australia, and various islands of the Indian Ocean[32] It is also called *currant bush* or, more ambiguously, *native currant* or even *black currant*. In India, it is called wild karanda or wild karavanda. It grows as a multi-stemmed shrub, 0.5 to 3 metres in height. The leaves are glossy green, opposite, narrow ovate to lanceolate and 1–5 cm in length[33]. The branches bear thorns of 1–3 cm length and have white, star-shaped flowers 1 cm across are followed by ovate green berries, 1–2 cm in length, which turn black or dark purple when ripe.

The roots are chewed and the saliva swallowed, the root-sap being considered tonic and restorative for virility. The root decoction is used as an anthelmintic, especially against Taenia, chest-complaints, and as a cough remedy tonic. A piece of the root is sometimes fixed into a hut-roof as a snake-repellent and fly repellent. The roots also contain an active ingredient called 'carissin', which is useful in the treatment of cancer. The roots are put into water-gourds to impart an agreeable taste to the water, so that disguise the strong smell of groundwater. The roots are often added to the food of a sick person as an appetizer. The root bark is admixed with spices and used as enema for lumbago and other pains. Root-scrapings are used in the treatment of glandular inflammation. The whole plant is used as ‘bitter’ and expectorant. The raw Fruits or cooked and are used in jams. The leaf decoction is used in the treatment of intermittent fever, diarrhoea, oral inflammation and earache. The boiled leaves are applied as a poultice to relieve toothache. The unripe fruits are rich in tannins and are astringent and the ripe fruits are taken as an antiscorbutic and remedy for biliousness. The fruits are good source of tannins and are also used in dyeing.

MATERIALS AND METHODS:

Plant material:

Fresh plant of *Carissa spinarum* was collected from Tirupathi district, Andhra Pradesh, India in the month of August and authentified by Asst. Prof. Dr. K. Madhava Chetty, Taxonomist, S.V.University, Tirupati, A.P. Specimen vouchers (Ref No. MNRCOP/MPB/2019 – 94, 95) were deposited at Department of Biotechnology for further reference. The present work was carried out in the Department of Biotechnology MNR College of Pharmacy, Sangareddy, Hyderabad. The leaves of *Carissa spinarum* was washed thoroughly and dried under shade at 28±2^{o}C for about 10 days. The dried material was grounded well into fine powder in a mixer grinder and sieved in 43 mesh number. The powder was stored in air sealed polythene bags at room temperature until further use.

Chemicals:

1,1-Diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide, trichloroacetic acid, gallic acid (GA), rutin (RU), and Folin–Ciocalteu’s reagent, were purchased from Sigma Aldrich. Aluminium chloride, Sodium carbonate. All other chemical reagents used were of analytical grade.

Preparation of extracts:

The shade dried leaves of the plant of *Carissa spinarum* were reduced to fine powder (#40 size mesh) and around 2000g of powder was subjected to successive hot continuous extraction (soxhlet) with petroleum ether, chloroform, acetone, ethyl acetate and methanol. Finally, the drug was macerated with ethyl acetate and methanol. Each time before extracting with the next solvent the powdered material was air dried. After the effective extraction, the solvents were distilled off and the extract was then concentrated by distillation and solvent recovery. Finally, the extracts were concentrated on water bath dried up to constant weight and the extract obtained with each solvent was weighed. Percentage yield of the extract was calculated in terms of air dried weight of plant material. The colour and consistency of the extracts was noted. The obtained extracts were subjected to phytochemical investigation and pharmacological investigation.

Preliminary phytochemical Analysis of secondary metabolites:

All the extracts were subjected to preliminary phytochemical screening for the presence or absence of various secondary metabolites such as alkaloids, flavonoids, phenols, terpenoids, saponins, tannins, glycosides using analytical grade solvents and reagents. The respective yields and preliminary phytochemical investigation results given in Table 1.0 and 2.0.

Table No. 1: Extraction yield (%) of *Carissa spinarum with* various solvents by hot soxhlation

S. No.	Extract (200gm)	Color in daylight and consistency	% Yield
1	Petroleum Ether	Solid Greenish	7.305
2	Chloroform	Solid Dark Brown	6.021
3	Acetone	Solid Dark Brownish Black	9.145
4	Ethyl Acetate	Solid Dark Brown	19.912
5	Methanol	Semi-Solid Reddish Brown	24.028

Table No. 2: Preliminary Phytochemical investigation of various extracts of *Carissa spinarum*

Nature	Petroleum Ether	Chloroform	Acetone	Ethyl Acetate	Methanol
Alkaloids	-	+	-	++	+
Amino acids	-	-	-	-	+
Flavonoids	-	+	+	++	++
Anthraquinone Glycosides	-	-	-	-	+
Triterpenoids	+	+	-	-	+
Reducing Sugars	-	+	+	++	++
Gums	-	-	-	+	+
Tannins and Phenolics	-	+	+	++	++
Saponins	+	+	+	+	+
Fixed oils	+	+	+	+	+

+ = Present - = Absent

Determination of total phenolic content

Total phenolic content of methanolic extract of Carissa spinarum was measured using the Folin–Ciocalteu reagent method [34]. Briefly, from the stock solution of (1 mg/ml methanol) 200µl of both of the crude extracts were made up to 3 ml with distilled water then mixed thoroughly with 0.5 ml of Folin–Ciocalteu reagent for 3 min, followed by the addition of 2 ml of 20% (w/v) sodium carbonate. The mixture was allowed to stand for another 60 min in the dark and absorbance of the reaction mixtures was measured at 650 nm. Quantification was done on the basis of the standard curve of Gallic acid concentration range from 50 to 500 mg/ml (r^2 = 0.998). Total phenolic conent was calculated from the calibration curve and expressed as mg of gallic acid equivalent (GAE)/g dry weight.

Determination of total flavonoid content:

Total flavonoid content of both crude extracts was determined using the aluminium chloride colorimetric method [35]. In brief, from the stock solution of 1 mg/ml crude extracts, 50µl of

each extract was diluted up to 1 ml with methanol, then mixed with 4 ml of distilled water and subsequently with 0.3 ml of 5% $NaNO_2$ solution, 0.3 ml of 10% AlCl3 solution was added and leave for 5 min of incubation then allowed to stand for 6 min. This was followed by the addition of 2 ml of 1 M NaOH solution to the mixture and final volume of the mixture was brought to 10 ml by the addition of double distilled water. The mixture was allowed to stand for 15 min and absorbance was measured at 510 nm. Quantification was done on the basis of the standard curve of rutin concentration ranging from 50 to 500 mg/ml (r^2 = 0.999). Total flavonoid content calculated from a calibration curve was expressed as mg of rutin equivalent (RU)/g of dry weight, discussed in Table 3.0.

Table No. 3: Total phenolic and flavonoid contents of *Carissa spinarum*

S. No.	Extract	[a]Total phenolic content *GAE/g DW*	[b]Total flavonoid (GAE)/g DW content
1	Ethyl acetate	30..634 ± 1.06	28.36 ± 1.65
2	Methanolic Extract	36.120 ± 1.46	26.6 ± 1.20

Each value in the table was obtained by calculating the average of 3 experiments mean ± SD

SD stands for standard deviation for n = 3 observations.

[a]mg gallic acid equivqlent (GAE)/g DW.

GAE/g DW* stands for milligrams of gallic acid equivalents per *gram* of *dry weight

[b]mg rutin equivqlent (GAE)/g DW.

Qualitative assay:

Antioxidant activity was determined on the basis of their scavenging activity of the stable DPPH free radical and hydrogen peroxide radical scavenging activity. Commercially available thin layer chromatography (TLC) plates were used. A suitably diluted stock solutions were spotted on pre-coated silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extract. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethyl acetate and methanol. Bleaching of DPPH by the resolved

bands was observed for 10 min and the colour changes (yellow on purple background) were noted. DPPH forms deep pink colour when it is dissolved in ethyl acetate and methanol. When it is sprayed on the chromatogram of the extract, it forms pale yellow colour which indicates the presence of antioxidants.

***In Vitro antioxidant* activity:**

DPPH radical scavenging activity:

Radical scavenging activity of extract, fractions were performed against stable DPPH assay. The effect of antioxidants in the DPPH radical scavenged and by hydrogen donating capacity of a compound. When the radical form of DPPH is scavenged by an antioxidant through the donation of hydrogen to form a stable DPPH molecule, this leads to a color change from purple to yellow and a decrease in absorbance was measured at 517nm [36]. The radical scavenging activity of extracts was measured by method with slight modifications [37] stock solution (1mg/ml) of the petroleum ether, chloroform, acetone, ethyl acetate and methanol of extractions, the standard ascorbic acid was prepared in methanol. Finally, the ethyl acetate and methanolic extracts showed best results as compared to other extracts. They are employed for antioxidant activity comparing with standard Ascorbic acid solution (0.1ml) and different extracts upto 200µg/ml were added to 3ml of a 0.004% methanol solution of DPPH. An equal amount of methanol and DPPH served as control activity. After 30 minutes incubation in the dark, absorbance was recorded at 517nm and the antioxidant activity of the extract was expressed as IC50. The IC50 value was defined as the concentration in. For each concentration, a separate blank sample was used for background. The percentage inhibition activity was calculated from:

$$[(A0 – A1)/A0]x100,$$

where A0 is the absorbance of the control and A1 is the absorbance of extract/standard. The antioxidant activity of the extract was expressed as IC50. All the tests were performed in triplicate and discussed in Table 4.0 and In Fig 1.0. The graph was plotted with the average of three observations [38-40].

Table No. 4: Antioxidant activity of ethyl acetate, methanolic extracts of *Carissa spinarum* and ascorbic acid as standard by DPPH method

S. No.	Conc. of the Extract (µg/ml)	% Inhibition		
		Ethyl Acetate	Methanolic Extract	Ascorbic Acid
1	10	25.455 ± 0.621	11.615 ± 0.531	35.843 ± 0.891
2	20	27.340 ± 1.193	17.124 ± 0.700	36.431 ± 1.330
3	40	30.427 ± 0.734	18.000 ± 0.504	47.652 ± 1.248
4	60	31.612 ± 1.043	19.030 ± 0.318	53.794 ± 0.886
5	80	34.356 ± 0.639	20.857 ± 0.802	60.724 ± 0.683
6	100	37.567 ± 0.670	21.351 ± 0.438	70.685 ± 0.725
7	120	54.243 ± 0.663	34.240 ± 0.486	81.549 ± 0.897
8	140	61.464 ± 0.685	46.080 ± 0.607	85.126 ± 0.728
9	180	64.843 ± 0.642	51.783 ± 0.714	90.765 ± 0.485
10	200	69.994 ± 0.587	56.314 ± 0.639	93.968 ± 0.682
11	IC_{50}	114.440 ± 0.281	119.221 ± 0.342	48.120 ± 0.231

Conc. Of the Extract (µg/ml) Stands for Micrograms of extract in a microlitre

Each value in the table was obtained by calculating the average of 3 experiments mean ± SD.

SD stands for standard deviation for n = 3 observations.

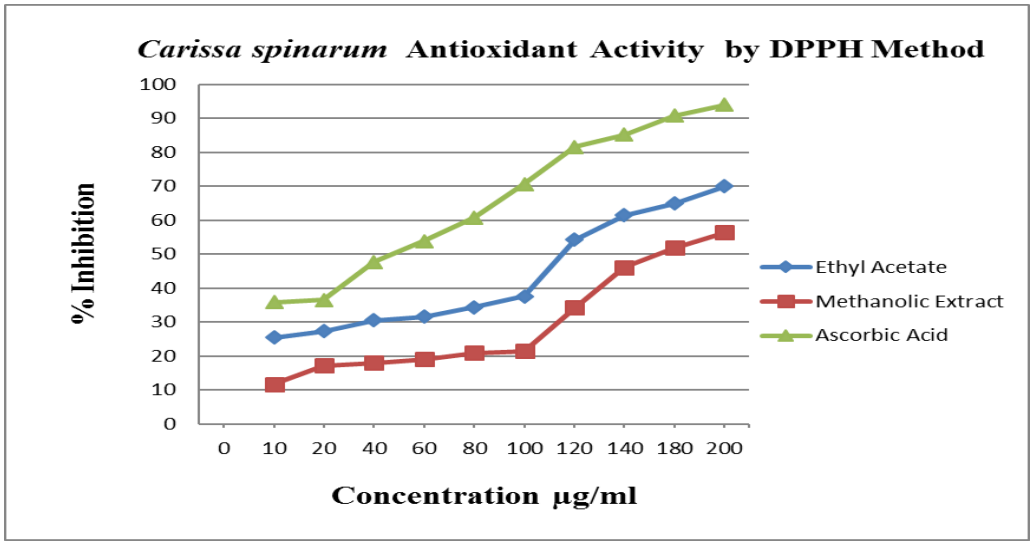

Figure No. 1: Effect of antioxidant activity of Ethyl acetate extract, methanolic extract of *Carissa spinarum* & ascorbic acid *by* DPPH method

DPPH radical scavenging activity of extracts was measured at different concentraions (10-200 µg/ml), and absorbance was recorded at 560 nm. The results are expressed as mean±SD. DPPH radical scavenging activity of standard ascorbic acid (green) ethyl acetate extract (blue) and methanolic extract (red) of *Carissa spinarum* extracts of radical scavenging activity.

Hydrogen peroxide radical scavenging activity:

A solution of hydrogen peroxide (20mM) was prepared in phosphate buffer saline (pH 7.4), different concentrations of plant extract and standard Ascorbic acid solution viz. 10,20,40,60,80,100,120,140,180,200 µg/ml in methanol (1 ml) where added to hydrogen peroxide solution (2 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background substraction. The antioxidant activity of the extract was expressed as IC50. The percentage inhibition activity was calculated from:

$$[(A0 – A1)/A0] \times 100$$

where A0 is the absorbance of the control and A1 is the absorbance of extract/standard. All the tests were performed in triplicate and discussed in Table 5.0 and the graph was plotted with the average of three observations [38-40].

Statistical analysis

Data were expressed as Mean ± SD. Statistical analysis was performed by SPSS 11.5. One-way analysis of variance (ANOVA) was utilized to evaluate differences.

Table No. 5: Antioxidant activity of ethyl acetate, methanolic extracts of *Carissa spinarum and* ascorbic acid as standard by H_2O_2 method

S. No.	Conc. Of the Extract (µg/ml)	% Inhibition		
		Ethyl Acetate	Methanolic Extract	Ascorbic Acid
1	10	1.002 ± 0.875	0.968 ± 0.364	1.185 ± 0.122
2	20	1.294 ± 1.077	3.824 ± 0.802	17.626 ± 0.563
3	40	13.614 ± 0.974	12.758 ± 0.626	21.071 ± 0.865
4	60	26.116 ± 0.972	15.149 ± 0.501	25.175 ± 0.960
5	80	29.019 ± 0.923	16.843 ± 0.250	26.270 ± 0.866
6	100	29.659 ± 1.457	18.677 ± 0.561	30.986 ± 1.212
7	120	47.290 ± 0.977	27.704 ± 0.734	48.960 ± 0.902
8	140	58.189 ± 0.722	37.281 ± 0.630	58.631 ± 0.616
9	180	69.490 ± 0.823	52.938 ± 0.617	72.293 ± 1.286
10	200	73.464 ± 0.602	62.871 ± 0.428	77.624 ± 1.706
11	IC_{50}	124.530 ± 0.942	164.560 ± 0.980	122.623± 0.610

Conc. of the Extract (µg/ml) Stands for Micrograms of extract in a microlitre

Each value in the table was obtained by calculating the average of 3 experiments mean ± SD. SD stands for standard deviation for n = 3 observations.

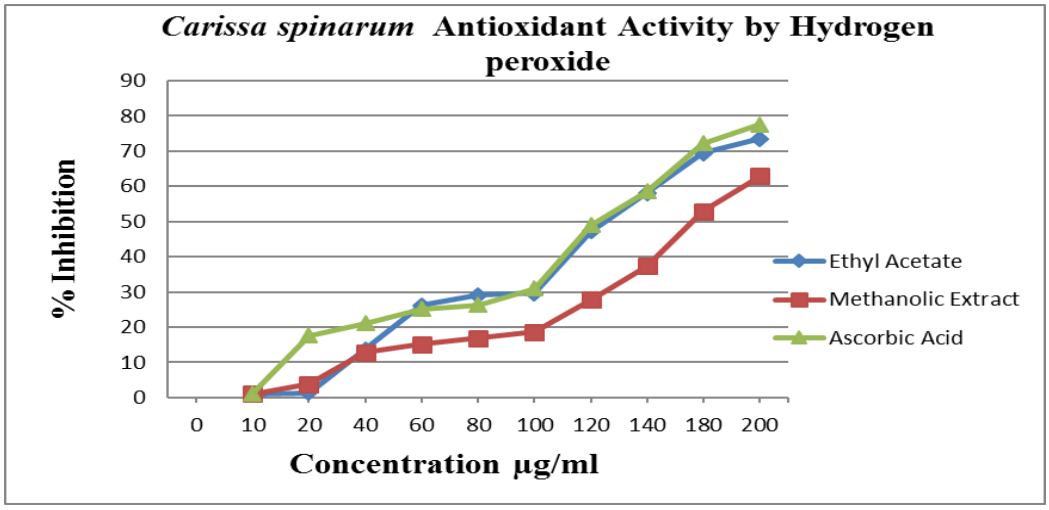

Figure No. 2: Effect of antioxidant activity of Ethyl acetate extract methanolic extract of *Carissa spinarum* & ascorbic acid *by* H_2O_2 method

Hydrogen peroxide radical scavenging activity of extracts was measured at different concentrate ions (10-200 µg/ml), and absorbance was recorded at 560 nm. The results are expressed as mean±SEM. Hydrogen peroxide radical scavenging activity of standard ascorbic acid (green), Ethyl acetate extract (blue) and methanolic extract (red) of *Carissa spinarum* extracts of radical scavenging activity.

RESULTS

Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials which are rich in phenolics and flavonoids, are increasingly being used in the food industry for their antioxidative properties and health benefits. In the present research study, the total phenolic and flavonoid content in *Carissa spinarum* has shown high phenolic and flavonoid contents. The leaf powder of *Carissa spinarum* was extracted by successive soxhlation with petroleum ether, chloroform, acetone, ethyl acetate, methanol. The results of yields were described in Table no.1.0. and the presence or absence of phytoconstituents in all the solvents are described in Table 2.0. The petroleum ether of *Carissa spinarum* extract was solid greenish colour and the yield was 7.305%w/w, chloroform extract was solid dark brown in colour and the yield was 6.02%w/w, acetone extract was solid dark brownish black in colour and the yield was 9.145%w/w, ethyl acetate was solid dark brown in colour and the yield was 19.912%w/w, methanolic extract was semisolid reddish brown in colour and the was 24.208%w/w. The total Phenolic (TPC) in ethyl acetate and methanol were 30.6341 and 36.120 and Total Flavonoid Content (TFC) in ethyl acetate and methanol 28.36 & 26.60 respectively

Phenolic compounds are important plant constituents because of their free radical scavenging ability facilitated by their hydroxyl groups and the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity [41]. Phenolic compounds are also involved in conferring plants with oxidative stress tolerance. Flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen and various other free radicals implicated in several diseases [42]. Flavonoids, on the other hand, suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species, and up-regulate and protect antioxidant defences [43].

The results Table 4.0 and in Fig 1.0 illustrates a significant decrease in the concentration of DPPH radicals due to the scavenging ability of successive ethyl acetate and methanolic extracts of *Carissa spinarum* and Ascorbic acid was exhibited 69.994±0.587,

56.314±0.639%, 93.968±0.682 % inhibition with 200 µg/ml respectively. The IC50 value DPPH radical scavenging model of ethyl acetate and methanolic extracts were found to be 114.400±0.281 and 119.221±0.342 and 48.120 ±0.231µg/ml respectively. The activity was attributed to the phytoconstituents in the plant.

The results in Table 5.0 and Fig 2.0 illustrates Hydrogen peroxide radical scavenging activity with a significant decrease in the concentration of free radicals due to the scavenging ability of successive extracts of *Carissa spinarum* and Ascorbic acid was exhibited as 73.464±0.602, 62.871±0.428, 77.624±1.706% inhibition with 200 µg/ml respectively. The IC50 value hydrogen peroxide radical scavenging model of ethyl acetate and methanolic extracts and ascorbic acid were found to be 124.530±0.942, 164.560±0.980, 122.654±0.610µg/ml respectively. Thus ethyl acetate extract of *Carissa spinarum* showed comparatively higher antioxidant activity than the methanolic extract of *Carissa spinarum*, which is in accordance with the total phenolic and flavonoid content of the two extracts, Thus ethyl acetate and methanolic extract of *Carissa spinarum* is having stronger antioxidant activity, the activity was due to the phytoconstituents in the plant.

DISCUSSION

Medicinal plants have become very popular because they have very few side effects comparing synthetic drugs. Phytochemical compounds were studied because they are highly abundant in nature and often used as parts of defence mechanisms in plants. Free radicals are often generated as by products of biological reactions or from exogenous factors. In present study, the total phenolic and flavonoid content of methanolic extract of *Carissa spinarum* was determined and the extract showed high phenolic and flavonoid content. Antioxidant activity of these crude extracts may be attributed to the high phenolic and flavonoid contents in the plant.

In this study, the ethyl acetate extract showed comparatively higher antioxidant activity than the methanolic extract of *Carissa spinarum*, which is in accordance with the total phenolic and flavonoid contents of the extract. In summary of results demonstrated that the ethyl acetate and methanol extracts of *Carissa spinarum* had high amount of flavonoids and phenolic contents which revealed that the *Carissa spinarum* has a potent antioxidant activity as measured by DPPH and hydrogen peroxide radical scavenging activity, of the extracts ethyl acetate and methanolic extracts showed good response towards antioxidant activity. Therefore ethyl acetate and methanolic extracts were potent plant extracts for the

development of new drugs for preventing cell damage caused by free radical exposures thus active ingredients in the polar solvent fractions are proved as biologically significant. Hence suggesting that the *Carissa spinarum* is a potential source for antioxidant molecules. The plant can be used as natural antioxidants and preservative in food industries as well as in non-food systems.

CONCLUSION

The results demonstrated that the ethyl acetate and methanolic extracts of *Carissa spinarum* has high amount of flavonoids and phenolic contents. The *Carissa spinarum* has a potent antioxidant activity revealed by DPPH and hydrogen peroxide radical scavenging activity, of ethyl acetate and methanolic extract. The plant would be helpful as an antioxidant and as a free radical scavenging agent, therefore ethyl acetate and methanolic extracts are potent plant extracts for development of new drugs in preventing cell damage caused by free radical exposure. The results are more specific and showed good response towards antioxidant activity. A positive, significant linear relationship was established between antioxidant activity and compounds in the plant. The present study revealed the significant antioxidant activity of the plant *Carissa spinarum.*

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION STATEMENT

1. Conception or design of the work- PRATHIBHA BHARATHI MARE *
2. Data collection- PRATHIBHA BHARATHI MARE and SARITHA KALAMALLA
3. Data analysis and interpretation- PRATHIBHA BHARATHI MARE and ALAGARSAMY VEERACHAMY
4. Drafting the article- SIVA PRASAD SAGILI
5. Critical revision of the article - VISHAKA KULKARNI

6. Final approval of the version to be published - PRATHIBHA BHARATHI MARE

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