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ANTIOXIDANT ANALYSIS OF *CARISSA CARANDAS* LINN PLANT LEAVES

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Abstract:

Many chemical species are involved in the control of oxidative chemical processes in all biological systems, and these are often referred to as an antioxidant. Oxygen is an essential component for all living organism. A part of oxygen taken into living cells is converted into a severe harmful reactive oxygen species(ROS) and free radicals. The excessive production of free radicals or deficiencies in antioxidant defenses leads to the appearance of "oxidative stress". These harmful effects are balanced by the action of enzymic and non-enzymic antioxidants. In this study we analyzed the presence of enzymic antioxidants like catalase, peroxidase and glutathione-s transferase and non-enzymic antioxidant content like ascorbic acid, tocopherol, total carotenoids, lycopene, total phenols, reduced glutathione and chlorophyll. The results of the present study showed Carissa carandas leaves possessed considerable levels of both enzymic and non-enzymic antioxidants.

Keywords: *Oxidative stress,Antioxidants,Enzymic and Non-enzymic antioxidant, Carissa carandas leaves.*

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INTRODUCTION:

Medicinal plants have been found as important contributors to the pharmaceutical, agriculture and food industries. With the onset of the synthetic era, pharmaceutical industries are producing a lot of synthetic drugs that help to alleviate the chronic diseases. With the passage of time many problems associated with frequent use of synthetic drugs like severe side effects and microbes are resistance to these synthetic drugs. In recent times research on medicinal plants has been intensified all over the world. The natural pharmaceuticals are receiving extraordinary importance and popularity as safe, efficacious, cost effective medicines with benefits due to combination of medicinal ingredients with vitamins and minerals.[1] Recently there is an emerging trend in research to support the biological activities of medicinal plants. Many scientific researches have been reported about the efficacious and chemotherapeutic role of medicinal plants in the treatment of diverse diseases like cancer, diabetes mellitus and cardiovascular diseases.[2]Free radicals are continuously produced by the body's normal use of oxygen. The excessive production of free radicals or deficiencies in antioxidant defenses leads to the appearance of "oxidative stress".[3] Antioxidants are substances that prevent damage to cells caused by free radicals and search for free radicals, lend them electron which stabilizes the molecule, thus preventing damage to other cells. They also have the ability to repair previous damage to cells. These antioxidants are found naturally in fruits and vegetables.[4] An ideal antioxidant should be readily absorbed and quench free radicals and chelate redox metals at physiologically relevant levels.Dietary antioxidants may be required to maintain optimal cellular function. The most efficient enzymatic antioxidants involve glutathione peroxidase, catalase and superoxide dismutase. Non-enzymatic antioxidants include vitamin-E, vitamin-C, thiol antioxidants, melatonin, carotenoids, saponins, flavonoids and tannins.[5] With this backdrop, the present study was formulated to analyze the antioxidant potential of *Carissa carandas* leaves.

Carissa carandas Linn is a member of Apocynaceae family and has climbing shrub, usually growing to 10 or 15 feet (3-5 m) high.This plant is widely cultivated throughout the tropical and sub-tropical regions. In Jharkhand, Bihar, Rajasthan and other near states commonly known as ‘Karunda’or ‘Jasmin flower Carissa’ has been proven multipurpose tree.[6]

Carissa carandas is traditionally used for the treatment of poor digestion, acidity and wounds. The roots are used as an anthelmintic, stomachic and anti-scorbutic and for the treatment of intestinal worms, scabies and pruritus; they are also used to reduce high blood pressure. The pharmacological properties of *Carissa carandas* have reported to show anticonvulsant,[7] analgesic, anti-inflammatory, antipyretic, [8] antibacterial, antifungal,[9]hepatoprotective, [10] acute hypotensive, [11] and anti-cancer activities,[12] Most antioxidant studies were conducted with roots, fruit and bark, whereas leaves are less explored. This study was designed to investigate the antioxidant properties of *Carissa carandas* leaves.

MATERIALS AND METHODS:

Plant material:

The plant *Carissa carandas* was collected from the University of Agricultural Science, GKVK, Hebbal, Bengaluru. The leaves were procured fresh for the estimation of each parameter. They were washed free of surface contaminants in running water and blotted dry between the folds of soft tissue paper.

Preparation of sample:

For the enzymatic and non-enzymatic antioxidant content, the fresh leaves extract of Carissa *carandas Linn* are prepared by using simple maceration method.

ENZYMIC ANTIOXIDANT

The enzymic antioxidants analyzed were Catalase, Peroxidase and Glutathione S-transferase(GST).The enzyme-catalyzed decomposition of H_2O_2 was measured spectrophotometrically by the method of Luck (1974).[13] The peroxidase activity was assayed by the method of Reddy *et al.* (1985).[14] The method proposed by Habig *et al.* (1974)[15] was adopted for assaying the activity of GST.

NON-ENZYMIC ANTIOXIDANTS

The non-enzymic antioxidants analyzed in the leaves of *Carissa carandas* are ascorbic acid, tocopherol, total carotenoids, lycopene, total phenols, flavonoids, reduced glutathione and chlorophyll. The ascorbic acid levels were estimated by the method of Roe and Keuther (1943).[16] The tocopherol content was estimated using Emmerie- Engel reaction as explained by Rosenberg (1992).[17]The method described by Zakaria *et al.* (1979) [18] was followed for the estimation of total carotenoids and lycopene.The levels of total phenols were assayed using the method of Mallick and Singh (1980).[19]The flavonoid content was quantified by the method of Cameron *et al.* (1943).[20]Moron *et al.* (1979)[21] have proposed a method for the determination of reduced glutathione, which was adopted for this study.The chlorophyll content was estimated by the method proposed by Witham et al. (1971). [22]

RESULT AND DISCUSSION:

In recent years there is an upsurge in the areas related to newer developments in prevention of disease especially the role of antioxidants.[19] So medicinal plants are the richest source of antioxidants with lot of health benefits without side-effects. Our aim is to bring the unexplored plants to the light and utilize it fully to solve health issues.The present study was formulated to generate information about the potential antioxidants (enzymic and non-enzymic).

ANTIOXIDANT STATUS IN THE LEAVES OF *Carissa carandas*

The enzymic (CAT, POD and GST) and non-enzymic (ascorbate, tocopherol, total carotenoids, lycopene, reduced glutathione, chlorophyll, total phenols and flavonoids) antioxidants were analyzed in the leaves of *Carissa carandas* and the results obtained are presented in Table 1 and Table 2.

TABLE 1: ENZYMIC ANTIOXIDANT ACTIVITIES IN THE LEAVES OF *Carissa carandas*

Enzymes	*Carissa carandas* leaves
Catalase (U#/g)	14.82 ± 0.66
Peroxidase (U@/g)	1.74 ± 0.017
Glutathione s transferase(U$/g)	0.0085 ± 5.77

The values are Mean ± Standard deviation of triplicates

#1 Unit = Amount of enzyme required to decrease the absorbance at 240 nm by 0.05units/minute

@ 1 Unit = Change in absorbance at 430 nm /minute

$1 unit = nmoles of CDNB conjugated / minute

Catalase is a tetramer of four polypeptide chains it decomposes hydrogen peroxide into oxygen and water($2H_2O_2 \rightarrow O_2 + 2H_2O$).[23]It is present in all prokaryotes and eukaryotes and it is believed to play a role in cellular antioxidant defense mechanisms by limiting the accumulation of H_2O_2[24] and also fight against oxidative stress *Coffea arabica* and *Coffea canephora* of green coffee samples has been reported to have high catalase activity[25]whereas it's activity is declined in *Triticum aestivium* leaves upon senescence.[26]

The leaves of *carissa carandas* plant in the present study, showed considerable activity of catalase which play an important role in cellular oxidation and fight against oxidative stress.

Glutathione-S-transferase is a glutathione-dependent antioxidant enzyme that shows high activity with lipid peroxides.[24]This enzyme plays a critical role in protecting tissues against the products of oxidative stress and electrophiles and the protective effect of many naturally occurring chemo preventive agents against carcinogenesis have been ascribed to decreased bioavailability of potential DNA damaging entities and their destruction into excretable metabolites, facilitated through the induction of GST.

The leaves of *carissa carandas* plant in the present study, showed substantial activity of peroxidase and glutathione S-transferase which play an important role in eliminating free radicals.

TABLE 2: NON-ENZYMATIC ANTIOXIDANTS LEVELS IN THE LEAVES OF *Carissa carandas*

Parameters	***Carissa carandas* leaves**
Ascorbic acid (mg/g leaf)	4.51 ± 0.01
Alpha Tocopherol (µg/g leaf)	278 ±0.57
Carotenoids (mg/g leaf)	127 ± 0.85
Lycopene (mg/g leaf)	1.16 ±0.06
Total Phenols (mg/g leaf)	109.4 ± 0.4
Flavonoids(mg/g leaf)	14.7 ± 0.07
Reduced Glutathione(nmoles/g leaf)	235.2 ± 0.29
Chlorophyll (mg/g leaf)	16.9 ± 0.04

The values are Mean ± Standard deviation of triplicates

Ascorbic acid (vitamin C) is an important water-soluble antioxidant and thus works in aqueous environments of the body. Vitamin C cooperates with Vitamin E to regenerate α-tocopherol from α-tocopherol radicals in membranes and lipoproteins [28]and also raises intracellular glutathione levels thus playing an important role in protein thiol group protection against oxidation.[29] Methanolic extracts of *Cissus quadrangularis* showed high levels of ascorbic acid(479mg/100g).[30]

In the present study the plant *Carissa carandas* leaves contain 4.51 ± 0.01mg/g leaf of ascorbic acid,which plays an important role by protecting against oxidation.

Tocopherol is a fat-soluble vitamin existing in eight different forms. In humans, α-tocopherol is the most active form, and is the major powerful membrane bound antioxidant employed by the cell [31]. The main function of Vitamin E is to protect against lipid peroxidation.[32] and there is also evidence to suggest that α-tocopherol and ascorbic acid function together in a cyclic-type of process.During the antioxidant reaction, α-tocopherol is converted to a α-tocopherol radical by the donation of labile hydrogen to a lipid or lipid peroxyl radical, and the α-tocopherol radical can therefore be reduced to the original α-tocopherol form by ascorbic acid.[33]In the present study the plant *Carissa carandas*leaves contains 278 ±0.57µg/g leaf of alpha tocopherol.

Lycopene and β-carotene are compounds called carotenoids, which are highly colored pigments that help protect to plants against damage from sunlight. Carotenoids are important to humans because they have antioxidant activity and prevent free radicals from causing harm to the body. Lycopene is a carotenoid present in many fruits and vegetables that has potent antioxidative properties. Intake of vegetables and fruits rich in carotenoids, including lycopene might be a protective factor against hyperglycemia.

In the present study the plant *Carissa carandas* leaves contains carotenoids 127 ± 0.85mg/g of leaf and lesser amount of lycopene that is 1.16 ±0.06mg/g of leaf.

Phenolic compounds or polyphenols are ubiquitous in plants with more than 8000 structure reported. Antioxidant mechanisms of polyphenolic compounds are based on hydrogen donation abilities and chelating metal ions.[34] However, phenolic compounds act as pro-oxidants under certain conditions, such as high concentrations of phenolic compounds or metal ions, and high pH. Chemical structures also affect the antioxidant activities.Flavonoids are phenolic substances isolated from wide range of vascular plants.The antioxidant activity of flavonoid is due to their ability to reduce free radical formation.

In the present study the plant leaves show 109.4 ± 0.4mg/g leaf of phenolic compounds and also exhibit 14.7 ± 0.07mg/g of flavonoid, which plays important role in reducing free radical formation and to scavenge free radicals.

The reduced form of glutathione is GSH, glutathione, whilst the oxidized form is GSSG, glutathione disulphide. The antioxidant capacity of thiol compounds is due to the Sulphur atom, which can easily accommodate the loss of a single electron.[35]

The present study of plant *Carissa carandas* leaves shows highest levels of reduced glutathione that is235.2 ± 0.29nmole/g and 16.9 ± 0.04mg/g of chlorophyll.

CONCLUSION:

The present study mainly focused on *in vitro* methods of antioxidant evaluation. Our results revealed that the *Carissa carandas* leaves were found to be a rich source of both enzymic and non-enzymic antioxidant.

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