INTERNATIONAL JOURNAL OF ADVANCE RESEARCH AND DEVELOPMENT

(Volume 4, Issue 1)

Available online at: www.ijarnd.com

Anti-oxidant, anti-diabetic activity and DNA damage inhibition activity of carissa carandas fruit

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ABSTRACT

Carissa carandas (C. carandas) fruits are well known ancient natural medicine owning high antioxidant and antidiabetic activity. It is a straggly, woody, climbing shrub rich in white gummy latex with sharp thornsand belonging to the family Apocynaceae. Because of its eminent medicinal property, the present study was conducted to investigate the antioxidant and antidiabetic properties of C. carandas fruit along with DNA damage inhibition assay. C. carandas type 1 (T1) and type 4 (T4) fruits extract were prepared in methanol. In vitro, antioxidant activity was evaluated by four vitro assays viz. DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay, FRAP (Ferric Reducing Antioxidant Power) assay and ABTS (2, 2'-azinobis (3-ethyl benzothiazoline-6-sulfonic acid) radical cation depolarization assay. Further anti-diabetic activity was also investigated by performing Alpha-Glucosidase Inhibitory Assay. The extracts scavenged DPPH radicals efficiently. The scavenging effect was observed in case of T1 extract little higher than T4 extract. FRAP scavenging activity and also ABTS assay for methanolic extract of C. carandas were also analyzed, where result showed to be the activity of T1 was observed more than T4. The effect of C. carandas fruit extract on the activities of α-amylase and β–glucosidase were evaluated to determine anti-diabetic activity. The T1 fruit extract was shown formidable inhibition of β–glucosidase and alpha-amylase activity over T4. Green and pink coloured fruit extracts were used in DNA damage inhibition assay, resulted in green coloured fruit has more antioxidant activity over pink and showed potent inhibition of DNA damage.

Keywords— *Carissa carandas, Fruit extract, Anti-oxidant activity, Anti-diabetic activity, DNA damage inhibition*

1. INTRODUCTION

Diabetes mellitus (DM) is a major endocrine disorder, affecting approximately 5% of the world's population. Diabetes is characterized by abnormalities in carbohydrates, lipid and lipoprotein metabolisms, which not only lead to hyperglycemia but also causes many complications such as hyperlipidemia, hyperinsulinaemia, hypertension and atherosclerosis (C.Unachukwu *et al*.,2012). Various medicinal properties have been attributed to natural herbs. Medicinal plant constitutes the main source of new pharmaceuticals and healthcare products. The history of plants being used for the medicinal purpose is probably as old as the history of mankind. One of the surveys conducted by the WHO reports that more than 80% of the world's population still depends upon the traditional medicines for various diseases.

The use of herbs in the management of diabetes mellitus has been prevalent in Indian society from a long time and there are several medicinal plants have reported lowering the blood sugar level (V. Devmurari *et al*., 2010). Drugs have been derived either directly or indirectly from plants. Some plant product act by lowering the level of glucose in the blood while others act by inhibiting glucose absorption from the gut and hence prevent the surge in blood glucose that can occur immediately after the meal. Plants contain phyto molecules including alkaloids, flavonoids, saponins, glycosides, dietary fibres, peptidoglycans, polysaccharides, glycolipids, amino acids, carbohydrates and others which can act as potent hypoglycemic agents (Devmurari V. P. *et al*., 2010).

Carissa carandas (Apocynaceae), commonly known as Karanda, is a widely used medicinal plant. *C. carandas* is large dichotomously branched evergreen shrub with a short stem and a strong thorn in pairs. This species is a rank-growing, straggly, woody, climbing shrub, usually growing to 10 or 15 feet (3-5 m) high, sometimes ascending to the tops of tall trees (Chanchal Mishraa *et al*., 2013). The fruits, leaves, barks, and roots of *C. carandas* have been used for ethnomedicine in the treatment of human diseases, such as diarrhoea, stomachic, anorexia, intermittent fever, mouth ulcer and sore throat, syphilitic pain, burning sensation, scabies, and epilepsy. The prominent biological activities have also been reported. That includes antidiabetic, antimicrobial, cytotoxicity, anticonvulsant, hepatoprotective, antihyperlipidemic, cardiac depressant, analgesic, anti-inflammatory, antipyretic, and antiviral properties (Bhaskar *et al*., 2009, Hegde *et al*., 2009, Itankar *et al*., 2011, Agarwal T *et al*., 2012, Sumbul *et al*., 2012). Plant yielded major bioactive compounds, i.e., alkaloids, flavonoids, saponins, large amounts of cardiac glycosides, triterpenoids, phenolic compounds, and tannins. Roots yield volatile principles including 2-acetyl phenol, lignan, carinol, sesquiterpenes (carissone, carindone), lupeol, β-sitosterol, 16 β-hydroxybetulinic acid, α-amyrin, β-sitosterol glycoside, and des-

Nmethylnoracronycine. Leaves yield triterpenoid constitutes as well as tannins. Fruit is a rich source of iron, with a fair amount of vitamin C. Mature fruit, high in pectin, is useful for making jellies, jams, squash, syrup and chutney (Wani R. A *et al*., 2013). Thus *c. carandas* is a useful food and medicinal plant of India found to be widely distributed throughout the subtropical and tropical region. The plant has been used as a traditional medicine over thousands of years in the ayurvedic, unani, and homoeopathic system of medicine. Traditionally, whole plants and its parts were used in the treatment of various ailments (Debasish Panda *et al*., 2014).

2. TRADITIONAL USES

The *C. caranda* plant is commonly used as a condiment in Indian spices and cold beverages. The sweeter types may be eaten raw but the more acid ones are best stewed with plenty of sugar. Unripe fruit is a good appetizer; astringent, antiscorbutic, cooling, acidic, stomachic, anthelmintic and leaf decoctions are given in the commitment of remittent fever (Trivedi *et al*., 2004). Leaf extract is externally applied for curing leprosy. Two drops of plant oil are given with half a cup of honey for controlling worms of minors (Trivedi *et al*., 2007). Traditional healers of Chhattisgarh use the different plant parts to cover the cancerous wounds and to kill the maggots. Karonda is mainly used for making pickle, jelly, jam, squash, syrup and chutney at industrial scale. The ripe fruit emits gummy latex when it is cooked, but yields a rich red juice which becomes clear when it is cooled, so this is used as a refreshing cooling drink in summer. It is also sometimes substituted for apples to make an apple tart, with cloves and sugar to flavour the fruit. In many parts of India, fruits are commonly used with green chillies to make a tasty dish taken with chapattis (Siddiqui *et al*., 2003). In Konkan, India, the root is pulverized with horse urine, lime-juice and camphor as a remedy for the itch (Khare *et al*., 2007).

Ambika Chauhan *et al*., (2015), are demonstrated that daily intake of fruits is associated with the diminution of chronic degenerative disease and the current study aimed to make consumers aware about the effect of processing methods on the nutritional value of caranda fruit. In this study, two samples of sweet *C. carandas* fruit was taken for the detection of antioxidant activity, anti-diabetic activity and DNA damage inhibition assay. Samples were named as T1 and T4 on the basis of trees. There were 6 types of sweet *C. carandas* tree, which differ by the 6 types of accession numbers. Here we have undertaken the study that includes extraction of antidiabetic, antioxidant and DNA damage inhibition substance from C. *carandas* fruit.

3. MATERIALS AND METHOD

All the materials and chemicals were used as received. All the standard company chemicals having ISI mark were purchased from Jain chemicals Shimoga, Karnataka, India. Fruit samples were collected from Malnad regions of Shimoga district during the month of May 2016. The plant material was identified with the help of members from Department of Botany, Kuvempu University, Shankaraghatta and was stored in 4ºC for further studies.

3.1 Preparation of methanolic extract from the fruit of the *C. carandas*

20g of fruit material was homogenized with 80% methanol and kept it for overnight. Later the solution was centrifuged. The final volume was made upto150ml with distilled water.

3.2 Anti-oxidant activities of *Carissa carandas*

3.2.1 DPPH radical scavenging activity: 5g of the sample was homogenized with 20ml of methanol (80% v/v) in a pestle and mortar. To 0.2ml of extract, 0.3 ml of acetate buffer and 2.5 ml of DPPH solution were added. The reduction in colour was measured spectrophotometrically at 517nm (Ashwini U *et al*., 2014, B. Avinash *et al*., 2017). The absorbance of DPPH solution without sample was also measured. The difference in the absorbance of DPPH solution with and without sample was calculated. The decrease in absorbance with sample addition was used for calculation of anti-oxidant activity. The standard curve was prepared with different concentrations of ascorbic acid (20-100 µg/ml) and results were expressed as ascorbic acid equivalent antioxidant capacity (AEAC) (Raghavendra *et al*., 2018).

Calculation

$$\text{DPPH (Radical scavenging assay)} = \frac{\text{OD517} \times \text{Std. value} \times \text{Total volume} \times 100}{\text{Assay value} \times \text{Wt of sample (g)} \times 1000}$$

$$\%\ \text{inhibtion} = \frac{(\text{AC} - \text{AS})}{(\text{AC})} \times 100$$

Where,
AC= Absorbance of control
AS=Absorbance of sample

3.2.2 Frap assay: 5g of sample homogenized with 20ml of methanol (80%v/v) in a pestle and mortar. 0.2ml of extract were taken in test tubes, to that 1.8ml of FRAP reagent was added. The mixture was incubated at room temperature for 40 min and the absorbance was measured at 593nm. The standard curve was developed with different concentration of ascorbic acid (20-100 µg/ml). The result was expressed as AEAC (Md. Irshad *et al*., 2012).

Calculation

$$\text{Antioxidant capacity (mg AEAC/100g)} = \frac{\text{Std. OD} \times \text{Sample} \times \text{Total voume} \times 100}{\text{Assay volume} \times \text{Wt of the sample (g)} \times 1000}$$

3.2.3 ABTS (2, 2'-azinobis (3-ethyl benzothiazoline-6-sulfonic acid) radical cation depolarization assay: $ABTS^{+}$ radical cation was obtained by adding potassium persulfate solution to ABTS solution. This mixture was stored in dark at room temperature for 12-16 hours before use. Before the assay, the mixture was diluted with methanol to give an absorbance of 0.70+0.02 at wavelength 734nm (Shalaby *et al*., 2013). The radical is stable for more than two days when stored in dark at room temperature. Add 30µl of plant extract in methanol to 3ml of the ABTS solution and read the absorbance at 30ºC exactly 1 minute after initial mixing and up to 6 minutes. Samples have to be diluted approximately to get the % inhibition of 20-80%. Appropriate solvent blanks should be run in each assay. The analysis is done in triplicate. The percentage inhibition of absorbance at 734nm is calculated and plotted as a function of the concentration of trolox (Raghavendra *et al*., 2018).

$$\%\ \text{inhibition}\ (\lambda\ 734\text{nm}) = \frac{1 - \text{Absc}}{\text{Abs0}} \times 100$$

Where,
Abs0 – absorbance of an uninhibited radical solution
Absc – absorbance measured in 1 min after addition of compound to assay

The antioxidant activity of samples is expressed as ascorbic acid equivalent antioxidant capacity (AEAC). A standard curve can be developed using acid (0-20µg). The calculations of the samples were read against the standard curve expressed as µM AEAC /100g.

3.3 Antidiabetic activity of *Carissa carandas*

3.3.1 Alpha-Amylase Inhibitory Assay: This assay was carried out using a modified procedure of McCue and Shetty *et al*., (2004). A total of 250µl of extract (1.25-10 mg/mL) was taken in a tube and 250µ1 of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/mL) was added. This solution was pre-incubated at 25ºC for 10 min, after which 250µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals and then further incubated at 25ºC for 10 min. The reaction was terminated by adding 500µl of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 5 mL of distilled water and the absorbance was measured at 540nm using spectrophotometer (Wickramaratne *et al*., 2016). A negative control was prepared using the same procedure replacing the extract with distilled water. The amylase inhibitory activity was calculated as a percentage of inhibition formula.

$$\text{Inhibition}\ (\%) = \frac{\text{Abs 410 (control)} - \text{Abs 410 (extract)}}{\text{Abs 410 (control)}} \times 100$$

3.3.2 Beta-Glucosidase Inhibitory Assay: The effect of the plant extracts on glucosidase activity was determined according to the method described by Kim *et al*., (2005), using α-glucosidase from Saccharomyces cerevisiae. The substrate solution p-nitrophenylglucopyranoside (pNPG) was prepared in 20mM phosphate buffer, and pH 6.9. 100 µL of glucosidase (1.0 U/mL) was preincubated with 50µL of the different concentrations of the extracts (acetone, ethanol, and water) for 10 min. Then 50 µl of 3.0 mM (pNPG) as a substrate dissolved in 20 mM phosphate buffer (pH 6.9) was added to start the reaction. The reaction mixture was incubated at 37ºC for 20min and stopped by adding 2mL of 0.1 M Na_2CO_3. A negative control was prepared using the same procedure replacing the extract with distilled water. The glucosidase activity was determined by measuring the yellow-coloured para nitrophenol released from pNPG at 405nm (Telagari *et al*., 2015).

3.4 DNA damage inhibition

Free radicals can both initiate and exacerbate several diseases. Hydroxyl free radicals are well known to damage cellular DNA in humans, and even partial damage to DNA can make a cell cancerous. DNA damage inhibition by the methanolic extract of *C. carandas* leaves is shown in fig.6, which is the electrophoretic pattern of pBR322 DNA after UV photolysis of H_2O_2 in the absence (control C and R) and presence (sample) of the extract. Control pBR322 showed a band of supercoiled and open circular plasmid DNA on agarose gel electrophoresis (Verma *et al*., 2015). UV photolysis of H_2O_2 damages the entire DNA (no band visible). The extract displayed considerable protective activity, with resulting bands of supercoiled and open circular plasmid DNA (Sanjay Guleria *et al*., 2017). The result infers that UV-photolyzed H_2O_2(3%) treatment of pBR322 entirely obliterated the DNA (in R), while 50g of methanolic extract of *C. carandas* leaves protected against free radical-mediated DNA damage(S). These results demonstrated the DNA damage inhibition potential of this extract, which might, therefore, be used in future to prevent cancer. Other plants have been reported to protect against free radical-mediated DNA damage (Shameem *et al*., 2015).

4. RESULT AND DISCUSSION

4.1 Anti-oxidant activity

4.1.1 DPPH: DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radical is determined by the decrease in absorbance at 517nm induced by antioxidants (Aniruddha Sarma *et al*., 2015). The extract can able to reduce the stable radical DPPH to the yellow colored diphenypicrilhydrazine solution (Wickramaratne *et al*., 2016).

Table 1: Percentage inhibition of DPPH in *C. carandas* fruit extract

Sample	DPPH % inhibition
Control	69.4
T1	34.11
T4	33.14

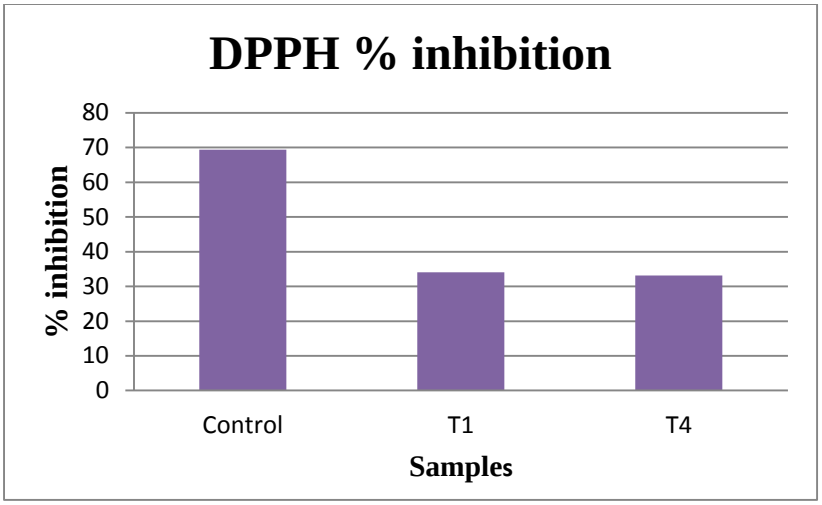

Fig 1: DPPH assay

DPPH activity in a methanolic extract of T1 was found to be more compared to T4 and it is expressed in mg/100g FW. It is demonstrated that H-donor activity where it reduces the radical to the corresponding hydrazine. DPPH radicals react with a suitable reducing agent, the electron becomes paired off and the solution loses color stoichiometrically depending on the number of electrons taken up. The DPPH radical scavenging activity for control, T1 and T4 sample of fruit showed the inhibition of 69.4%, 33.14% and 34.11% respectively. Ascorbic acid was taken as standard. Verma *et al*., (2011) reported the increased mount of activity when we increase the concentration of extract. They worked on karanda leaf extract but fruits are preferable one. That we proved in our work. According to Jacobek *et al*., (2007), the antioxidant activity of investigated fruits, blueberry, elderberry and black currant can be regarded as good candidates in the production of health beneficial functional foods. The result obtained in this study are more efficient and higher when compare to other fruits like strawberries in different regions that are explained by Dragović-UzelacVerica *et al.,* (2007).

4.1.2 FRAP

Table 2: FRAP inhibition assay in *Carissa carandas* fruit extract

Sample	FRAP(mg/100g FW)
Control	97.3
T1	90.85
T4	83.57

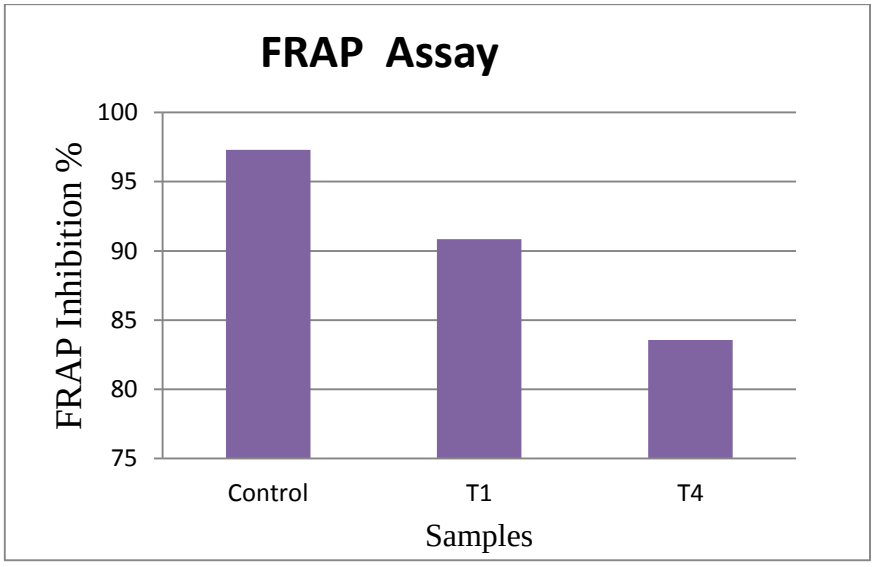

Fig. 2: FRAP assay

Ferric reducing anti-oxidant reducing assay (FRAP) was examined in the methanolic fruit extracts using the FRAP reagent and is expressed in terms of ascorbic acid equivalent. The values obtained from the FRAP assay are expressed as mg of ascorbic acid/g of extract and are in table 2.FRAP scavenging activity in a methanolic extract of *Carissa carandas* was analyzed, where T1 was observed more than T4. This assay gives an indication of the reducing ability of free radical in the fruit extract. This was a colorimetric method based on the reduction of ferric tripyridile triazine (TPTZ) complex to its ferrous form. This reduction gives an intense blue complex with an absorption maximum at 593nm. It inhibits free radical formation. Dragović-UzelacVerica *et al*., (2007) conducted studies on the antioxidant capacity of strawberries and obtained FRAP results are not much efficient when compare to ABTS assay. We can conduct further purification process to our sample in order to get increased activity and that will be much more efficient when compared to the control. Because the obtained results were eminent that is very near to the values of control though we performed studies using crude extract.

4.1.3 ABTS (2, 2'-azinobis (3-ethylbenzothiozoline-6-sulfonic acid) radical cation depolarization assay

Table 3: ABTS inhibition assay in the fruit of *Carissa carandas* fruit

Sample	ABTS (inhibition %)
Control	98.8
T1	95.6
T4	75.94

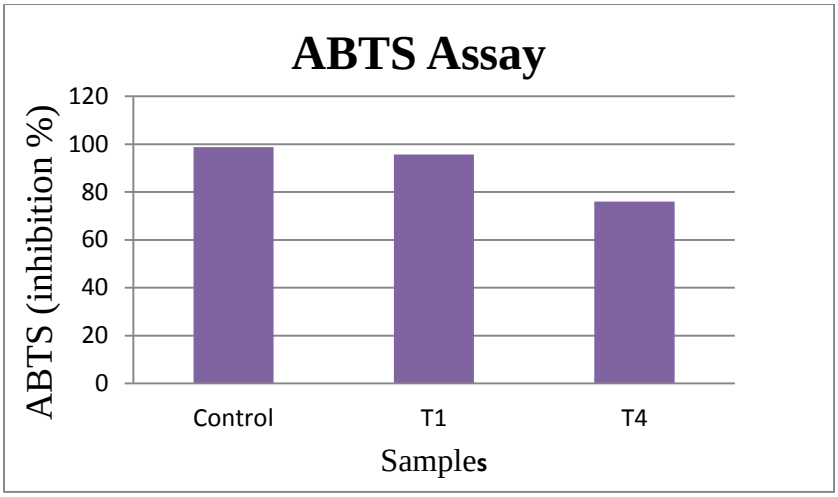

Fig. 3: ABTS inhibition assay

ABTS inhibition assay for methanolic extract of the fruit sample is in table 3. ABTS assay was examined in the methanolic fruit extract using the ABTS solution and is expressed in terms of ascorbic acid equivalent. Figure 3 shows the ABTS inhibition assay for the methanolic extract of *Carissa carandas*, where percentage inhibition of T1 was 95.6% and T4 was 75.94%.

ABTS is also frequently used by the food industry and agricultural researcher to measure the antioxidant capacities of foods. In this assay, ABTS is converted to its radical cation by the addition of sodium persulfate. This radical cation is blue in color and absorbs light at 734nm. The ABTS radical cation is reactive toward most antioxidants including phenolics, thiols, and vitamin C. During this reaction, the blue ABTS radical cation is converted back to its colorless neutral form. The reaction may be monitored spectrophotometrically. This assay is often referred to as the various antioxidants tested are compared to that of trolox which is a water-soluble analog of vitamin E (Stephanie *et al*., 2009). Fruits are having high radical scavenging activity. This result was agreed with the Chew *et al*. (2011), who studied the antioxidant activity of *Asiatic pennywort* extract. But the values obtained for T1 crude extractare nearly equal the values of control, as well as T4, also having nearer value to the T1. So that the activity could be raised by further purification of this extract compare to standard and control.

4.1.4 Antidiabetic activity

Table 4: β-glucosidase inhibition of *Carissa carandas* fruit extract

Sample	β –glucosidase inhibition %
Control	0.0
T1	82.51
T4	81.30

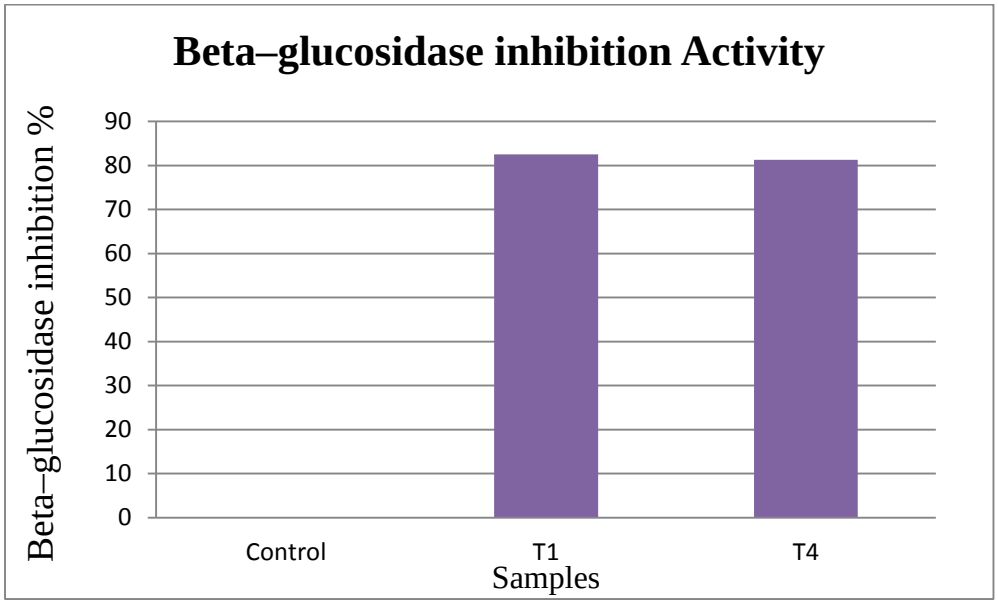

Fig. 4: β –glucosidaseinhibition Activity

Table 5: Amylase inhibition of *Carissa carandas* fruit extract

Sample	Amylase inhibition %
Control	0.0
T1	34.87
T4	30.28

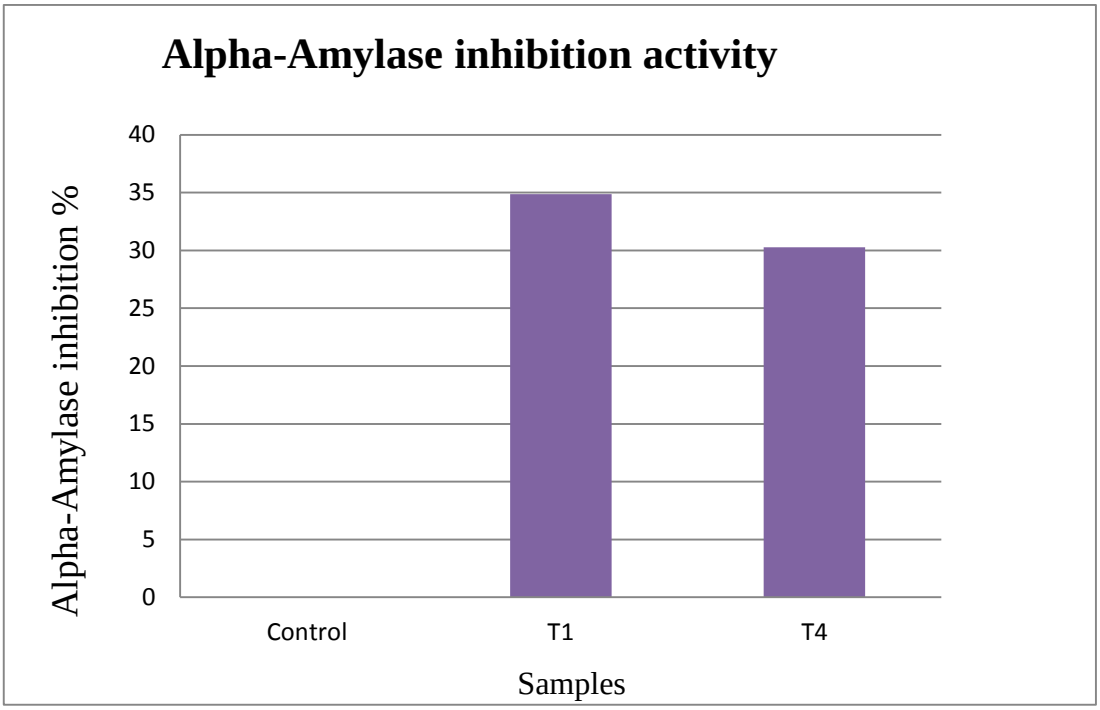

Fig. 5: Alpha-Amylase inhibition Activity

Alpha-amylase is a prominent enzyme found in the pancreatic juice and saliva which breaks down large insoluble starch molecules into absorbable molecules Inhibitors of α-amylase and β-glucosidase delay the breaking down of carbohydrates in the small intestine and diminish the postprandial blood glucose excursion. An effective means of lowering the levels of postprandial hyperglycemia has been offered by α -amylase and β-glucosidase inhibitors. β glucosidase, α-amylase and inhibition potential of the *Carissa carandas* extracts were shown in the Figure-4 figure-5 Values obtained for the inhibition activity of β-glucosidase and α amylase are given in the table-4 and table-5. Inhibitors of α-glucosidase delay the breaking down of carbohydrate in the small intestine and diminish the postprandial blood glucose excursion in a person suffering from diabetes (Itankar *et al*., 2011). One of the strategies and methods adopted to cure diabetes mellitus involves the inhibition of carbohydrate-digesting enzymes such as α-amylase and β-glucosidase in the gastrointestinal glucose absorption thereby lowering postprandial glucose level. This is an attempt to search for alternative drugs from medicinal plants with increased potency and lesser adverse effects than existing drugs. The results were expressed as a percentage of the blank control. Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined.

In this study, the effect of *C. carandas* fruit extract on the activities of α-amylase and β–glucosidase was evaluated. The fruit extract showed potent inhibition of β–glucosidase activity but α–amylase inhibition activity was not observed. As for β–glucosidase, the aqueous extract exhibits strong inhibition towards the activity of the enzyme. Natural β–glucosidase inhibitors from plants had been shown to have a strong inhibition activity against β–glucosidase and therefore could be potentially used as an effective therapy for postprandial hyperglycemia with minimal side effects. Result and the work are parallel to the data of Itankar *et al*., (2011), who studied on the anti-diabetic activity of karanda fruit.

4.1.5 DNA damage inhibition assay

The estimation of DNA damage inhibition in green unripe carandas fruit was found to be 14.98% but in pink sour fruit, there was no DNA damage inhibition. This result revealed that green fruit (GUR) has more antioxidant activity compared to pink fruit (PUR). Here the untreated DNA used as a control.

Table 6: DNA damage inhibition

	PUR	GUR	Control (Untreated DNA)
DNA damage inhibition in %	0.0	14.987%	100%

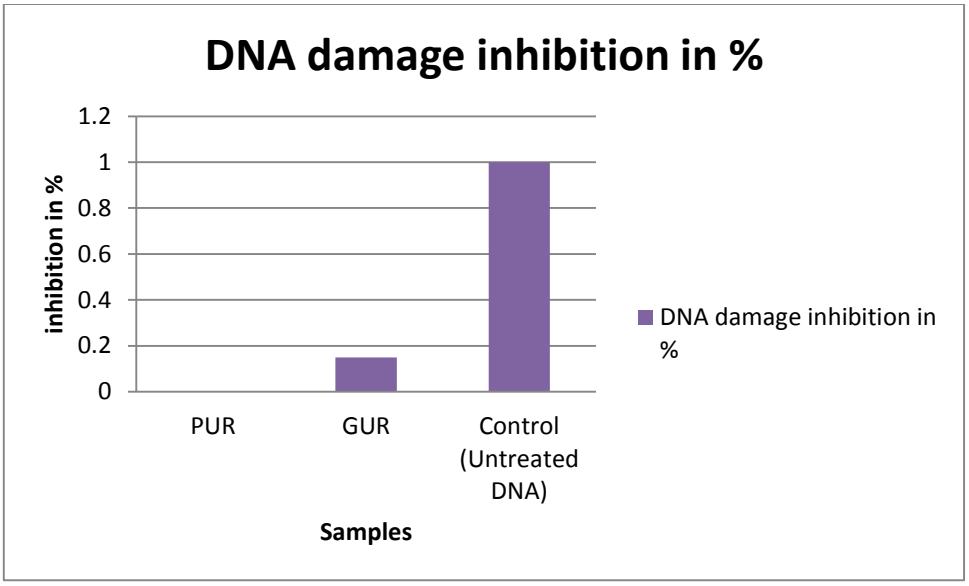

Fig. 6: DNA damage inhibition assay

The above results revealed that if there is more DNA damage inhibition then the fruit sample has more antioxidant property. Here the GUR fruit extract shows DNA damage inhibition, but in PUR fruit DNA damage inhibition was completely nil. Verma *et al*., (2011) demonstrated the antioxidant as well as DNA damage inhibition assay of *C. carandas* leaf extract but the result obtained by us with fruits are eminent than the activity of leaf extract.

5. CONCLUSION

In this study, *C. carandas* fruit was selected for the analysis of antioxidant activity, anti-diabetic activity and DNA damage inhibition activity. Two samples of sweet variety in the fruit of *C. carandas* are selected, which were indicated as T1 and T4 which differ by their tree accession number. This fruit is checked for its anti-oxidant activity like DPPH, FRAP, ABTS assays. These fruits could contribute to the antioxidant capacity of underutilized fruit. The biological activities of these two samples were analyzed for promising anti-diabetic activity by enzyme inhibition assays. The study also showed the DNA damage inhibition potential of the extract, which could be used in cancer prevention. The overall study needs further purification of active substances from this fruit. But this preliminary study of *C. carandas* extract takes us a step ahead in the process of taking a new validated treatment of a traditionally used medicinal plant. The present investigation is encouraging and could be used as an effective reference data for the standardization of medicinal properties of *C. carandas* fruit.

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