Research Article

Evaluation of Antiulcer and Antioxidant Activity of Leaves Extract of *Carissa Carandas Linn.*

Taslima Begum[1]*, Sumsunnahar Shifa[1], Rayhana Begum [1], Tamanna Sharmin Tonny[1],Farzana Afroze [1], Faruquzzaman [1], Sultana Jahan [1], Priyanka Rani Das [1], Arghya Prosun Sarkar[2]

[1]Department of Pharmacy, Primeasia University, Banani, Dhaka-1213, Bangladesh.

[2] Department of Pharmacy, Islamic University, Kushtia-7003, Bangladesh.

***Corresponding author's E-mail:** taslima.begum@primeasia.edu.bd

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ABSTRACT

Besides many other activities the necessity of investigation of antiulcer as well as antioxidant activity from natural origin has also been increased due to having certain side effects, adverse effects, drug interaction of allopathic drugs. The main objective of the present study includes investigation of antiulcer and antioxidant activities of 60 % ethanolic leaves extract of Carissa carandas Linn. Antiulcer activity was investigated using ethanolic acid induced gastric ulcer model. Carissa carandas Linn. Showed protection index of 42.45%, 47.17 % and 64.15 % at the dose of 100, 200 and 400 mg/kg respectively while standard drug omeprazole also showed a protection index of 73.59 % which were statistically more significant. The antioxidant activity was investigated using DPPH free radical scavenging assay. The IC50 values of extract as well as ascorbic acid were 15.01 µg/ml as well as 3.56 µg/ml respectively. Further studies can be carried out for identification and isolation of compounds of Carissa carandas Linn. which are responsilble for such activities.

Keywords: Carissa carandas *Linn*., antiulcer, antioxidant, ascorbic acid, DPPH.

INTRODUCTION

Treatment of various human disorder employing herbs is an old tradition. There is justification of the use of herb for the treatment of various human disorder. Due to having certain side effects, adverse effects, drug interactions of allopathic medicines the need of inquiring the traditional drugs and making them available in the market has been increased gradually.[1] From bark, leaves, roots, fruits, fruit rind, seeds etc plant parts various natural constituents can be derived. [2] Folk medicinal practitioners in Bangladesh confide on medicinal plants for major components of formulations. *Carissa carandas Linn.* is commonly found in Bangladesh.[3] It *is* an evergreen shrub of Apocynaceae family. [4] Common name of *Carissa carandas Linn.* is Koromcha in Bangla and Karanda in English.[5] Traditionally all parts of this plant are used to treat various disorder. Fruits possess astringent, antiscorbutic properties. Intermittent fever, diarrhoea, oral inflammation and earache are treated by leaves. It possesses certain notable biological properties such as cardiotonic, analgesic, antipyretic, anti-inflamatory, antirheumatic, antibacterial, antiviral, hepatoprotective, anticonvulsant, free radical scavenging, and histamine releasing properties. [6] In the treatment of mouth ulcer fruits, leaves, barks and roots have been used. [7] A common disorder of alimentary tract is peptic ulcer. [8] Proton pump inhibitors and H_2 receptor blocker are most frequently used drugs for the treatment of peptic ulcer. The development of new antiulcer drugs has rationality increased concern due to the side effects. [9] The element which scavenges free radicals is called antioxidant. By inhibiting free radicals it prevents various diseases caused by free radicals.[10] Antioxidant compounds such as phenolic acids, polyphenols, flavonoids may be obtained from natural source.[11] The main objective of the present study is to investigate antiulcer and antioxidant activities of 60 % ethanolic leaves extract of *Carissa carandas Linn.*

MATERIALS AND METHODS

Sample selection and collection

At first mature leaves of *Carissa carandas Linn.* Were selected and brought from the district of Comilla, Bangladesh to study antiulcer and antioxidant activities. After authentication of the plant, from Bangladesh National Herbarium, Dhaka, Bangladesh, an acknowledgement memorandum was stored in Bangladesh National Herbarium, Dhaka, Bangladesh (Specimen Number : 46724).

Sample preparation and extraction

The fresh mature leaves were umbrage dried for seven days to generate a grindable state. 50 gm of powdered material was drabbled into petroleum ether for 24 hrs to remove chlorophyll from the leaves. After filtration the residue powdered material was air dried and then drabbled into 60 % ethanol for another seven days. Then the from the filtrate the solvent was removed using rotary evaporator. 6.9 gm i.e. 13.8 % of crude extract was obtained which was stored in airtight container for further investigations.

Antiulcer activity

The antiulcer activity was investigated following the method of Mostofa et al, 2017.[12] Ethanolic acid was used to induce gastric ulcer and omeprazole was used as standard drug.

Animals

Wister albino rats were culled from Jahangirnagar University, Savar, Dhaka, Bangladesh. The weights of the rats were between100-140 gm. They were kept in the experimental laboratory for adjustment. 20 ± 2^0 C temperature, 44-56 % RH, light and dark cycles of 10 and 14 hours respectively were maintained in the laboratory. The rats were provided with foods prepared by Animal store of Jahangirnagar University. No unusualness was observed. The animals were fasted 12 hours before the experiments. But they were provided with water.

Making groups of animals

There were six groups having three rats in each named as follows:

Group I: Normal control

Group II: Ethanol control

Group III: Omeprazole 20 mg/kg

Group IV: 100 mg/kg *Carissa carandas Linn.*

Group V: 200 mg/kg *Carissa carandas Linn.*

Group VI: 400 mg/kg *Carissa carandas Linn.*

Induction of gastric ulcer using ethanol

60 % ethanol and 40 % 0.3 M HCl solutions were mixed to prepare ethanolic acid that was orally administered to induce gastric ulcer. The rats of group I was given 5 ml/kg distilled water. The rats of group II were given 5 ml/kg distilled water. The rats of group III, group IV, group V and group VI were given 20 mg/kg omeprazole, 100 mg/kg extract, 200 mg/kg extract and 400 mg/kg extract respectively. 30 minutes after pretreatment with the above mentioned treatment the rats of each group except group I were given 25 ml/kg of freshly prepared ethanolic acid.

Sacrificing and clipping of stomach

90 minutes after administration of distilled water to the rats of group I and 60 minutes after induction of gastric ulcer the rats of group II, group III, group IV, group V and group VI were sacrificed and the stomachs were instantly clipped and then each stomach was opened along with the greater curvature. After washing with distilled water the scoring for ulcer was made using magnifying glass.

Antioxidant activity

The antioxidant activity was investigated using DPPH (1,1-diphenyl-2- picryl hydrazyl) free radical scavenging assay following the method of Hossain et al, 2010. [13] Ascorbic acid was used as standard.

Preparation of stock solution

In 10 ml of ethanol 8 mg extract was dissolved. Few drops of twin 80 was added for better dissolution of extract. The concentration of this solution was 0.8 mg/ml i.e. 800 µg/ml which was serially diluted to have solutions of concentrations of 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml and 25 µg/ml serially.

Preparation of standard solution

In 10 ml of ethanol 8 mg of ascorbic acid was dissolved. The concentration of this solution was 800 µg/ml which was serially diluted to have concentration of 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml and 25 µg/ml serially.

Preparation of control solution

4 mg DPPH was dissolved in ethanol and the final volume was made upto 100 ml. The concentration of the solution (0.004 %) was 40 µg/ml. 3 ml of this freshly prepared solution was used as negative control.

Preparation of sample and taking absorbance at 517 nm

Within each premarked test-tube 2 ml of 0.004 % DPPH solution and 1 ml solution of extract was added from each concentration individually to make the final volume of 3 ml. Then the mixtures were kept in room temperature in a dark place for 30 minutes. After that absorbance was taken at 517 nm using UV spectrophotometer. Same procedure was followed for standard ascorbic acid solution.

The percentage inhibition was calculated from the following equation

$$\% I = \{(A_0 - A_1)/A_0\} \times 100$$

Here A_0 is the absorbance of control, A_1 is the absorbance of extract or standard solution.

RESULTS AND DISCUSSION

Ethanol induced gastric ulcer

After inquiry of gastric mucosa using magnifying glass scoring for ulcer was made from which mean ulcer index of respective group was prepared. % protection was determined from the mean ulcer index by using the following formula:

$$\%\text{Protection} = \frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

The antiulcer effect of 60 % ethanolic extract as well as standard drug omeprazole is tabulated in table 1.

Statistical analysis was done using One way ANOVA: Dunnett's test (n=3). The Mean ulcer indexwere represented as mean ± S.E.M. The results were considered as statistically significant, more significant and extremely significant when $p<0.05^{*}$, $p<0.01^{**}$, $p<0.001^{***}$ respectively. a= when compared with nomal control and b= when compared with ethanol control. Here DW= Distilled water, EA= Ethanolic acid.

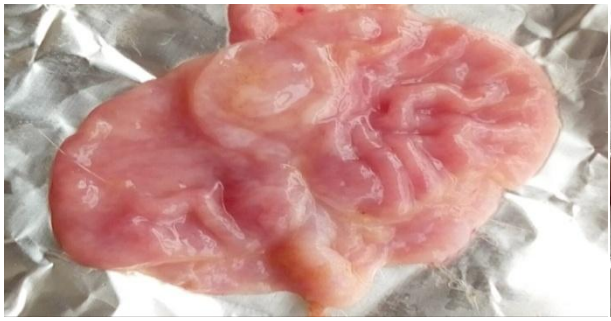
Ethanol speedily penetrates into gastric mucosa and causes damage to the plasma membrane superficially. Besides enormous build up of calcium leads to the damage of cells. As a consequence gastric ulcer results. [14, 15]

Table 1: Antiulcer effect of *Carissa carandas Linn.* compared with standard drug omeprazole

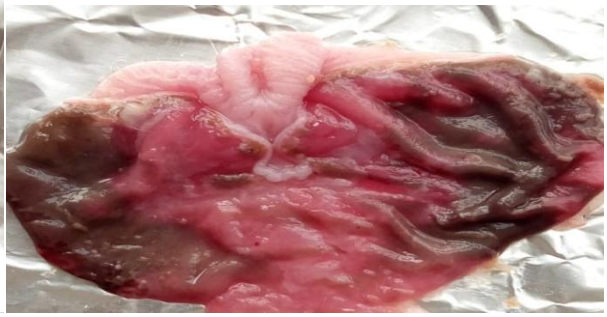
Groups	Treatment	Dose	Mean Ulcer Index	% Protection
I	DW	5 ml/kg	6.333±0.333	-
II	EA	25 ml/kg	35.333±0.882^{a**}	-
III	Omeprazole+ EA	20 mg/kg + 25 ml/kg	9.333±0.333^{b**}	73.59
IV	Extract+EA	100 mg/kg+ 25 ml/kg	20.333±0.333^{b**}	42.45
V	Extract+EA	200 mg/kg + 25 ml/kg	18.667±0.333^{b**}	47.17
VI	Extract+EA	400 mg/kg + 25 ml/kg	12.667±0.333^{b**}	64.15

In ethanol control rats characteristic lesions such as haemorrhagic streak, perforation, thick, black and dark red spot and lesions were observed which was reduced in rats pretreated with extract as well as standard drug. *Carissa carandas Linn.* Showed dose dependent more significant protection index of 42.45 %, 47.17 % and 64.15 % at the dose of 100, 200 and 400 mg/kg p.o. respectively whearas standard drug omeprazole also showed more significant protection index of 73.59 % at the dose of 20 mg/kg p.o. The gross figures of opened stomach according to group are mentioned in the following:

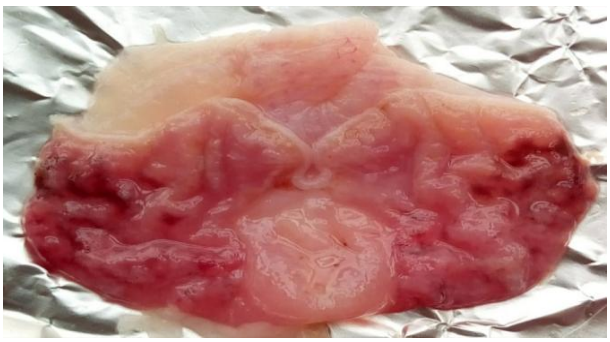
A. Control group



B. Ethanol control group

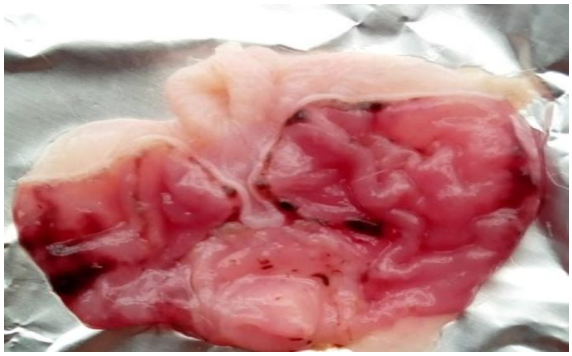


C. Standard group



D. 400 mg/kg extract group

E. 200 mg/kg extract group



F. 100mg/kg extract group

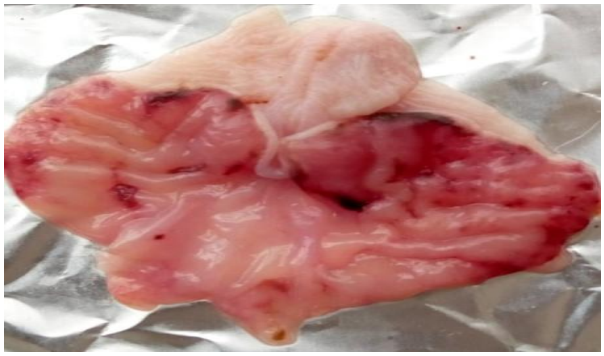


Figure 1: Antiulcer effect of 60 % ethanolic extract of *Carissa carandas Linn.* Compared with Omeprazole in Wistar albino rats

Antioxidant activity

In the present study DPPH produced purple color solution which was retained until the addition of extract solution or ascorbic acid solution of various concentrations. After keeping the solution mixture for 30 minutes in dark place it had been observed that the purple color had been turned into colorless gradually with the increased concentration of extract as well as ascorbic acid in the solution mixture while the purple color of control solution was unchanged.

The scavenging effects of 60 % ethanolic extract as well as standard ascorbic acid on DPPH free radical are tabulated in table 2.

Table 2: Effect of *Carissa carandas Linn.* extract as well as ascorbic acid on DPPH free radical

Concentration (µg/ml)	Log C	*Carissa carandas Linn.*		Ascorbic acid	
		% I	IC_{50}	% I	IC_{50}
25	1.398	44.82±0.1039	15.01	68.87±0.07937	3.56
50	1.699	70.89±0.05196		74.61±0.07937	
100	2	73.96±0.03000		79.14±0.03000	
200	2.301	78.18±0.1277		88.96±0.07937	
400	2.602	83.51±1308		96.93±0.02667	
800	2.903	85.83±0.03000		99.09±0.03333	

Statistical analysis was done using One way ANOVA: Dunnett's test (n=3). The % inhibitions are represented as mean ± S.E.M. Here, % I is percentage inhibition and IC_{50} is the concentration which is required for 50 % inhibition. From the table it can be clearly said that similar to the standard ascorbic acid, the extract showed dose dependent % inhibition. The % Inhibition was plotted against Log C in Microsoft Office Excel. Two straight lines were obtained from which IC_{50} value was determined. The graphical representations are mentioned in the following figure.

Figure 2: Effect of *Carissa carandas Linn.* extract on DPPH free radical

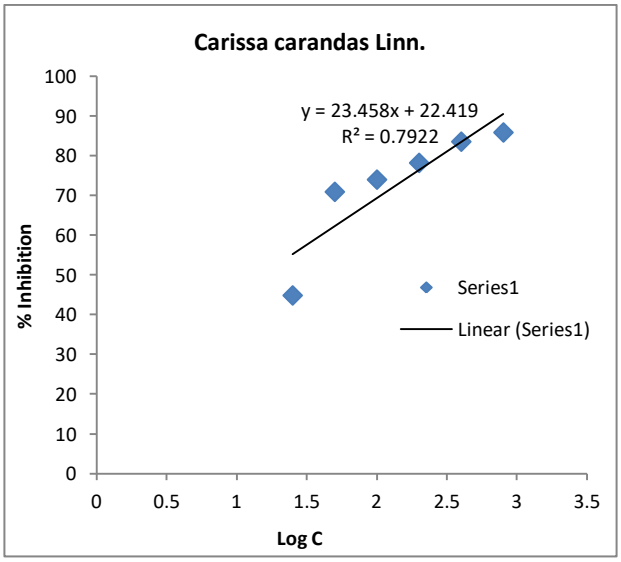
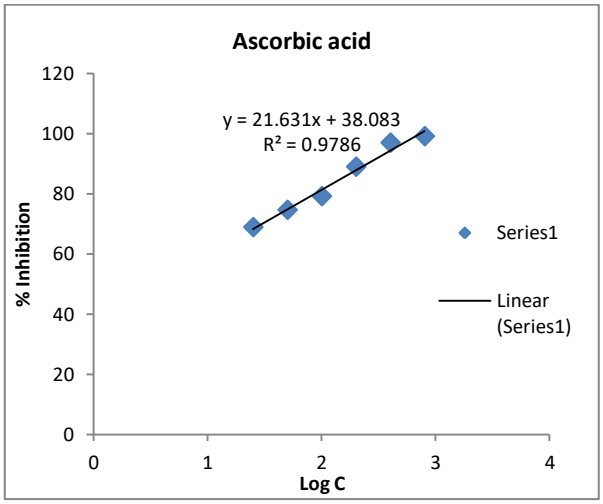


Figure 3: Effect of Ascorbic acid on DPPH free radical



The constituents responsible for having antioxidant property includes phenolic compounds and flavonoids.[11] The IC_{50} value of 60 % ethanolic leaves extract of *Carissa carandas Linn.* and standard ascorbic acid were 15.01 µg/ml and 3.56 µg/ml respectively. The antioxidant activity might be obtained due to presence to these phyto ingradients. On the basis of the results it can be said that the extract possesses good free radical scavenging capacity.

CONCLUSION

Present study clearly concluded that the 60 % ethanolic leaves extract of *Carissa carandas Linn.* showed significant antiulcer as well as good antioxidant activity compared to respective standard. Further studies can be carried out to identify and separated the components that are responsible for these activities.

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