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Antioxidant, α-amylase inhibition, DNA protection and antidepressant activity of *Caralluma edulis* extracts

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Caralluma edulis (*C. edulis*), also known as *Chunga*, is a valuable xerophyte herb with diverse phytochemical groups and a number of proven biological activities. Presently, n-hexane, chloroform and methanol extracts of *C. edulis* was tested for antioxidant activity by Ferric thiocyanate assay, Ferric reducing antioxidant power assay, phosphomolybdenum method and DPPH radical scavenging assay, along with its α-amylase inhibition potential against standard drug acarbose, DNA protection activity against Fenton's reagent and anti-depressant action by forced swimming assay, tail suspension and rearing method in albino mice. The results suggest that the methanol extract showed the maximum antioxidant activity in all models followed by chloroform extract. In *in-vitro* anti-diabetic model, comparable results with concentration dependent effects were observed. In DNA protection assay all extracts revealed protection against Fenton's reagent. Anti-depressant activity was also observed chiefly by the *n*-hexane extract of the plant supported by GC-MS analysis. It is concluded that the plant contains valuable antioxidants, and the constituents may produce their anti-diabetic effect by preventing carbohydrates absorption similar to acarbose. The herb is beneficial in protecting DNA against harmful free radicals and can prevent diseases involving nucleotides, and can combat depression associated with chronic ailments.

Keywords: *Caralluma edulis*, Antioxidant, Phosphomolybdenum, Ferric thiocyanate, α-amylase, DNA protection, Anti-depressant

INTRODUCTION

Herbal medicine is a prevailing system owing to its affordability and broad margin of safety. It is considered safe as they are natural and nontoxic (Ishtiaq et al. 2017; Ishtiaq et al. 2019).

Caralluma edulis (Edgew.) Benth. ex Hook. f. (*C. edulis*) is an ethnomedicine belongs to family Apocynaceae. Its common name is *Chunga* in Pakistan. It is used in the management of Alzheimer's disease, fever, gastric disturbances, hypertension, rheumatism, leprosy and parasitic infections. It is also used as carminative, febrifuge and stomachic (Ali et al. 2011; Adnan et al. 2014). In several studies it was found to be used for diabetes (Ahmad et al., 2006; Ahmad et al., 2009; Yaseen et al. 2015; Malik et al. 2015). It is

scientifically proven to possess antioxidant, antidiabetic, antinociceptive, anti-inflammatory, anti-hyperlipidemic, and hunger suppressant effects (Aslam et al. 2019). The chemical composition of C. edulis disclosed β-sitosterol, magasatgmane, glycosides, steroidal glycosides, saturated pregnane steroids, flavonoids (Memon et al. 1984; Abdel-Sattar et al. 2008).

The current project was taken up to establish antidiabetic mechanism by testifying its extracts by their α-amylase inhibition. Alongside DNA damage protection and antidepressant action was assessed to ascertain its role in prevention of mutagenic diseases and minimizing stress associated with chronic ailments.

MATERIALS AND METHODS

Plant material

The plant was procured in February from Attock, Punjab. It was verified by Prof. Dr. Zaheer-ud-Din, at GC University Lahore. A sample specimen of the plant was deposited with a voucher code GC Herb.3503 in the herbarium of Botany department at GC University, Lahore.

Chemicals and instruments

All the solvents *n*-hexane, chloroform and methanol were of analytical grade. The chemicals were procured from Merck Germany.

Experimental animals

Young healthy Swiss Albino mice, 6–8 weeks old, weighing about 21–24 g were used in this study. The animals were procured from UVAS Lahore, Pakistan. The animals were kept under standard conditions of environment (Temperature 23–25°C, humidity 70–75%, 12h/12h light/dark cycle). The animals had open access to standard pelleted diet, water *ad libitum*. Animals were familiarized to lab environment for a few days prior to conduct the experiments. The study design used was approved by the Animal Ethical Committee of The University of Lahore framed under National Institute of Health Guide for the Care and use of Laboratory Animals. Efforts were made to reduce the number of animals used and to minimize animals' distress in the experiments (National Institute of Health, 1985).

Preparation of extracts

The plant was garbled and washed with distilled water to remove the dust. Garbling was followed by shade drying of the material. The dried plant part was then pulverized with the aid of an electrical crusher. The dry powdered plant material was macerated by using analytical grade n-hexane, as solvent at 37°C for 15 days and then it was sifted through muslin cloth as well as with Whatmann No. 1 filter paper. Thus, the extract was obtained. It was further concentrated by using rotary evaporator at 50rpm, at 40°C under reduced pressure. A thick paste was poured into petri dish and percentage yield of extract was calculated. The residue obtained after filtration was shade dried. The procedure is then again repeated with other menstruums and percentage yield of *C. edulis* extracts were calculated.

$$Percentage\ yield = \frac{Actual\ yield}{Theoretical\ yeild} \times 100$$

Antioxidant activity

Ferric thiocyanate assay

The antioxidant potentials of the different fractions of the *C. edulis* on the inhibition of linoleic acid peroxidation was analyzed by using Ferric thiocyanate assay (Siddiqui et al. 2017). 100µL of each extract was mixed with 2.5mL of the linoleic acid emulsion and 2.0mL of phosphate buffer (0.02mM and pH 7.0). The linoliec acid emulsion was prepared by mixing 00.28g of linoleic acid, 00.28g of tween 20 as emulsifier and phosphate buffer 50mL. The reaction mixture was incubated at temperature 40ºC for a period of 5 days. The mixture devoid of plant extract was used as control/ blank. Then, the 100μL from this mixture was taken out and mixed in 100μL of 30% ammonium thiocynate, (20mM), 100μL of ferrous chloride in 3.5% HCl and 5mL of 75% methanol. The mixture was then allowed to stand for 10 min at 25 º C. After 10 min ferrous chlorides ($FeCl_2$) was also added and wavelength was noted at 500 nm. Percentage inhibition was calculated and considered as antioxidant activity

$$Inhibition\ of\ lipid\ peroxidation\ (\%) = \frac{1 - Absorbance_{sample}}{Absorbance_{control}} \times 100$$

Ferric reducing antioxidant power assay (FRAP)

The abilities of ferric reduction of different fractions of extracts were determined by the standard procedure (Mraihi et al. 2013). The principle of this assay is centered on the reduction of a ferric-tripyridyltriazine complex to its ferrous oxidation state, in the presence of antioxidants. FRAP reagent was prepared by adding 25mL of acetate buffer (300mM and pH 3.6), 20 mM of ferric chloride (2.5mL in 40mM HCl) and 2.5mL of

10mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) solution. Aliquots of 40*µ*L sample supernatant were mixed with 0.2mL distilled water and 1.8mL FRAP reagent. The absorbance was measured at 593nm wavelength. FRAP value was calculated by the standard 1mM $FeSO_4$ curve. TPTZ solution was used as blank. The final results were presented as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1mM $FeSO_4$.

Phosphomolybdenum method

The total antioxidant activity of *C. edulis* extracts was calculated by using phosphomolybdate complex formation method (Banu, 2017). A quantity of 500*µ*g/mL of each fraction was taken and mixed with 2mL of phosphomolybdate reagent (28mM Sodium phosphate:4mM Ammonium molybdate:0.6M Sulphuric acid) in clean test tubes. They were incubated in a water bath at 95°C temperature for a period of 90 minutes. After incubation, the test tubes were allowed to cool and absorbance of the sample mixtures was measured at 695nm against blank *i.e.* Phosphomolybdate reagent–3mL. Total antioxidant activity was expressed with reference to BHT.

DPPH free radical scavenging assay

The ability of the various fractions to scavenge DPPH free radicals was estimated by the standard method adopted with suitable modifications (Ishtiaq et al. 2014). The stock solution of each sample was prepared in methanol to achieve the concentration of 1mg/mL. Dilutions were made to obtain concentrations of 1000,500,250,125 and 62.5*µ*g/mL, respectively. Diluted solutions (100*µ*L each) were mixed with 3 mL of methanolic solution of DPPH reagent (0.01mM). The test mixtures were shaken vigorously and allowed to incubate for 45min, in dark place at room temperature 25°C. The absorbance was recorded at 517nm against methanol as blank. Lower value of absorbance indicated higher radical scavenging potential of the particular sample. Percentage inhibition was calculated using the following formula;

$$Percentage\ inhibition\ (\%) = \left(\frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}}\right) \times 100$$

IC_{50} values were estimated from the percentage inhibition vs. concentration plot, using a non-linear regression algorithm. Ascorbic acid was used as standard in this method.

Antidiabetic activity

***In-vitro* α-amylase inhibitory activity**

In-vitro antidiabetic potential of n-hexane, chloroform, and methanolic extracts of *Caralluma edulis* was carried out by the describe method (Kumar et al. 2011). Take 1mL of plant extract /standard in a test tube. 1mL of a-amylase solution and 2mL of sodium phosphate buffer (pH 7.4) solution were mixed.Incubated the mixture for 5min at 37°C. 1mL of starch solution was mixed to the mixture along with their incubation time.Once again incubated the mixture at 25°C for 15min. At the same point α-amylase activity of reaction mixture was terminated by adding 1 mL of DNS reagent followed by heating at 80°C to 90°C for 8min. Let the mixture cool at 37°C temperature. Finally added 4 mL of distilled water and then make up the volume of each test tube 10mL. In the last absorbance was measured at 540nm by using UV-Vis spectrophotometer. Control solution was made by adding 1mL of distilled water in place of plant extract rest of the procedure continued as it is. Blank solution was prepared by adding 1mL of plant extract with 9mL of distilled water and carrying out the same procedure as categorized above. Following expression was used to calculate percentage inhibition of plant extracts against α–amylase.

$$Percentage\ Inhibition(\%) = \left[\frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}}\right] \times 100$$

The activity was performed in triplicates and results were expressed as mean±SD. IC_{50} values were determined by plotting graph of percentage inhibition against extract concentrations.

DNA Protection activity

The DNA damage protection activity of the extract was evaluated by procedure adopted by Golla and Bhimathati (2014) with slight modifications. Positive control was made by mixing 4*µ*L of DNA with 16*µ*L of distilled water and Negative control was prepared by mixing 4*µ*L of DNA, 3*µ*L of Fenton's reagent with 13*µ*L of distilled water. The samples were prepared by mixing 4*µ*L of DNA, 3*µ*L of Fenton's reagent (30mM H_2O_2, 80mM $FeCl_3$ and 50mM ascorbic acid) followed by the addition of 4*µ*L solutions of n-hexane, chloroform and methanol extract in DMSO and the final volume of the mixture was brought up to 20*µ*L using distilled water. The samples were incubated for 30min at 37°C. After 30min incubation, 5*µ*L bromophenol blue dye (0.25 in 50% glycerol) was added to each sample.

The samples (20μL) were loaded on 1% agarose gel (1g agarose in 100mL of 1x TAE Buffer) and electrophoresis was carried out at 100V for 1h followed by staining with ethidium bromide. The results were visualized and analyzed using Gel Documentation system.

Acute toxicity study

The mice were deprived of food for 24h before to the beginning of this test. Ten mice (five males & females each) were supplied with a single oral dose of 2000mg/kg of the extracts. All the animals were under strict observation, 30min after dosing and intermittently during first 24h and daily for consecutive 72h. Mice were noticed for different autonomic effects *i.e.* piloerection, lacrimation, salivation, central nervous system effects *i.e.* drowsiness, tremors, convulsions, skin, body weight, food and water consumption and mortality (Ishtiaq et al.2017).

Antidepressant activity

For the pharmacological investigation, extract was dissolved in distilled water with 1% Tween 80w/v. The doses were calculated individually for mice in milligrams of dried extracts per kg body weight. The treatments were administered orally. Dose of 600mg/kg of extracts were selected based on the results of preliminary experiments. Readings were taken, 1h after the administration of the doses. The positive control received fluoxetine (10mg/kg body weight) while, the negative control was given with suitable vehicle.

Forced swimming test

Mice were forced to swing in a glass cylinder of 30cm height and 12cm diameter and filled with water. The temperature of the water was maintained at 25±1°C. The animals were trained for swimming for 15min, 24h before the execution of the test and the test was done for 5min. The water was changed after each subject. The results were recorded via time sampling method to score the different behaviors *viz.* swimming, climbing and immobility. Immobility was considered when the mouse was almost in a straight posture on the surface, doing only those movements that were needed to keep it buoyant (Liu et al. 2012).

Tale suspension test

The mice both acoustically and visually isolated and were hung by the tail with a clip/tape for 6min and immobility was recorded as a failure to make any struggling efforts (Surana and Wagh, 2017).

Open field test

Mice were individually placed in a wooden box with the floor divided into nine squares 1h after the administration of the dose. The number of rearings, number of crossings and total immobility time were noticed for 5min. The box was cleaned after every subject with 10% alcohol, to eliminate the hangover of former subjects. Control and experimental animals were intermixed to reduce the possible impacts of circadian variations on mice open-field behavior (Wang et al. 2010).

GC-MS analysis

The GC-MS analysis of *C. edulis* was performed using Agilent GC-MS. DB-5 MS split and split-less mode column (30nm X 0.25mm) was used. The diameter of the column was 0.25μm. The operation was started at 70eV. Helium gas was used as carrier gas kept at a pressure of 11.66Psi and flow rate of 1.0 mL/min. The operating temperature ranges for the injector was 45°C–350°C. The oven temperature was set to increase as follows 50°C at 6°C/min to 200°C (5min) at 6°C/min to 325°C (10min). The temperature was kept maintain for 5 min in the starting of the process and at the end of sample run. With the help of filtration syringe the n-hexane extract of *C. edulis* was filtered through 0.45μm filter. The analysis was carried out utilizing splitless mode, injecting 2.00μL of the analyte sample at 50°C. A mass of range 35–500amu was perused and further evaluated with the help of GC-MS Lab-Solutions Software NIST-417 LIB, for identification and characterization of sample (Goyindaraian et al. 2016; Aslam et al. 2017). The name, molecular formula of the components was established by using homologous series of compounds, the retention times and similarity indexes for each compound were evaluated.

Statistical analysis

All calculations were conducted in triplicates and the results are expressed as mean±SD. For statistical analysis Microsoft Excel 2016 and GraphPad Prism 7 was used. The difference of means and least significant difference was calculated by applying one-way analysis of variance (ANOVA) followed by Tukey's test. Statistical significance was declared at $p<0.05$.

RESULTS

Percentage yield:

About 600 g of dried *C. edulis* was extracted

with n-hexane, chloroform, and methanol by cold maceration process. The methanol extract gave maximum yield followed by chloroform and n-hexane (Table 1).

The antioxidant potentials of the various extracts *i.e.* n-hexane, chloroform and methanol were evaluated by Ferric thiocyanate assay, Ferric reducing antioxidant power assay, phosphomolybdenum method and DPPH radical scavenging assay and the results are tabulated.

In Ferric thiocyanate assay and FRAP assay, the methanol extract showed the lead with highest antioxidant values followed n-hexane. While,by phosphomolybdenum method methanol extract showed maximum antioxidant potential followed by chloroform extract. All results were comparable with BHT standard (Table 2).

DPPH radical scavenging assay:

In case of DPPH assay, antioxidant potential of different polarity extracts of *C. edulis (*methanol, chloroform and n-hexane) were evaluated at different concentrations (62.5, 125, 250, 500 and 1000 µg/mL). The percentage inhibition and IC_{50} values of all extracts were also calculated. It was found that all the extracts were less active as compared to standard ascorbic acid (IC_{50} 7.00 µg/mL).While; the extracts demonstrated appreciable antioxidant activity (Table 3).

Similarly the IC_{50} values were also calculated and methanol showed the smallest value among the extracts (Table 4) also shown in the Figure 1.

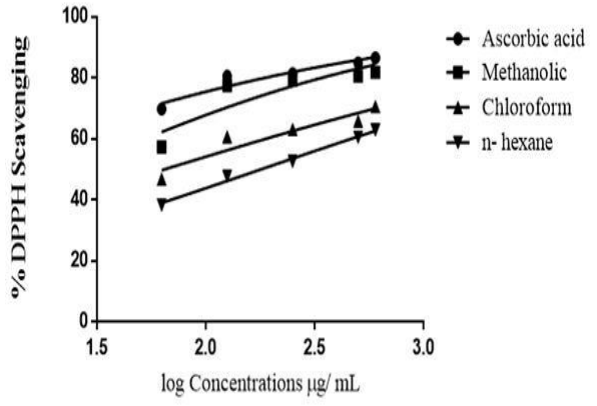

Figure 1: Percentage inhibition of *C. edulis* extracts against ascorbic acid by DPPH free radical scavenging assay

***In-vitro* α-amylase inhibition assay:**

The α-amylase activity of different extracts of *C. edulis* was measured at the concentrations of (100, 200, 300, 400 and 500 µg/mL). Acarbose was taken as reference standard. The obtained results concluded that methanol extract of plant presented higher α-amylase inhibition than chloroform and n-hexane extract of plant. However, these all three extracts were less effective than standard drug acarbose. The percentage inhibition was calculated (Table 5).

Similarly the IC_{50} values were also calculated and methanol showed the smallest value among the extracts (Table 6) also shown in the Figure 2.

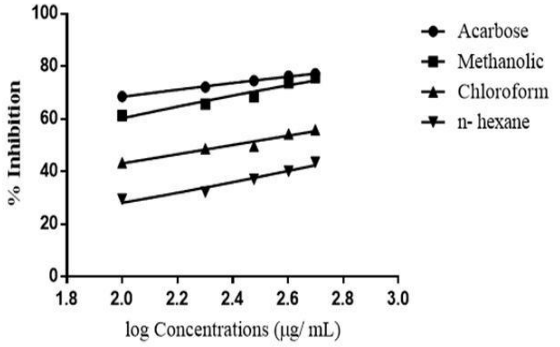

Figure 2: Percentage inhibition by *C. edulis* extracts against acarbose

DNA damage protection activity:

In DNA damage protection assay all extracts showed good protection against Fenton's reagent comparable DNA bands were observed with positive control (Figure 3). The dark band was visible with methanol extract followed by slightly lighter band with chloroform extract. On the other hand diffused band was visible with n-hexane extract.

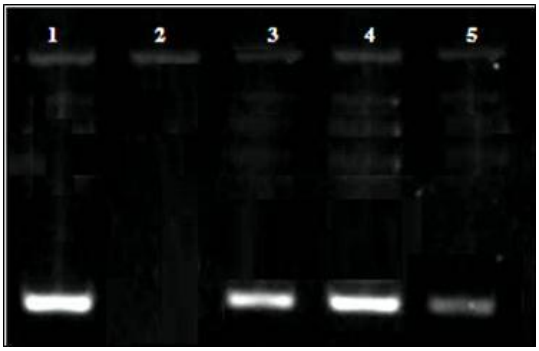

Figure 3: DNA damage protection activity by *C. edulis* extracts against Fenton's reagent

1.Positive control; 2.Negative control; 3.Chloroform extract; 4.Methanol extract; 5.n-Hexane extract

Acute toxicity study:

In acute toxicity study design, all the mice were carefully noted for any abnormal behavior or the appearance of any noxious signs or symptoms, at periodic time lapses of 0, 30 min, 1, 2, 4, 6, 8, 12 h and then every day for duration of 3 days. There wasn't any harmful or lethal signs were observed in clinical parameters. However, frequent water intake was predominant in all mice throughout the acute toxicity study. Mild drowsiness was observed in groups treated with n-hexane at the end of the study. The observations suggests that the LD_{50} of all the extracts are higher than 2000 mg/kg/day body weight.

Antidepressant activity:

Forced swimming test:

In forced swimming test, the mice treated with n-hexane and methanol extract showed significant reduction in the immobility time as compare to the vehicle (Figure 4).

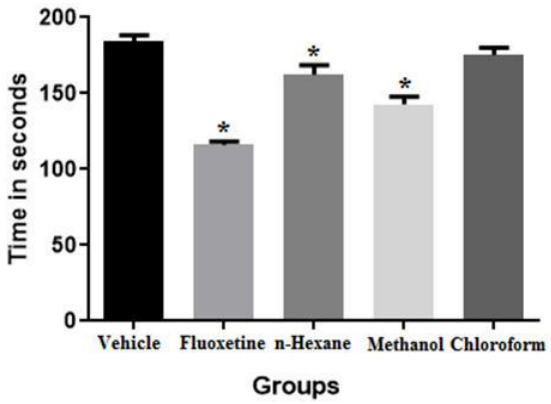

Figure 4: Comparison of the immobility time (seconds) in mice by the *C. edulis* extracts.

All the groups are compared with vehicle. Graphpad prism 7 was used and one way ANOVA with post-hoc Tukey's test was performed for multiple comparisons. *represents $p<0.05$.

Tail suspension test:

In tail suspension test, all the treatment groups showed significant reduction in the immobility time as compared to the vehicle (Figure 5).

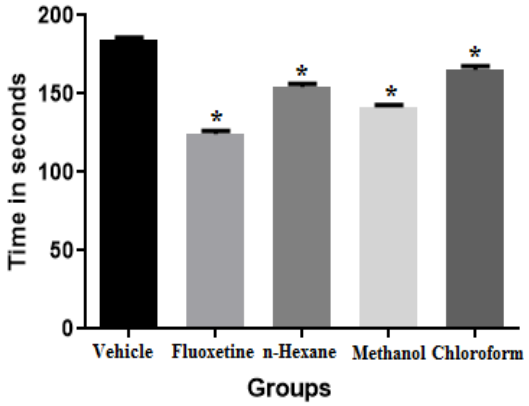

Figure 5: Comparison of the immobility time (seconds) in mice by the *C. edulis* extract.

All the groups are compared with vehicle. Graphpad prism 7 was used and one way ANOVA with post-hoc Tukey's test for multiple comparisons was applied. *represents $p<0.05$

Open field test:

The administration of the *C. edulis* extracts (n-hexane, chloroform and methanol) didn't significantly affected the locomotor activity of the mice in open field test as compared to control group over a period of 5 min. Yet, the fluoxetine treated group showed improved mobility performance as compared to control group.

GC-MS analysis:

The GC-MS analysis of *n*-hexane extract of *C. edulis* was carried out to figure out the chemical profile of the extract. The process was run for 30 min and compounds were identified. The obtained compounds are arranged in the way as they were eluted from the column (Figure 6). A total of 16 peaks were identified and quantified representing the chromatogram. Most of the compounds were nitrogen containing.

The main constituents are nortriptyline, cyclopropene, allene, propyne, heptane, 2-Butenedioic acid and 1, 3, 5-triazine (Table 7).

The list of the identified compounds with their molecular weight, chemical formulas, percentage areas and similarity indexes are given in the table below;

Table 1: Percentage yield of *C. edulis* extracts

Sr. no.	Extract	Dry Weight (g)	Percentage yield (%w/w)
1	n-Hexane	15.4	2.56
2	Chloroform	20.3	3.38
3	Methanol	30.1	5.01

Table 2: Antioxidant activity of *C. edulis* extracts

Antioxidant assays	n-Hexane	Chloroform	Methanol	BHT
Ferric thiocyanate assay (%)	47.44±0.003	46.71±0.01	59.38±0.025	61.82±1.08
FRAP assay (Eq. 1mM $FeSO_4$)	231.5±0.001	172.5±0.001	288.5±0.001	–
Phosphomolybdenum method	0.324±0.002	0.530±0.003	0.814±0.001	0.958±0.06

The results are expressed as mean±SD.

Table 3: Percentage inhibition of *C. edulis* extract against ascorbic acid by DPPH free radical scavenging assay

Percentage inhibition of *C. edulis* extract against ascorbic acid				
Concentrations (µg/mL)	**Ascorbic acid**	**Methanol**	**Chloroform**	**n-Hexane**
62.5	69.78±0.008	57.40±0.002	46.75±0.011	38.31±0.065
125	80.51±0.002	77.48±0.025	60.60±0.01	47.70±0.04
250	81.38±0.002	79.22±0.02	63.07±0.05	52.64±0.04
500	84.84±0.003	80.50±0.036	65.80±0.015	60.51±0.01
1000	86.58±0.001	81.81±0.04	70.56±0.09	62.94±0.05

*Values are expressed as mean ± SD.

Table 4: IC_{50} values of *C. edulis* extracts against ascorbic acid

Samples	IC_{50} values (*µ*g/mL)
Ascorbic acid	7.00
Methanol	24.07
Chloroform	64.37
n-Hexane	178.8

Table 5: α-amylase inhibition by *C. edulis* extracts against acarbose

Percentage inhibition of *C. edulis* extracts against acarbose				
Concentration (µg/mL)	**Acarbose**	**Methanol**	**Chloroform**	**n-hexane**
100	68.53±0.003	61.29±0.001	43.38±0.003	29.42±0.001
200	72.14±0.003	65.64±0.002	48.69±0.006	32.24±0.007
300	74.58±0.003	68.46±0.008	49.67±0.005	37.04±0.008
400	76.26±0.001	73.82±0.006	54.31±0.004	40.09±0.008
500	77.20±0.002	75.75±0.000	55.85±0.009	43.58±0.005

*values are expressed as mean ± SD.

Table 6: IC_{50} values of *C. edulis* extracts against acarbose

Samples	IC_{50} values (*µ*g/mL)
Acarbose	6.218
Methanol	36.38
Chloroform	248.1
n-hexane	1091

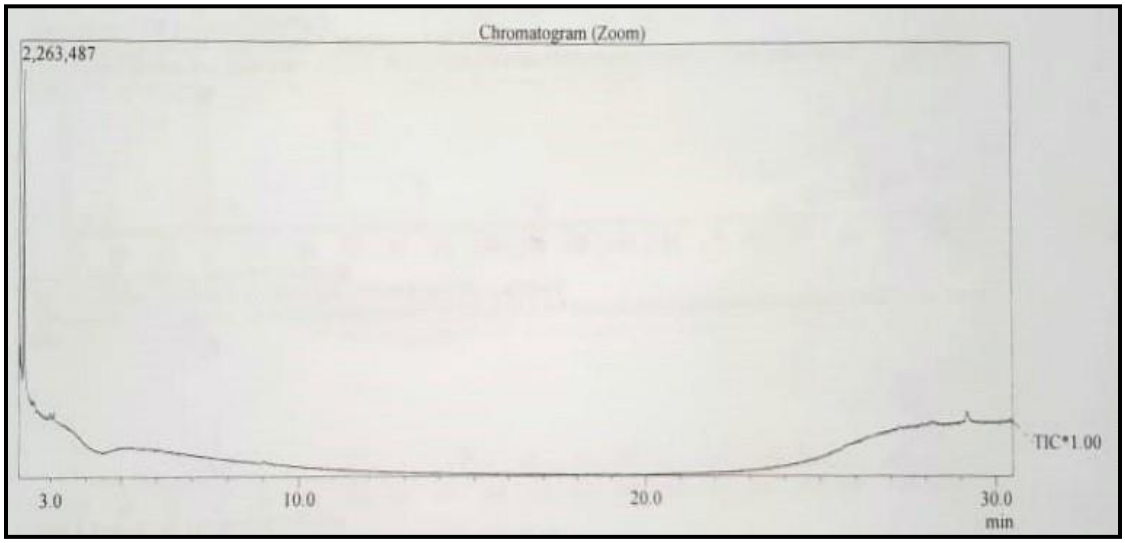

Figure 6: GC-MS chromatograph of n-hexane extract of *C. edulis*

Table 7: GC-MS analysis of n-hexane extract of *C. edulis*

Sr. No.	Compound Name	Molecular Formula	RT	Similarity Index	Mol. Wt.
1	2-Butyne-1,4-diol	$C_4H_6O_2$	2.125	76	86
2	2-Butenedioic acid	$C_5H_6O_4$	2.125	73	130
3	1,3,5-triazine	$C_9H_6N_6O_3$	2.125	73	246
4	Hydroperoxide	$C_7H_{16}O_2$	2.125	73	132
5	2-Hexen-1-ol	$C_6H_{12}O$	2.125	72	100
6	Heptane	C_7H_{16}	2.225	94 93 92 91	100
7	Hexane,3-methyl-	C_7H_{16}	2.225	91 88	100
8	Cyclopropene	C_3H_4	2.967 3.075	84 82	40
9	Propyne	C_3H_4	2.967 3.075	83 81	40
10	Allene	C_3H_4	2.967 3.075	82 81	40
11	Argon	Ar	2.967 3.075	80 78	40
12	Borane carbonyl	CH_3BO	2.967 3.075	79 78	42
13	Nortriptyline	$C_{19}H_{21}N$	29.167	72	263
14	Dimethyl amino	$C_7H_{19}N_3$	29.167	71	145
15	1-Methyldecyl amine	$C_{11}H_{25}N$	29.167	70	171
16	2-Amino nonadecane	$C_{19}H_{41}N$	29.167	70	283

*RT–retention time.

DISCUSSION

Oxidative stress is one of a major mother of multiple derangements in the body that covers a huge range of abnormalities, leading one to a number of health problems. Body need fee radical terminator for the purpose.It has also been discussed and studied that the lipid peroxidation is the main reason for the occurrence of cardiovascular and cancer diseases. Lipid peroxidation is studied with many kinds of biological harms which makes up the chain of free radical mediated reactions (Aqil et al. 2006).

Therefore the extracts were tested for their antioxidant power by their ability to scavenge the free radicals. It was observed methanol extract showed the maximum antioxidant activity followed by chloroform extract. While n-hexane showed the lowest (Table 2 & 3). It is due the presence of polyphenolics and flavonoids in the plant extracts. These are polyhydroxy species and encounter various metabolic disorders and preventive to oxidative stress. High content of these agents in plants render them appreciable antioxidants. High value of methanol and chloroform as compared to n-hexane is because of their polar nature. IC_{50} was also calculated for DPPH activity (Table 4, Figure 1). Phenolic species and flavonoids are primary antioxidants and free radical terminators and these species have the capacity to hunt oxygen free radicals because of their electron donating nature (Javanmardi et al. 2003).

The antioxidant potency is indicated by inhibiting of the peroxidation by plant extracts. Cycle of regeneration of new radicals can be break by reaction of hydrogen donating antioxidant by the lipid peroxide. The amount of lipid peroxide can be evaluated by the Ferric thiocyanate assay in which peroxides react with ferrous chloride in order to form ferric ions. To give red colored Ferric thiocyanate red pigment the ferric ions will unite with Ammonium thiocyanate (Ismail et al. 2010). Greatest value for percentage inhibition of fractions of lipid peroxidation have primary compounds of antioxidants which are capable of reacting with free radicals, only hydroxyl radicals which cease the radical chain reaction and prevent the hydroxyl peroxide production (Duh et al. 1999). Another simple, programmed test calculating the ferric reducing ability of plasma, the FRAP assay, is presented as an innovative technique for measuring “antioxidant power.” Ferric (Fe^{+3}) to ferrous (Fe^{+2}) ion reduction at low pH causes a dyed ferrous-tripyridyltriazine complex to form (Benzie & Strain 1996). The total antioxidant assay by phosphomolybdenum method is based on the reduction of Phosphate-Molybdenum (VI) to Phosphate-Molybdenum (V) by the plant extract. It shows that the plant phytochemicals have the ability to convert the reactive free radical species into their stable form (Prieto et al. 1999). Likewise, DPPH radical scavenging is a calorimetric assay and can be used to measure the DPPH capacity for extracts and targeted compounds in short time period. Phenolic compound is the cause of the decrease of absorbance of DPPH radical which is the cause of the reaction between free radicals and antioxidant molecules, the result of which is the discoloration from purple to yellow color as a result of the hydrogen donation by scavenging of radical (Cheung et al. 2003).

The importance of the antioxidant activity of this plant cannot be neglected as it can be utilized as an effective remedial therapy to prevent deleterious effects resulting from the free radicals. Absence of the antioxidants leads to the development of oxidative stress within the body that consequences in Parkinson’s disease, Alzheimer’s disease and cardiovascular diseases *i.e.* heart failure and myocardial infarction (Mecocci et al. 2004).

Additionally, in *in-vitro* α-amylase inhibition assay was performed to check the anti-diabetic potential of the extracts of *C. edulis* against standard drug acarbose (Table 5). A-amylase is considered as a chief enzyme produced by pancreas and slivery glands to convert macromolecules of polysaccharides such as starch into digestible forms like monosaccharides. Another enzyme in small intestine, α-glucosidase is responsible for the conversion of large molecules of carbohydrate into forms easily absorbable by the body. Inhibitors of these enzymes are also referred as starch blockers (Sundarram and Murthy, 2014; Benalla et al. 2010). The methanol extract again stood first in combating the diabetes against acarbose by *in-vitro* α-amylase assay at all tested concentrations with peak effect at 500 µg/mL with 75.75 ± 0.00 µg/mL and lowest IC_{50} value 36.38 µg/mL followed by chloroform (55.85 ± 0.009 µg/mL) and n-hexane (43.58 ± 0.005 µg/mL) (Table 5 & 6; Figure 2).

DNA damage protection was ascertained by the ability of extracts to counter hydroxal ($^{\bullet}OH$) free radicals produced by Fenton’s reagent. Free radicals can both initiate and fuel a few diseases involving DNA. These radicals react with nitrogen base and sugar moiety in the strands of DNA and

results into its relaxed and open circular forms. It ultimately causes DNA damage and denaturation of nucleic acids (Sonntag, 1987). The extracts, due to their antioxidant potentials, stopped the free radical mediated DNA damage as presented in the results (Figure 3).

In acute toxicity study of the plant extracts, it was found safe at the dose of 2000mg/kg body weight. Acute toxicity study helped us to device doses for antidepressant model. In anti-depressant study was assess via three models. In forced swim test, the immobility of mice is regarded as a behavioral despair and the response is considered as depression. A normal mouse will tend to move actively throughout to keep himself afloat. (Porsolt et al.1978). Similarly, in tail suspension test a depression free mouse will show struggling behavior to get himself out of the panic (Steru et al. 1985). Antidepressant drugs certainly reduced the immobility period. In this study a significant decrease in immobility was observed after the administration of 300 followed by 100 and 600 mg/kg body weight as compared to the vehicle, respectively (Figure 4 & 5). The results were comparable to fluoxetine.

In open field test the mice were noticed for 5 min for their actions. There was no alterations observed. This behavior model is incorporated to evaluate exploratory and motor activity mainly to analyze the general activity of the animals after the administration of extract (Herrera-Ruiz et al. 2006). Results shows that the extract do not intervene the normal CNS pathways, but perhaps modify the CNS mechanisms involved with this attitude. GC-MS profiling of n-hexane extract (Figure 6) justifies that the significant reduction in the immobility time in forced swim test and tail suspension test which was due to the antidepressant mechanisms as the extract was found to contain nortriptyline (Table 7).

Nortriptyline, a tricyclic antidepressant is an active metabolite of amitriptyline. Apparently, it has a lower frequency of related adverse effects, which makes it a preferable choice (Gillman, 2007). It is commonly employed for short-term treatment of several forms of depression. In CNS it produces its action at presynaptic receptors by blocking the norepinephrine, hence blocking the reuptake of this neurotransmitter and results in elevated concentration in the synaptic cleft. It also has affinity for α-adrenergic, histaminergic and cholinergic receptors (NCBI, 2019). Depression has also been associated with the disturbances of brain 5-HT activity. Nortriptyline is also reported to block 5-HT receptor activity which is also believed to mediate its antidepressant actions. (Fuxe et al.1977). Additionally nortriptyline is also indicated in chronic pain (including neuropathic pain), myofascial pain, burning mouth syndrome, anxiety disorders, attention-deficit/hyperactivity disorder (ADHD), enuresis, adjunctive therapy for smoking cessation (Derry et al. 2015; Ghanizadeh and Haghighat, 2012; Otasowie et al. 2014). Hence, this bioactive extract revealed valuable compounds and results suggest that *C. edulis* is effective as an antidepressant medicinal plant as well.

CONCLUSION

C. edulis is a valuable herb and is associated with multiple health outcomes. In the current project it was found to be efficient agent in treating diabetic through inhibition of α-amylase enzyme and it was assessed against a standard drug acarbose. It also proved its efficacy as DNA protectant and antidepressant, which makes it more chief herbal drug and can be subjected as future prospect of anticancer agent.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

IA, MSKA, & MSKA designed and performleed the experiments and also wrote first draft of the manuscript. WS, SP, SS, SI performed in-*vitro* and in-*vivo* studies and performed statistics and data analysis. HHF and NK perfomed the other part of practical research and reviewed the manuscript. All authors read and approved the final version.

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