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Pharmacological Evaluation of *Trianthema portulacastrum*

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Abstract

Trianthema portulacastrum is known for its important biological activities and its various parts are used in the folk medicine system to treat various diseases. The extracts of the plant have shown antioxidant, antibacterial, antifungal, antithelmintic, antiglomerulosclerosis anticancer, anti-inflammatory, hepatotoxic and hypolipidimic activities. Our investigations on the extracts of the plant have shown that they possess promising antioxidant activity. The extracts also showed moderate antibacterial activity indicating that they have substantial DPPH and ABTs free radical scavenging capabilities besides producing morphological changes in the bacteria. The macerated methanolic extracts of the plant have also shown significant antiglycation activity, thus further indicating that they have significant cabalibilities to suppress the AGEs formation and can therefore act as an antiglycation agent.

Keywords: *Trianthema Portulacastrum*, Antioxidant Activity, Antibacterial Activity, Antiglycation Activity, DPPH Assay, ABTS Assay, AWD Assay, Glucose-BSA Assay

Introduction

The plants extracts have been used for the treatment of chronic and infectious diseases since ages [1]. Nowadays traditional system of medicines is prevalent in a number of societies to treat chronic diseases in a cost effective manner [2]. In comparison to synthetic products, natural products are considered as safe and have potential to cure various diseases without much side effect [3].

The protein glycation is the key molecular basis of several pathological conditions such as skin diseases, atherosclerosis, rheumatism and diabetic complications i.e. retinopathy, nephropathy, neuropathy and cardiovascular diseases [4]. The advanced glycation end products (AGEs) are source of free radical species like reactive oxygen species (ROS) and reactive carbonyl species (RCS). These free radical species alter the normal redox status of lipids, proteins, nucleic acids their biological molecules to increase the oxidative stress [5]. The disruption of the balance between pro-oxidant and antioxidant, oxidative stress leads to damage biological molecules. It is intimately related with reductive stress which can cause potential damage [6, 7]. The inhibition of free radicals and proteins modification is considered as one of the mechanisms for antiglycation process [4, 8, 9]. Recently it has been considered that antiglycation is a very effective strategy to slow down aging and disease development. This fact was established by various researchers in many *in vitro* experiments which showed that biosynthesis of the AGEs can be inhibited by natural products present in plant extracts through their antioxidant properties, metal chelating properties, protein interaction or by blocking the receptors for the AGEs [10]. A spontaneous, nonenzymatic reaction between aldehydic or keto groups of glucose or reducing sugars and amino group of proteins termed as glycation. The AGEs are compounds that are characterized by a brown color, fluorescence and intra or inter-molecular crosslinking [4, 11]. The protein glycation and oxidative stress are directly related to progression of diabetes-related complication. During diabetes, the AGEs formation rate increased due to increased level of glucose in the blood [12].

Various chemical constituents besides polyphenols, flavonoids, alkaloids, terpenes and quinones are reported as having broad spectrum defense against plant pathogens [13, 14]. In a biological system, free radicals are produced inevitably as well as encountered exogenously causes oxidative stress leads to multiple degenerative disorders such as cardiovascular disorders, carcinogenesis, mutagenesis and ageing [15]. Generation of free radicals can be reduced and controlled by the antioxidants by reducing considerable oxidation rate of lipids and other molecules by inhibiting the oxidative chain reactions and are naturally synthesized in the body as well as supplied by nutraceuticals and dietary sources, repair and hence prevent body from damages done by reactive oxygen species [16, 17].

The plant *Trianthema portulacastrum* Linn, a glabrous herb locally known as 'Itsit' belongs to the Aizoaceae family [18]. The plant find use for treatment of various diseases including jaundice, fever, rheumatism, wounds and fever [20]. The plant contains essential nutrients like proteins, fibers, vitamins, carbohydrates, lipids, vitamins and minerals. Several phytochemicals such as terpenoids, terpenes, hydrocarbons, phytoecdysone, alcohols, flavonoids, polyphenols and alkaloids have also been reported from the plant [19-27].

Different biological activities such as antioxidant, antimicrobial activity and anthelmintic activities of the plant have been reported by several researchers [28-31]. The methanolic extract of the plant has shown hypolipidimic and antiglomerulosclerosis activity [32]. The extract of the leaves of the plant has been examined for hepatotoxic activity hence can be used to suppress the number and progression of mammary tumors [33-35]. It is a carcinopreventive agent and can be used to cure an early stage breast cancer and liver cancer [12, 36]. The diuretic property of the extracts has also been indicated in albino rats [37]. It showed remarkable anti-inflammatory activity due to presence of bioactive tetraterpenoid and trianthenol [38, 39].

It is reported that hydrolysates fractions of the stem, leaves and roots of the plant in acidified methanol contain 50.75~98.09 mg gallic acid equivalents/g dry weight of total phenolic contents and have substantial DPPH radical scavenging capabilities, reducing potential and abilities to inhibit peroxidation of linoleic acid [26].

During the literature review of biological activities of medicinal plants, it is observed that antioxidant and antiglycation activities are parallel to each other [40]. The present study is directed at investigating the plant extracts for their *in-vitro* antiglycation activity along with other biological activities. This would be the first attempt to evaluating the *in vitro* antiglycation activity of macerated methanolic extracts of various parts of the plant.

Experimental

Collection of Plant Material: The fresh plant material was collected from surroundings of the Village New Jabber near Chechian in Tehsil and District Mirpur of Azad Jammu and Kashmir. The parts of the plant were separated, washed and air dried under shade at room temperature. The leaves, stems and roots were separately crushed into finely divided powdered form.

Preparation of Extracts: A standard protocol was followed for preparation of the plant extracts [41]. The powdered materials were soaked in methanol up to double volume of plant material at room temperature. The infusions were filtered after 6 days and residues were re-extracted with methanol after another 6 days. Both filtrates were mixed and subjected to rotary evaporator under vacuum at 45 °C to remove solvent. The dried extracts were refrigerated at 4 °C for further use.

Evaluation of Antioxidant Potential: The reported protocol was adapted with slight modifications to evaluate the DPPH radical scavenging activity [16]. The stock solutions were prepared by dissolving plant extracts in methanol (50 mg/ 5 ml). The 2 mM stock solution of the DPPH was prepared in methanol and incubated for 30 minutes under dark. The 1000 µl of the DPPH stock solution and various concentrations of the plant extracts taken from stock solution ranging from 100 µl-500 µl were mixed in test tubes. Methanol was added to each test tube to make up the volume to 4 ml and incubated in dark for twenty minutes at room temperature. The intensity of absorbance was measured by spectrophotometer (T 60-U) and recorded at 517 nm. All experiments were carried out thrice. Similar procedure was used for ascorbic acid (standard drug) studies. The % radical scavenging efficacy was calculated with standard formula.

ABTS Radical Scavenging Assay: The ABTS 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) activity was tested using a modification of the reported protocol [42]. The 2 mM ABTS solution was prepared in deionized water. For complete oxidation of the ABTS solution to generate the $ABTS^{+\cdot}$ 0.5 mM solution of potassium persulphate was added then incubated the solution for 16 hours at 25 °C. The distilled water (3 ml) was used as blank and for standard set up 2 ml of the $ABTS^{+\cdot}$ and 1 ml of solvent was used. For the evaluation of the ABTS radical scavenging activity 2 ml of the ABTS stock solution was poured to 5 test tubes then added various concentrations of extracts solutions ranging from 20 µl-100 µl. The resulted reaction mixtures were incubated for 30 minutes at 25 °C and decrease in the absorbance was monitored at 734 nm. The percentage inhibition of reaction mixtures was calculated by the standard formula.

Procedure for Antimicrobial Assay

Bacterial Isolates: The methanolic extract of *Trianthema portulacastrum* were evaluated against four fresh bacterial culture, two were gram positive strains, *Escherichia* coli and *Bacillus pumilus* and two were gram negative strains, *Pseudomonas aeroginosa* and *Staphylococcus aureus*.

Nutrient Agar Media Preparation: The nutrient agar media was prepared by adding 1 g tryptone, 0.5 g yeast extract, 0.5 g NaCl and 100 ml deionized water. The pH was adjusted to 7. The agar media was autoclaved for 15 mins at 121 °C.

Testing of Antibacterial Susceptibility by Using Agar Well Diffusion Assay: For agar well diffusion assay, a slight modification of the reported method to test the ability of extracts was followed [43]. Under laminar flow, agar media was transferred to autoclaved petri plates and allowed them to solidify at room temperature. The entire surface of nutrient agar plates was inoculated with respective fresh bacterial inoculums by streaking evenly with the help of cotton swab. At equal distance four wells of 4 mm size were cut into the agar surface by using a cork borer sterilized with alcohol. The 40 µl of extracts was dispensed into respective well with the help of micropipette. In the central 4^{th} well, the undiluted medicament (rifamycin) was dispensed and considered as positive control. All plates were incubated for overnight at 37°C. The experimental design was carried out with three replications to minimize errors. After incubation period, the inhibition zones were measured in millimeter.

Antiglycation Assay: The antiglycation activity was assessed by adapting a standard protocol with minor changes [44]. First of all phosphate buffer solution (PBS) was prepared and pH was adjusted to 7.4 by drop wise adding NaOH or HCl solution. The 0.1 mM sodium azide solution was added to this buffer solution to avoid any bacterial contamination. The Bovine Serum Albumin solution (50 mg/ ml) and 2 mM glucose solution were prepared in the PBS and plant extracts solutions were prepared in DMSO. The reaction mixtures were prepared in test tubes by adding

500 µl of the BSA solution, 1000 µl of glucose solution, 1000 µl of the PBS and 1000 µl of different concentration of extracts solution ranging from 600 µl -1000 µl. The reaction mixtures were incubated at 60 °C for 3 days. After incubation, the reaction was stopped by adding 10 µl of 100% trichloroacetic acid. The reaction mixture taken as negative control was prepared with plant extract and kept in refrigerator at 4 °C. A standard drug, aminoguanidine was used as positive control. The suppression of AGE's production was evaluated by using *Photoluminescence Spectrometer* (Shimadzu RF-600) at the fluorescence intensity of excitation 340 nm and emission 440 nm. The % glycation inhibition of each of the reaction mixtures was measured by the standard formula.

Results and Discussion

DPPH Free Radical Scavenging Assay: The antioxidant efficacy of various parts of *T. portulacastrum* is expressed as percentage scavenging activity. Various concentrations in range of 100 µL to 500 µL of each of the extracts were taken from stock solutions. The methanolic extracts of the roots, stems and leaves showed excellent activity. The results are represented in Fig 3, Fig 4 and Fig 5. The %age scavenging activity increases with increase in the concentration of the extracts. The DPPH free radical scavenging activity of the leaves, stems and roots is similar to the %age scavenging activity of ascorbic acid shown in the Fig 2. The results are shown in Table-1. The leaves, stems and roots showed 75%, 74% and 81% inhibition, respectively.

ABTS Free Radical Scavenging Assay: The proficiency of substances to neutralize free radicals can be ascertained by the ABTS assay. In order to determine the capability of *T. portulacastrum* to inhibiting the ABTS radical cation, the reaction mixtures comprises of various concentrations ranging from 20 µl to 100 µl were used. The excellent percentage inhibition potential, 88 %, 81 % and 87 % was shown by the extracts of leaves, stems and roots, respectively. The percentage inhibition was enhanced with increase in concentration of the each extract of plant.

The ABTS antioxidant activity of the plant extracts were found similar to the ascorbic acid which showed that it possesses excellent radical scavenging activity (95%). The results are shown in the Table-2 and presented in Fig 6, Fig 7, Fig 8 and Fig 9. It is concluded that the plant possessing the ability to scavenge free radical due to presence of antioxidant agent like polyphenols and flavonoids.

Evaluation of Antiglycation Potential: The antiglycation activity of *T. portulacastrum* was carried out to visualise its potency to stop cross linking of glucose and protein. Advanced glycation end products are responsible to deactivate the natural antioxidants present in the body and damage tissues and organs. The plant contains antiglycation agents like alcohols and flavonoids reduce the effects of the AGEs. The extracts of the leaves and stems showed excellent activity in stoping the Maillard reaction while extracts of the roots showed no significant activity.

The antiglycation activity was assessed by the photoluminence approach. The fluorescence values of extracts were found smaller than the control, BSA/ glucose. The decrease in fluorescence was observed with increase in concentration which indicates that the sample has the potency of controling the AGEs formation. The potential of the extracts of the leaves and stems of the AGEs suppression is similar to aminoguanidine, standard drug. The results are summarized in the Table-3 while graphical representation of the antiglycation activity of the extracts of the leaves and stems are presented in the Fig 11 and Fig 12, respectively. This showed that the plant posseses excellent antiglycation activity.

Antibacterial Activity: The extracts of *T. portulaacstrum* were screened for the antimicrobial activity by using the AWD method against *Escherichia coli, Bacillus pumilus, Staphylococcus aureus* and *Pseudomonas aeruginosa*. The clear zones of inhibition for the extract of the leaves and stems were observed in case of *S. aureus* and *P. aeruginosa* whereas no activity indicated in case of *E. coli* and *B. pumilus*. The extract of the root was found inactive for the selected gram positive and gram negative bacteria. The inactivity of the plant extracts against bacterial strains suggested that these parts of plant would lack interaction ability with the membrane proteins or lipids of the chosen microorganisms. The mean diameters of inhibitions zones are presented in the Table-4.6. An effective medicament, rifamycin was used as positive control. The results indicated good activity against the selected bacterial strains. The antibacterial activities of the plant extracts and rifamycin antibacterial activity are shown in the Fig 13 and Fig 14.

Table 1: DPPH Free Radical Scavenging Assay of Various Parts of *T. portulacastrum*

Concentration (µL)	% Inhibition of Ascorbic Acid	% Inhibition of Leaves Extract	% Inhibition of Stems Extract	% Inhibition of Roots Extract
100	24	29	14	30
200	51	40	30	47
300	65	57	46	68
400	70	68	61	80
500	80	75	74	81
IC_{50} (µL)	**173**	**264**	**317**	**209**

Table 2: ABTS Free Radical Scavenging Assay of Various Parts of *T. portulacastrum*

Concentration (µL)	Percentage Inhibition of Ascorbic Acid	Percentage Inhibition of Leaves Extract	Percentage Inhibition of Stem Extract	Percentage Inhibition of Root Extract
20	43	29	32	18
40	59	53	46	40
60	73	64	60	59
80	83	79	70	81
100	95	88	81	87
IC_{50} (µL)	**24**	**36**	**46**	**50**

Table 3: Antiglycation Inhibition Assay of the Extracts of *T. portulacastrum*

Concentration (µL)	% Inhibition of Aminoguanidine	%Inhibition of Leaves Extract	%Inhibition of Stems Extract	% inhibition of Roots Extract
600	48	16	33	0
700	52	32	41	0
800	56	40	52	0
900	64	65	77	0
1000	75	84	91	0
IC_{50} (µL)	**678**	**826**	**763**	**0**

Table 4: Antibacterial Activity of Various Parts of *T. portulacastrum*

Bacteria	Diameter of Inhibition Zone of Control (mm)	Diameter of Inhibition Zone of Leaves Extract (mm)	Diameter of Inhibition Zone of Stems Extract (mm)	Diameter of Inhibition Zone of Roots Extract (mm)
P. aeruginosa	4	2	3	0
S. aureus	3	4	6	0
E. coli	2	0	0	0
B. pumilus	3	0	0	0

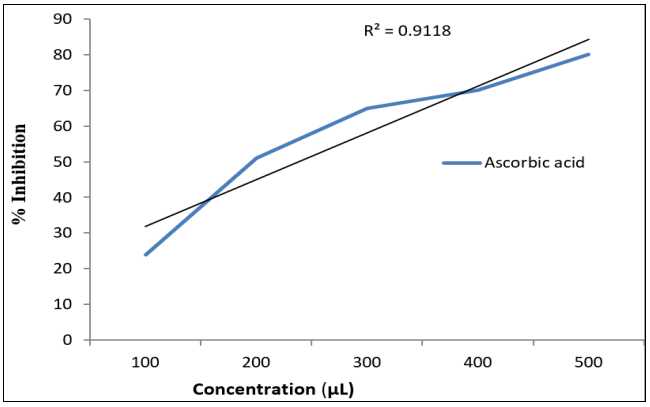

Fig 2: Dose Response of the Ascorbic Acid against DPPH Free Radical Scavenging Activity

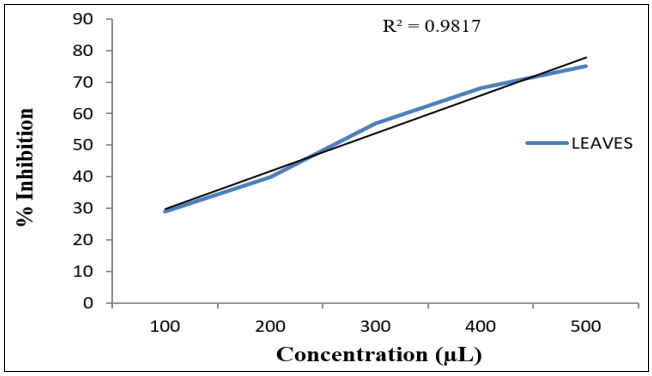

Fig 3: Dose Response of Leaves of *T. portulacastrum* against the DPPH

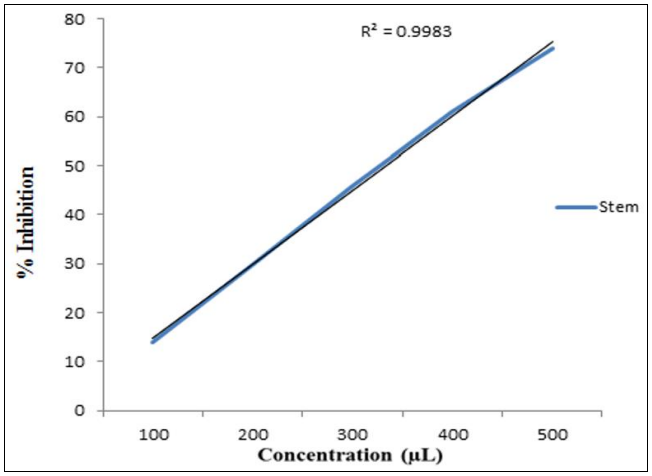

Fig 4: Dose Response of Stems of *T. portulacastrum* against DPPH

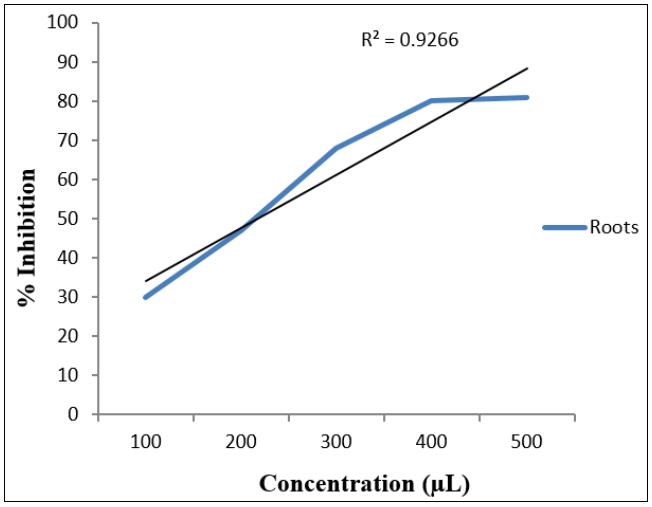

Fig 5: Dose Response of Roots of *T. portulacastrum* against DPPH

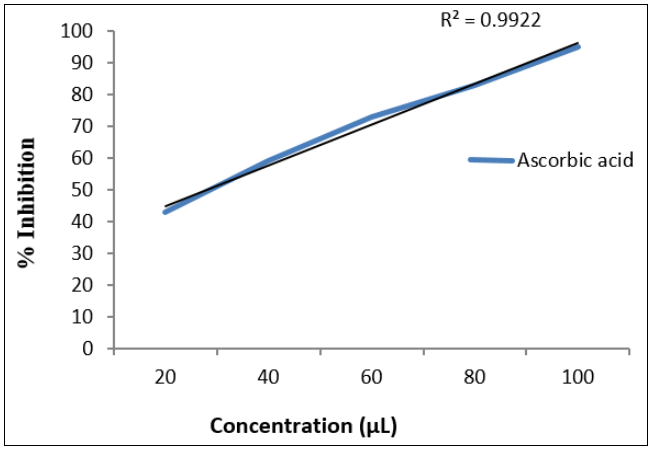

Fig 6: Dose Response of Ascorbic Acid against the ABTS

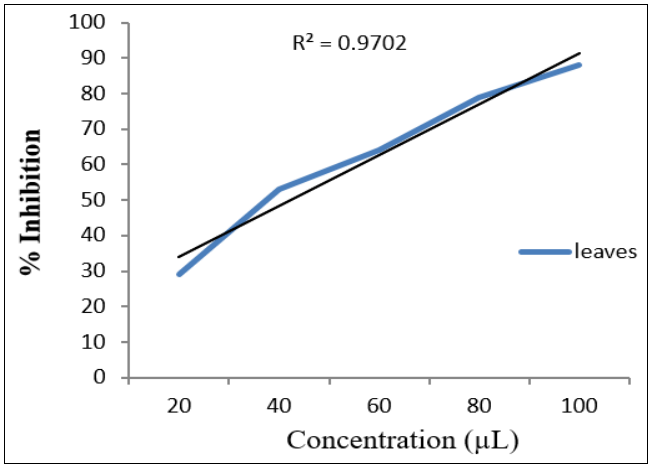

Fig 7: Dose Response of Extarcts of Leaves of *T. portulacastrum* against ABTS Free Radical Scavenging Assay

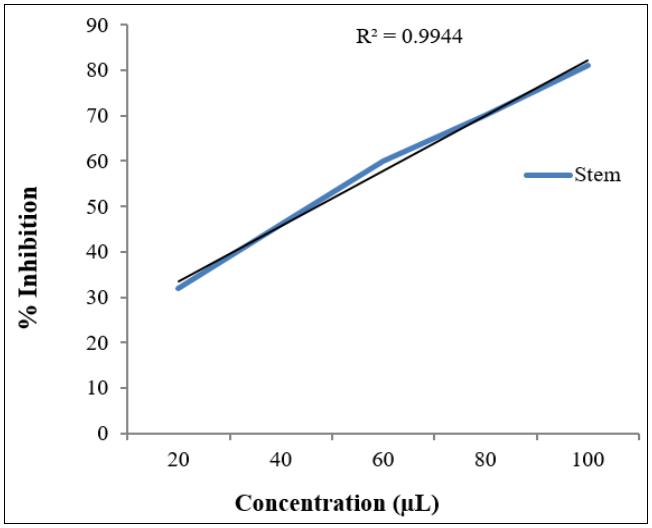

Fig 8: Dose Response of Stems of *T. portulacastrum* against the ABTS

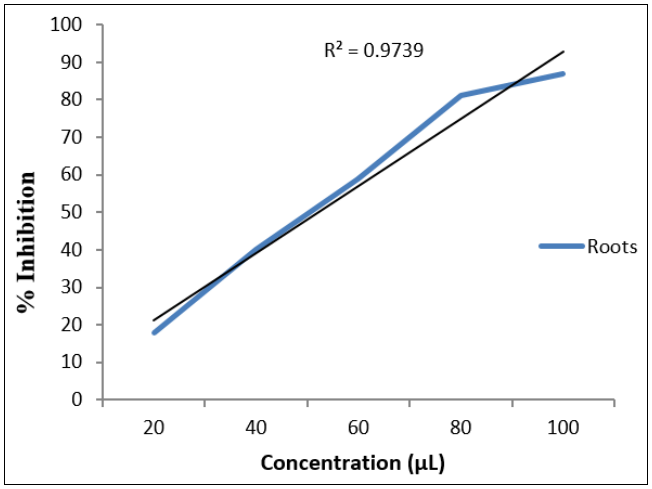

Fig 9: Dose Response of Roots of *T. portulacastrum* against the ABTS

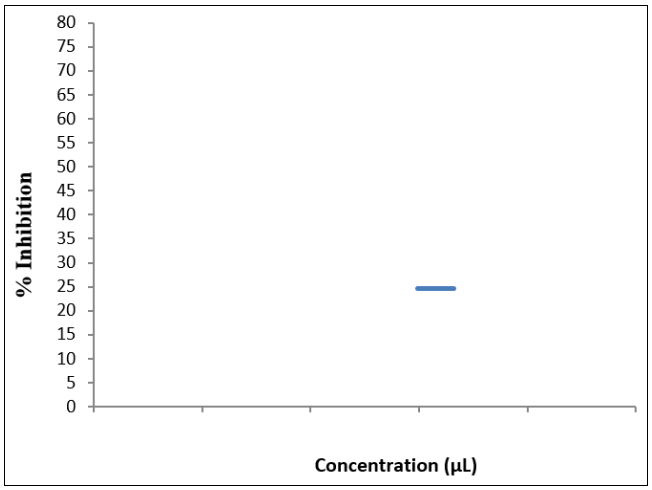

Fig 10: Dose Response of Aminoguanidine in Antiglycation Assay

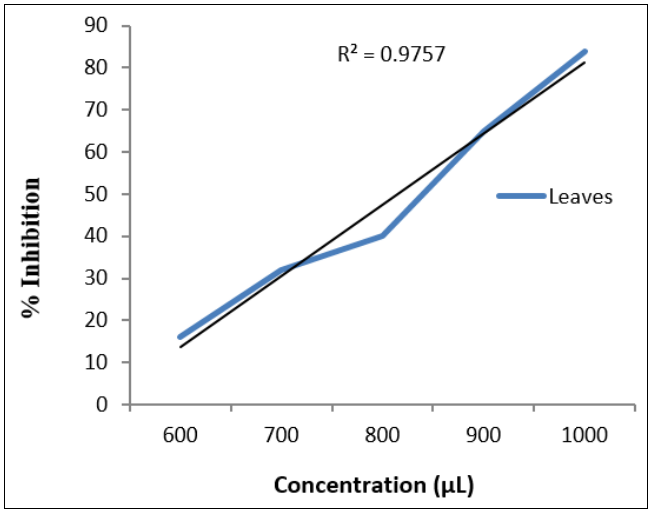

Fig 11: Dose Response of Leaves Extracts of *T. portulacastrum* in Antiglycation Assay

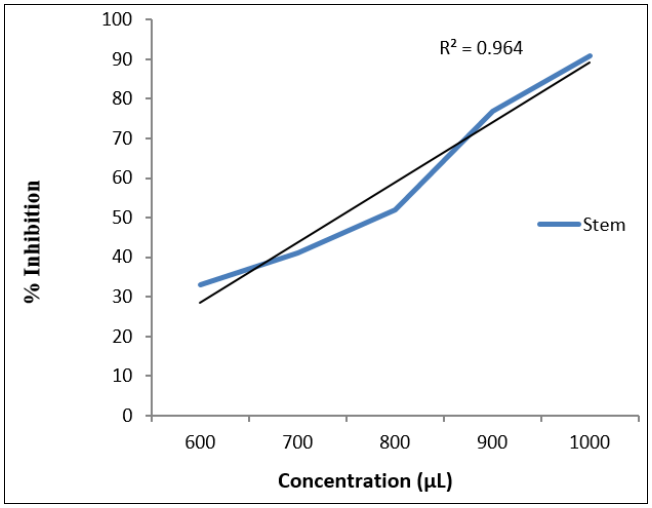

Fig 12: Dose Response of Stems of *T. portulacastrum* in Antiglycation Assay

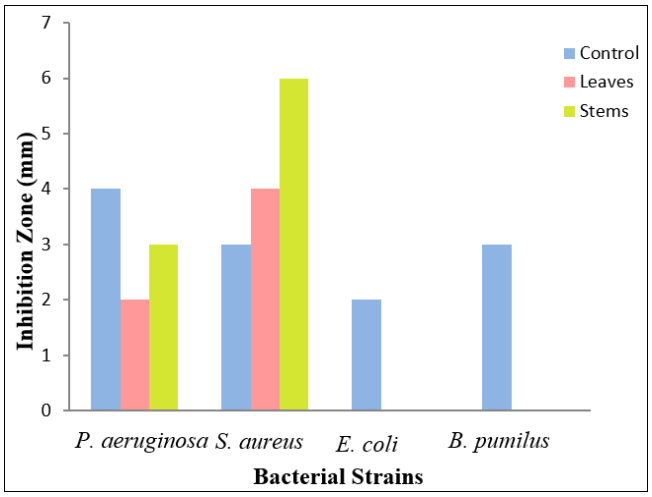

Fig 13: Graphical Representation of Antibacterial Activity of *T. portulacastrum*

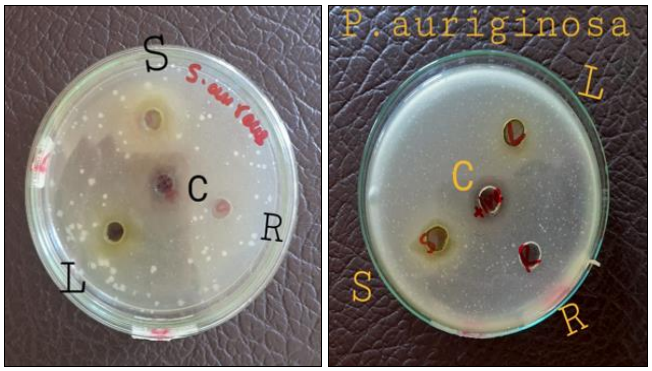

Fig 14: Antibacterial Activity of Different Extracts of the Plant and Positive Control

Conclusion

T. portulacastrum contains various secondary metabolites such as alcohol, alkaloids and terpenoids that are responsible for its biological activities. The methanolic extracts of various parts of plant showed activity against free radicals and proved to be an excellent antioxidant agent. The plant extracts were also evaluated for the antiglycation potency. The extracts of the leaves and stems showed excellent activity whilst the extracts of the roots showed no detectable activity. The plant extracts showed antibacterial activity due to presence of various secondary metabolites such as alcohol, flavonoids, alkaloids and terpenoids. The methanolic extracts of various parts of plant showed antioxidant activity. The plant extracts were also evaluated for their antiglycation activity. The extracts of the leaves and stems showed excellent activity whilst the extracts of the roots showed no detectable activity. The extracts of the plant were tested for their antibacterial activity and found to possess the antibacterial activity. The extracts of the plants were also evaluated for their antidiabetic activity and no detectable activity was observed.

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