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PHYTOCHEMICAL EVALUATION AND IN-VITRO ANTIOXIDANT EFFICACY OF VARIOUS EXTRACTS OF WHOLE PLANT OF *TRIANTHEMA PORTULACASTRUM*

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ABSTRACT

This study was designed to examine the presence of phytoconstituents, total phenol and flavonoid content, the antioxidant potential of various extracts of *Trianthema portulacastrum L* whole plant using various in-vitro assays. The dried plant material was subjected to successive extraction with various solvents based on polarity (Pet Ether (PE), Chloroform(CE), Ethyl Acetate(EAE),Ethanol and by hot continuous extraction in Soxhlet's apparatus and method of maceration was allowed for Aqueous(AE) extraction. The resultant crude extracts were dried . The total phenolic content and flavonoid content of Ethyl acetate extract of plant was found to 82.58±0.629 and 143.77 ±0.509 mg of GAE and Quercetin equivalents respectively. Antioxidant potential of the various extracts were studied by DPPH, nitric oxide scavenging and FRAP methods and results were quantified by colorimetric methods. The Ethyl acetate extract exhibited potent antioxidant activity as determined by 2,2-diphenyl-1-picrylhydrazyl(DPPH), nitric oxide scavenging and ferric reducing antioxidant power assays(FRAP). The IC_{50} value for the Ethyl acetate extract of *Trianthema portulacastrum L* whole plant *was* found to be 179.974±0.996 µg/ml and 166.926±0.122 µg/ml by DPPH and nitric oxide scavenging assays respectively. A strong interrelationship was observed between antioxidant measurements, total phenolic content and their total flavonoid content. It is recommended that, phenolic compounds were a major come up with the antioxidant properties of plant extract. Based on the results, the Ethyl acetate extract of *Trianthema portulacastrum L whole plant* can account for the promising new source of natural compounds with antioxidants potential.

INTRODUCTION

Compounds obtained from natural products have served as either templates or specific agents for the treatment of a number of different types of diseases. Today, use of natural products is of widespread in traditional and folkloric systems of medicine worldwide, particularly in developing countries where access to modern therapies may be challenging or expensive. Specifically, India is considered to be one of the largest producers of medicinal herbs. Much work has been carried out in recent years on the beneficial effect of phenolic compounds which act as natural antioxidants and help to counteract free radicals. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions, quenching singlet and triplet oxygen, or decomposing peroxides[1]. The antioxidant effect is mainly due to components such as flavonoids [2], phenolic acids, and phenolic diterpenes[3]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals,

Many of these phytochemicals possess significant antioxidant capacity that may be associated with lower incidence and lower mortality rates of cancer in several human population [4]. Flavonoids and phenolics are well known for their antioxidant activity and are specific compounds that protect human, animal and plant cells against the damaging effects of free radicals in addition an imbalance between antioxidants and free radicals results in oxidative stress, will lead to cellular damage [5]. Oxidative stress is a detrimental condition that occurs when there is an excess of ROS and decrease in antioxidant levels, this may caused tissue damage by physical, chemical which leads to tissue injury in human and causes different diseases [6]. Researchers revealed that the antioxidants of plant origin with free-radical scavenging properties could have enormous importance as therapeutic agents in diseases caused due to oxidative stress [7]. *Trianthema portulacastrum* Linn.also known as horse purslane, has historically been valued by Indian and African cultures for its numerous medicinal effects [8]. The plant is capable of growing in sunny desert areas and also grows abundantly as a "weed" in well irrigated and high-rainfall areas, particularly in India and neighboring countries. The dehydrated plant is reportedly used against throat troubles and anti-fungal agent. The plant is alexiteric, analgesic, stomachic, laxative, alterative; cures "Kapha," bronchitis, "Vata," piles and ascites. Extract of the roots are lithotriptic, cardio protective, diuretic, ascetic, analgesic, laxative, alterative and reputedly used as an emmenagogue. Based on the traditional knowledge and recent pharmacological studies, the objective of the present study was to investigate the chemical constituents and antioxidant potential of various extracts of *T. portulacastrum* by various in vitro assays.

METHODS

Collection of Plant material and extraction: The whole plant of *Trianthema portulacastrum* was collected from the forests of Maisammaguda, Secunderabad situated in the state of Telangana (India) and shade dried and powdered mechanically. The plant specimen was authenticated by botanist of Osmania University and authenticated voucher specimen Number 145 of the plant has been preserved in department for future reference. The dried plant powder was extracted with various solvents based on polarity (Pet ether, Chloroform, Ethyl acetate, Ethanol) by hot continuous extraction in Soxhlet's apparatus and method of maceration was allowed for aqueous extraction. the extracts were evaporated to dryness under vacuum and dried in vacuum desiccators, stored in refrigerator.

Phytochemical Evaluation: Phytochemical screening of alkaloids, saponins, carbohydrates, tannins, phenolics, flavonoids, steroids and glycosides were carried out for various extracts of plant using standard protocols.

Total phenol content Estimation: The total phenolic content of *T. portulacastrum* was assessed using Foline-Ciocalteau phenol reagent method described by Singleton et al [9]. Briefly, 1.0 mL of the extract at various concentrations was mixed with 2.5 mL of 10% Foline-Ciocalteau reagent and 2.5 mL of 7.5% sodium carbonate. The contents were thoroughly mixed and allowed to stand for 30 minutes. The absorbance was read at 750 nm in a spectrophotometer. The total phenol content was expressed as gallic acid equivalents in milligram per gram of the extract.

Total Flavonoid content estimation: The flavonoid content of *T. portulacastrum* was determined using aluminium chloride colorimetric method described by Chang et al [10]. Briefly, 0.5 mL of the extract at various concentrations was mixed with 3mL of 95% methanol, 0.1 mL of 10% (weight/volume) aluminium chloride, 0.1 mL of 1M potassium acetate, and 2.8 mL of distilled water. The reaction mixture was allowed to stand at room temperature for 30 minutes and absorbance was measured at 415 nm against a blank sample. A calibration curve was prepared using quercetin in methanol. The flavonoid content was expressed as quercetin equivalents in milligram per gram of the extract.

IN-VITRO ANTIOXIDANT ACTIVITY:

DPPH antiradical capacity: The antiradical potential of *T. portulacastrum* was determined spectrophotometrically as described by Ilahi et al [11]. Five different concentrations of various extracts of plant material (100, 200, 400, and 800 and 1000 µg/ml) were mixed with 100 **µ**L of DPPH radical solution in a 96-well microplate and incubated for 20 min at room temperature. The resultant mixture was read

spectrophotometrically at 517 nm against a methanol blank and the following equation was used to calculate the % inhibition of each extract:

%inhibition

$$=(Acontrol-Asample)/(Acontrol)\times 100$$

Where *Acontrol* and *Asample* indicate the absorbance of the DPPH solution and the reaction mixture respectively. The IC_{50} represented the concentration of the extract that inhibited 50% of DPPH radical.

Nitric oxide scavenging activity: Nitric oxide radical scavenging activity was determined according to the method reported by Garrat [12]. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess-Ilosvay reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 25°C for 180 min. Fro the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. finally, 1.0 ml naphthyl ethylene diamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min, the absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated.

Reducing power Determination: The Fe^{2+} reducing power of plant extract was determined by the method of Oyaizu [13] with slight modification. Various concentrations of plant extract(0.75mL) was mixed with 0.75 ml of phosphate buffer (0.2 mole, pH 6.6) and 0.75 mL of potassium ferricyanide $K_3Fe(CN)_6$ (1%w/v), followed by incubating at 50°C for 20 mins. The reaction was stopped by adding 2.5mL of 10% (w/v) trichloroacetic acid followed by centrifugation at 3000 rpm for 10min. Finally, 1.5mL of the upper layer was mixed with 1.5mL of distilled water and 0.5mL of $FeCl_3$ (0.1%) and the absorbance was measured at 700 nm. Higher the absorbance of reaction mixture indicated greater the reducing power. Ascorbic acid is used as reference compound.

RESULTS AND DISCUSSIONS:

Phytochemical Constituents: Phytochemical screening was carried out for various extracts of *T.portulacastrum* by preliminary tests. From the results it was evident that, Carbohydrates, Tannins, Phenols, Flavonoids, Proteins and Amino acids were present in all extracts (Pet. Ether, Chloroform, Ethyl acetate,Ethanol and Aqueous). Alkaloids were present in all four extracts except Ethyl acetate extract; Saponins were seen in all four extracts except chloroform extract; Glycosides and steroids were present in only chloroform extract. Results were shown in (Table 1). '+' indicates the presence and '-' indicates the absence of phytochemical constituents.

Total phenol content Estimation: Phenolic compounds are the key phytochemicals with high free radical scavenging activity. It has generated a great interest among the scientists for the development of natural antioxidant compounds from plants. In the current work, phenolic content of the various extracts of *T. portulacastrum* were measured and listed in (Table2). The Ethyl acetate extract of *T. portulacastrum* showed higher amount (82.58±0.629) of phenolic compounds when compared to other extracts. The concentration of the phenolic compounds was increased with increase in the dose. The results are described as Gallic acid equivalents (GAE) (Figure 1).

Total Flavonoid estimation: The flavonoid content of various extracts of *T. portulacastrum* was determined using aluminium chloride colorimetric method. In the current study, the total flavonoid content were measured and listed in (Table 2). The total flavonoid content in Ethyl acetate extract was found to be high (143.77±0.509) when compared to other extracts. The flavonoid content was expressed as quercetin equivalents in milligram per gram of the extract. (Figure 2).

DPPH antiradical capacity: The DPPH assay method is based on the reduction of DPPH, a stable free radical (purple colour) to the non radical form DPPH (yellow colour) in the presence of hydrogen donating antioxidants. The degree of decolorization (yellow colour) depends on the number of electrons captured.

Table 1: Phyto chemical Constituents in various extracts of *T. portulacastrum*

	Pet Ether	Chloroform	Ethyl Acetate	Ethanol	Aqueous
Alkaloids	+	+	-	+	+
Saponins	+	-	+	+	+
Glycosides	-	+	-	-	-
Carbohydrates	+	+	+	+	+
Tannins and Phenolics	+	+	+	+	+
Flavonoids	+	+	+	+	+
Steroids	-	+	-	-	-
Proteins and Amino acids	+	+	+	+	+

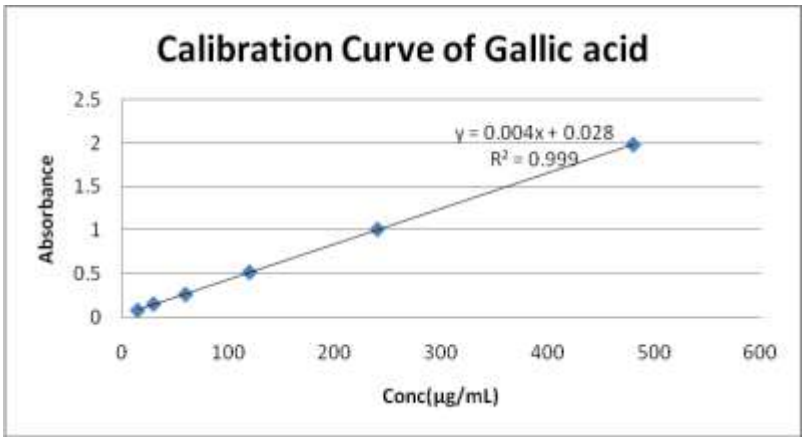

Figure 1.calibration Curve of gallic acid.

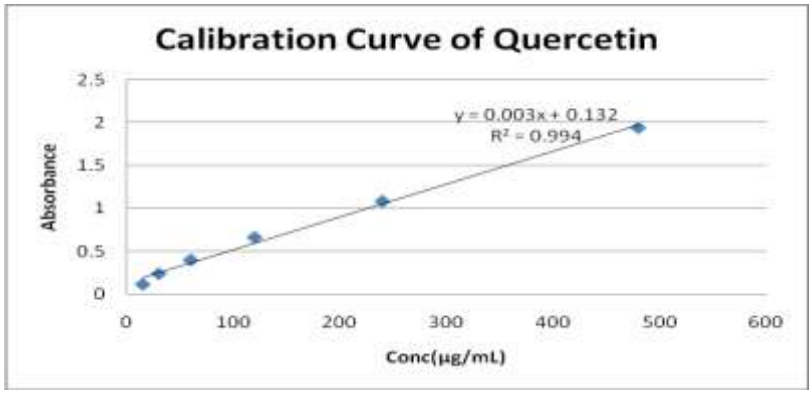

Figure 2. Calibration curve of Quercetin

Table 2: Total Phenolic and Total flavonoid content in various extracts of *T. Portulacastrum*

S.No	Extract	Total Phenols Content (Gallic acid equivalents in mg per gm extract)	Total Flavonoid Content (Quercetin Equivalents in mg per gm extract)
1	Pet Ether	55.08±0.877	27.88±0.838
2	Chloroform	24.66±0.629	10.77±0.693
3	Ethyl Acetate	82.58±0.629	143.77±0.509
4	Ethanol	19.58±1.040	2.88±0.838
5	Aqueous	72.08±0.946	70.66±0.881

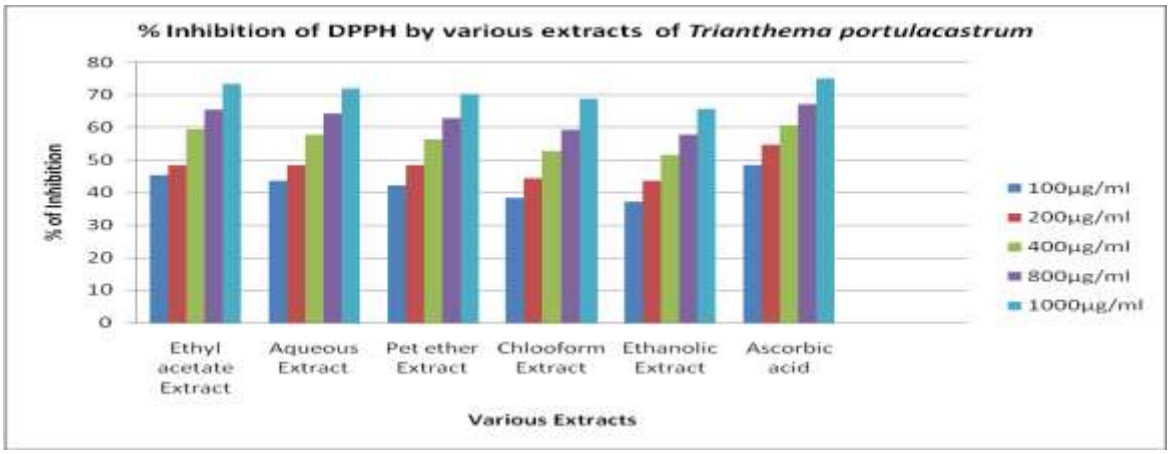

Figure 3(a): Representation of DPPH radical scavenging activity of various extracts of *T. portulacastrum* by MS-Excel

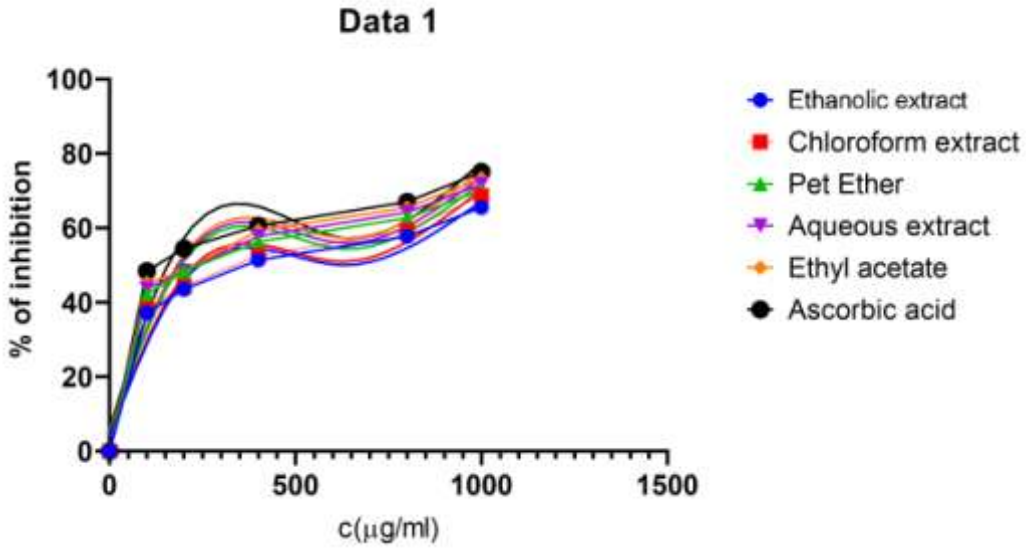

Figure 3(b): Representatoion of DPPH radical scavenging activity of various extracts of *T. portulacastrum* by GraphPad Prism 8.3.1

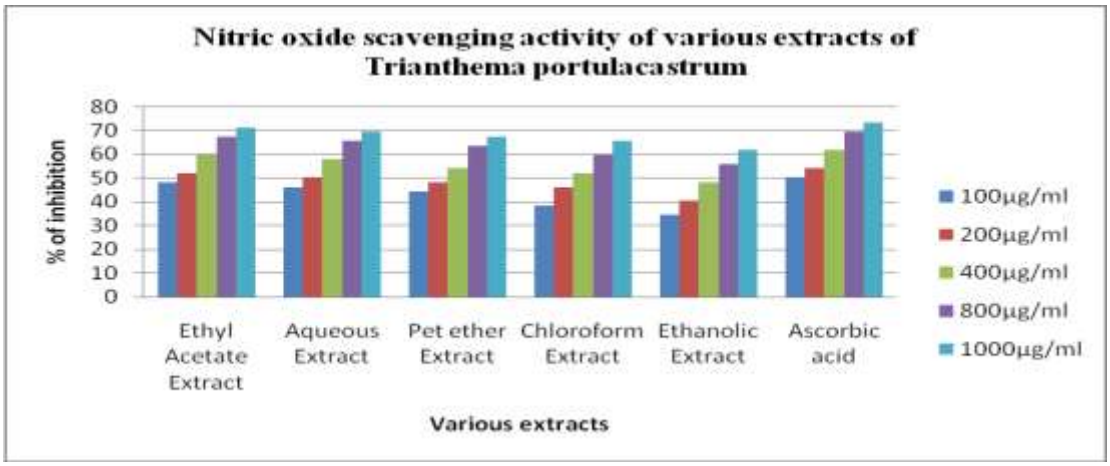

Figure 5(a): Representation of the nitric oxide scavenging activity of various extracts of *T. portulacastrum* by MS-Excel

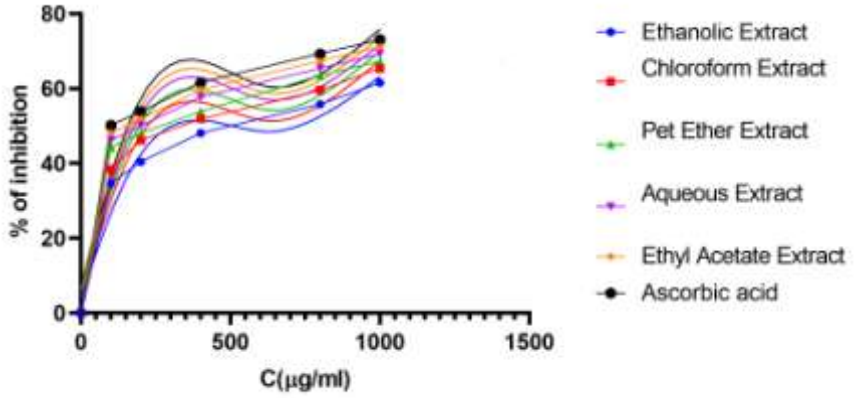

Figure 5(b): Representation of The nitric oxide scavenging activity of various extracts of *T. portulacastrum* by GraphPad Prism 8.3.1

Table 3: IC_{50} values for the various extracts of *T. portulacastrum*

S.No	Extract	DPPH Method (µg/ml)	Nitric oxide scavenging activity (µg/ml)
1	Pet Ether	190.988 ±0.858	195.173±0.552
2	Chloroform	226.657±1.623	221.422±0.151
3	Ethyl Acetate	179.974±0.996	166.926±0.122
4	Ethanol	240.345±3.363	302.013±1.782
5	Aqueous	185.617±1.352	178.014±0.117
6.	Ascorbic acid	155.868±1.022	156.471±0.470

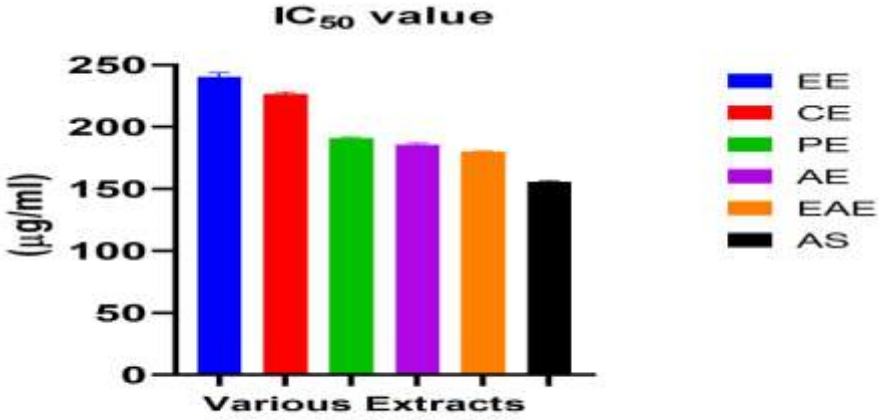

Figure 4: IC_{50} values of various extracts of *T. portulacastrum* by DPPH antiradical capacity

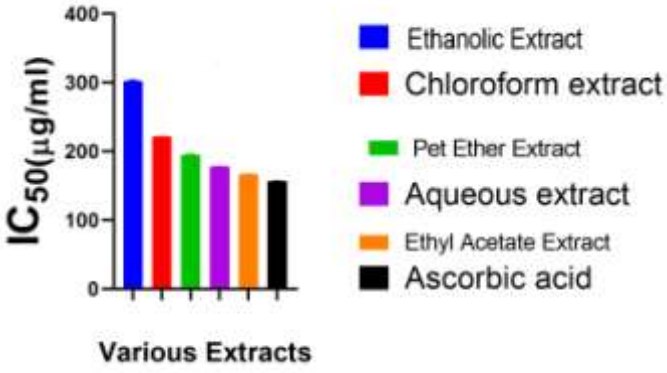

Figure 6: IC_{50} values of various extracts of *T. portulacastrum* by Nitric Oxide sca venging activity

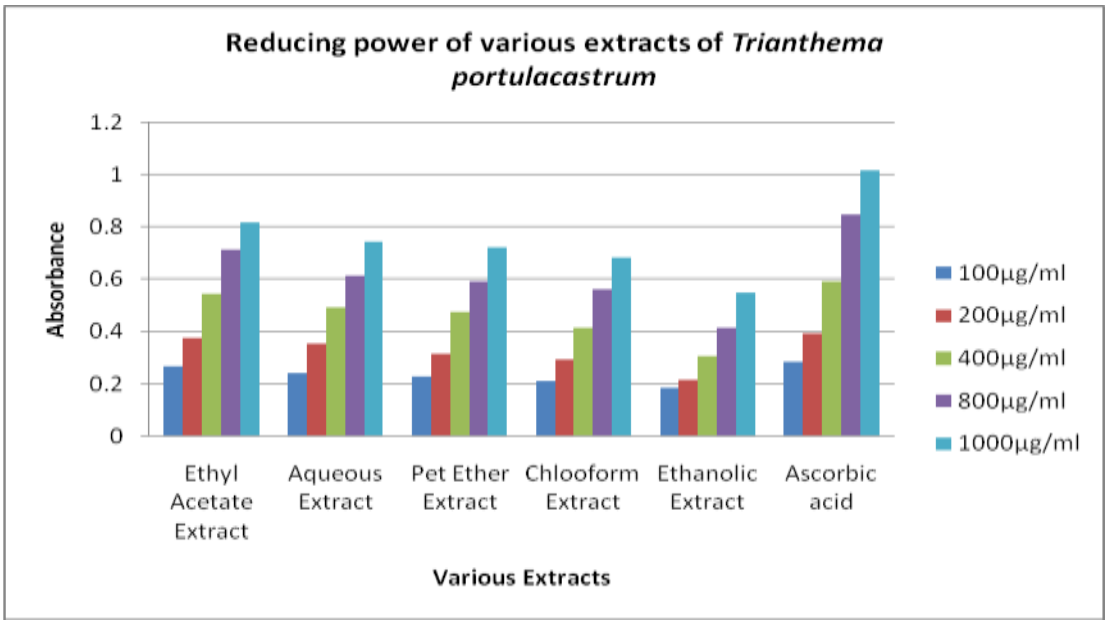

Figure 7: Reducing power of Various Extracts of *T. portulacastrum*

The decolorization of the DPPH therefore reflects the radical scavenging activity of the extracts, which can be quantitatively measured from changes in the absorbance at 517nm. DPPH radical scavenging activity of various extracts of *T. portulacastrum* were compared with ascorbic acid and are reported in (Figure 3a And 3b). IC_{50} values for the various extracts were calculated by using GraphPad Prism 8.3.1 and mentioned in (Table 3) and (Figure 4). Ethyl acetate Extract showed better antiradical activity than the remained extracts.

Nitric Oxide scavenging activity: Nitric Oxide is a potent chemical mediator of physiological processes playing vital role in various biological systems. Under aerobic conditions, NO reacts with oxygen to produce stable products (nitrate and nitrite). The quantities of which can be determined using Griess reagent. Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and is involved in the regulation of various physiological processes Excess concentration of NO is associated with several diseases. NO is generated in biological tissues by specific nitric oxide synthesis (NOSs), which metabolizes arginine to citralline with the formation of NO via a five electron oxidative reaction. These compounds are responsible for altering the structural and functional behaviour of many cellular components. Incubation of solutions of sodium nitroprusside in phosphate buffer solution at 25°c for 180 mins resulted in linear time dependent nitrite production, which is reduced by the extracts of the plants. NO scavenging capacity is determined by the decrease in the absorbance at 540 nm, induced by antioxidants. The nitric oxide scavenging activity of various extracts of *T. portulacastrum* in comparison with ascorbic acid are reported in (Figure 5a and 5b). IC_{50} values for the various extracts were mentioned calculated by using GraphPad Prism 8.3.1 and mentioned in (Table 3) and (Figure 6).

Reducing Power: Reducing power experiment is a good reflector of antioxidant activity of the plant. The plant having high reducing power generally reported to carry high antioxidant potential too. In this experiment, Ferric ions are reduced to ferrous ions, identified by colour change from yellow to bluish green. The results for ferric reducing power activity of various extracts of *T. portulacastrum* in comparison with ascorbic acid are reported in (Figure 7). Ethyl acetate extract showed high reducing power than that of other extracts. Reducing power potential of extracts increase with the dose, however the extracts exhibited low reducing power than that of ascorbic acid.

CONCLUSION:

Plant source is considered to be the good source of natural antioxidants for curing the diseases. Determination of natural antioxidant compounds will help to develop the new drug candidates for antioxidant therapy. The purpose

of this research was to study the total phenol, total flavonoid content and *in vitro* antioxidant efficacy of the plant *T. portulacastrum*. The plant is rich in phenolic and flavonoid content in it. It is evident that there exists a relationship between phenol, flavonoid content and antioxidant potency of the plant extract. Ethyl Acetate extract has shown better antioxidant profile when compared to the other extracts and less potent than the synthetic antioxidants like ascorbic acid and quercetin.

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