Available online at www.ijpsdronline.com
International Journal of Pharmaceutical Sciences and Drug Research 2010; 2(3): 219-223

Research Article ISSN 0975-248X

Antioxidant Potential Fractionation from Methanol Extract of Aerial Parts of *Convolvulus arvensis* Linn. (*Convolvulaceae*)

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ABSTRACT

Aerial parts of *Convolvulus arvensis* were subjected to extraction and further fractionation to obtain antioxidant rich fraction. Different concentrations of methanolic extract and its ethyl acetate fraction were subjected to antioxidant assay by DPPH method, nitric oxide scavenging activity and reducing power assay. The fractions showed dose dependent free radical scavenging property in all the models. IC_{50} values for methanolic extract and its ethyl acetate fraction were found to be 131.03 ± 2.46 and 43.21 ± 4.45 respectively in comparison to L-Ascorbic acid and Rutin as standard with IC_{50} values of 6.537 ± 0.235 and 5.437 ± 0.206 respectively in DPPH model. In nitric oxide scavenging activity the IC_{50} values were found to be 130.12 ± 2.46 and 57.5 ± 4.45, 21.06 ± 0.953 and 29.93 ± 0.324 for methanolic extract and its ethyl acetate fraction, L-Ascorbic acid and Rutin respectively. The fractions showed good reducing power with increasing concentration. However, the ethyl acetate fraction showed a good reducing power and better free radical scavenging activity as compared to methanolic extract thus its antioxidant potential is comparable to standards. HPTLC analysis of ethyl acetate fraction confirms the presence of flavonoids which are responsible for the antioxidant activity. This is the first report on antioxidant activity of *C. arvensis*.

Keywords: Antioxidant, *C. arvensis*, free radical.

INTRODUCTION

There is extensive evidence to implicate free radicals in the development of degenerative diseases. [1-2] Almost all organisms possess antioxidant defences that protect against oxidative damage and numerous damage removal and repair enzymes to remove or repair damaged molecules. However, the natural antioxidant mechanisms can be inefficient, hence dietary intake of antioxidant compounds become important. [3-5] Although, synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ) have been widely applied in food processing, they have been reassessed for their possible toxic and carcinogenic components formed during degradation. [6] In addition, it has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and incidence of number of human diseases. [7-8] Therefore, search into the isolation of natural antioxidant sources is important.

Convolvulus arvensis Linn. (*Convolvulaceae*) is an annual or sometimes perennial climber, commonly found as a weed throughout India. Reviewing the previous work of the *Convolvulus arvensis* L. was found to have very little chemical and biological studies. It was mentioned in folk medicine that the leaves of this plant have purgative activity [9], also used in asthma [10], jaundice [11] and as antihemorrhagic. [12] Some phytochemical studies were carried out on *Convolvulus arvensis* L. and showed that it contains alkaloids. [13-14] Phenolic compounds, sterols and resin, sugars. [15-18] So it was found that it is of interest to study this plant and check out scientifically its uses in folk medicine and traced any new biological activities.

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MATERIALS AND METHODS

Source of chemicals

All chemicals used were of analytical grade. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Chemical Co. Rutin, L- Ascorbic acid, Tri chloroacetic acid (TCA) and ferric chloride were procured from central drug house (CDH), new Delhi. Sulphanilic acid and napthylelene diamine dichloride from Rankem, Sodium nitroprusside and potassium ferricyanide were obtained from nice chemicals. Solvents used during the experiment were purchased from Rankem. Analysis of ethyl acetate fraction was done by HPTLC (CAMAG). The other chemicals used in this experiment were of the highest quality available. Absorbance was noted using UV/Visible Spectrophotometer (UV 1700, Pharmaspec, SHIMADZU, Japan).

Plant material

The aerial parts of the plant were collected from the road sides, waste land of Amritsar region and the collected plant material was authenticated by Dr. B.K Kapahi, Head of Department of Botany, IIIM, Jammu. A voucher specimen was retained and deposited at the crude drug repository of the herbarium of IIIM, Jammu (Vide CDR accession No.21583). The collected aerial parts were cleaned, dried under shade at room temperature, and powdered.

Extraction and fractionation

Coarsely powdered aerial parts (100 g) were defatted with petroleum ether (60-80°C) and dried marc was further extracted with 90% methanol using Soxhlet apparatus. The methanol extract so obtained was freed from solvent in a vacuum evaporator to obtain 12.5 g of extract. This was further extracted with chloroform, ethyl acetate and n-butanol to obtain their respective fractions. The percentage yields are 0.2 %, 2.5 % and 2.5 % respectively.

Phytochemical analysis

Methanol, chloroform, ethyl acetate and n-butanol fractions were subjected to phytochemical analysis for the presence of alkaloids, glycosides, tannins, flavonoids. [19] Ethyl acetate fraction was subjected to UV scanning from 190-400 nm by the help of HPTLC.

***In-vitro* antioxidant studies**

Methanolic extract and its ethyl acetate fraction were tested for their free radical scavenging property using different in-vitro models. All experiments were performed thrice and their results averaged. L-Ascorbic acid and Rutin were used as standard control in each experiment. IC_{50} values were calculated.

The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance is needed to inhibit a given biological process by half. In other words, it is the half maximal (50%) inhibitory concentration (IC) of a substance (50% IC, or IC_{50}). It is commonly used as a measure of pharmacological research. Sometimes, it is also converted to the pIC_{50} scale (-log IC_{50}), in which higher values indicate exponentially greater potency. According to the FDA IC_{50} represents the concentration of a drug that is required for 50% inhibition *in vitro*.

DPPH radical scavenging activity [20-21]

DPPH radical scavenging activity was performed according to the method of Blois. The 0.1 mM solution of DPPH in methanol (22.2 mg in 1000 ml) was freshly prepared. Different concentrations of extract and standards (5-250 µg/ml) were added at an equal volume to methanolic solution of DPPH. After 30 min at room temperature, the absorbance was recorded at 517 nm. Radical scavenging activity was calculated by the following formula (formula 1)

$$\% \text{ inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where, $A_{control}$ = Absorbance of control reaction and A_{test} = Absorbance in the presence of the samples of extracts.

Nitric oxide radical scavenging activity [22-23]

Nitric oxide radical scavenging activity was performed according to the method of Garrat. 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 (10-200 µg/ml) and the mixture incubated at 25°C for 150 min. From 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 naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm. The nitric oxide radicals scavenging activity was calculated by the formula 1.

Reducing power assay [24-25]

Reducing power assay was performed according to the method of Oyaizu. The extract (0.75 ml) at various concentrations (25-400µg/ml) was mixed with 0.75 ml of phosphate buffer (0.2 M, pH 6.6) and 0.75 ml of potassium ferricyanide (1%, w/v), followed by incubating at 50°C in a water bath for 20 min. The reaction was stopped by adding 0.75 ml of trichloracetic acid (TCA) solution (10%) and then centrifuged at 3000 r/min for 10 min. 1.5 ml of the supernatant was mixed with 1.5 ml of distilled water and 0.1 ml of ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power. Higher absorbance of the reaction mixture indicated greater reducing power.

Statistical analysis

All analysis was done in triplicate and results are expressed as ± mean S.D. IC_{50} values were determined by interpolations.

Table 1: IC_{50} values of free radical scavenging effect by DPPH method of various fractions and standards

S. No.	Fraction	IC_{50} (µg/ml)±S.D.
1.	Rutin	5.437±0.206
2.	Ascorbic Acid	6.537±0.235
3.	Methanolic extract	131.03 ± 2.46
4.	Ethyl acetate fraction	43.21 ± 4.45

(Values are mean ± S.D of 3 replicates)

Table 2: IC_{50} values of Nitric oxide scavenging activity of various fractions and standards

S. No.	Fraction	IC_{50} (µg/ml)
1.	Rutin	29.93 ± 0.324
2.	Ascorbic Acid	21.06 ± 0.953
3.	Methanolic fraction	130.12 ± 2.46
4.	Ethyl acetate fraction	57.54 ± 4.45

(Values are mean ± S.D of 3 replicates)

Table 3:- Reductive ability of C.arvensis and standards

Conc. (µg/ml)	Absorbance			
	Ascorbic acid	Rutin	Methanolic extract	Ethyl acetate frc.
25	0.075±0.003	0.062±0.062	0.055±0.003	0.061±0.002
50	0.347±0.004	0.313±0.004es	0.104±0.002	0.224±0.003
100	0.611±0.002	0.592±0.004	0.332±0.002	0.578±0.004
200	1.389±0.004	1.293±0.005	0.614±0.003	1.323±0.001
400	2.82±0.005	2.763±0.005	1.167±0.004	2.627±0.004

(Values are mean ± S.D of 3 replicates)

RESULTS

Phytochemical screening

Flavonoids were found in ethyl acetate fraction. HPTLC spectral analysis of ethyl acetate fraction showed absorbance peaks with λ_{max} of 263 nm, 251 nm, 234 nm which are characteristic absorbance of flavonoids.

Antioxidant studies

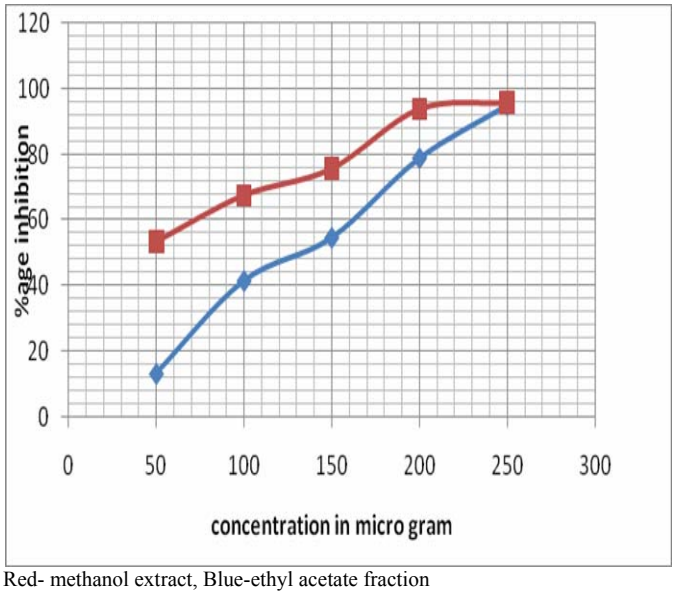
Several concentrations of methanolic extract and its ethyl acetate fraction ranging from (5-400 µg/ml) were tested for their antioxidant activity in different *in-vitro* models. . It has been observed that ethyl acetate fraction exerted higher antioxidant potential as compared to methanolic extract and its antioxidant activity is comparable to the standards Rutin

and L-Ascorbic acid. . The antioxidant activity increased with increasing concentration in all the models in both fraction and extract. The percentage inhibition of both standards and fractions in various models viz. DPPH and nitric oxide scavenging assay are shown in Fig. 1-11. IC_{50} values of both standards and extracts in the DPPH model and nitric oxide scavenging assay are shown in Table 1 and 2 respectively. Reducing power of fractions was good and increased with increasing concentration. Ethyl acetate fraction showed a significant reducing ability in comparison to Rutin and L-Ascorbic acid.

DISCUSSION

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The propagation of free radicals can bring about thousands of reactions and thus may cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals. [28-29] Antioxidants may offer resistance against oxidative stress by scavenging the free radicals and by their reducing ability.

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) is stable nitrogen centred free radical which can be effectively scavenged by antioxidants and shows strong absorbance at 517 nm. DPPH radical accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The change in absorbance of DPPH radical caused by antioxidants is due to the reaction between the antioxidant molecules and the radical, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Extent of DPPH radical scavenged was determined by the decrease in intensity of violet colour in the form of IC_{50} values. [30] Hence it has been widely used for rapid evaluation of the antioxidant activity of plant and microbial extracts relative to other methods. [31] DPPH is also considered as a good kinetic model for peroxyl radicals. [32] The methanolic extract and the ethyl acetate fraction showed significant DPPH scavenging activity (Table 1) (IC_{50}=131.03 ± 2.46 µg/ml and IC_{50}=43.21 ± 4.45 µg/ml) respectively when compared with the IC_{50} values of the standards Ascorbic acid and Rutin (IC_{50}=6.537 ± 0.235 and5.437 ± 0.206 µg/ml respectively).



Red- methanol extract, Blue-ethyl acetate fraction

Fig. 1: Free radical scavenging effect of the methanol extract and its ethyl acetate fraction of aerial parts of *C. arvensis* by DPPH

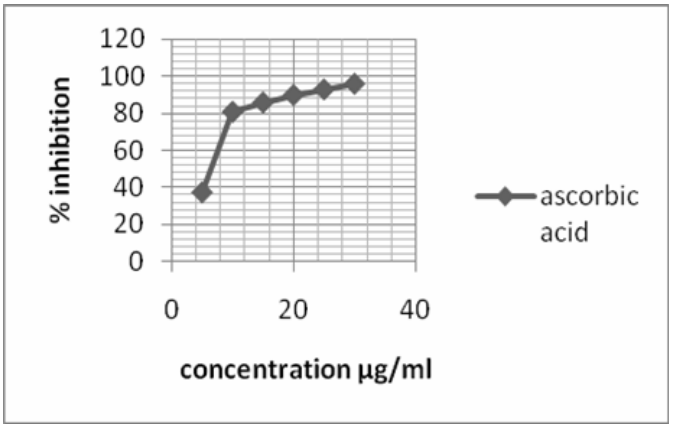


Fig. 2: Free radical scavenging effect of the ascorbic acid by DPPH

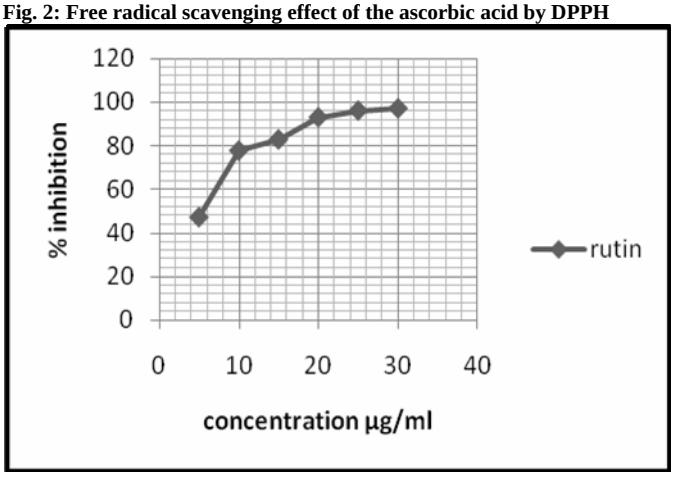


Fig. 3: Free radical scavenging effect of the Rutin by DPPH

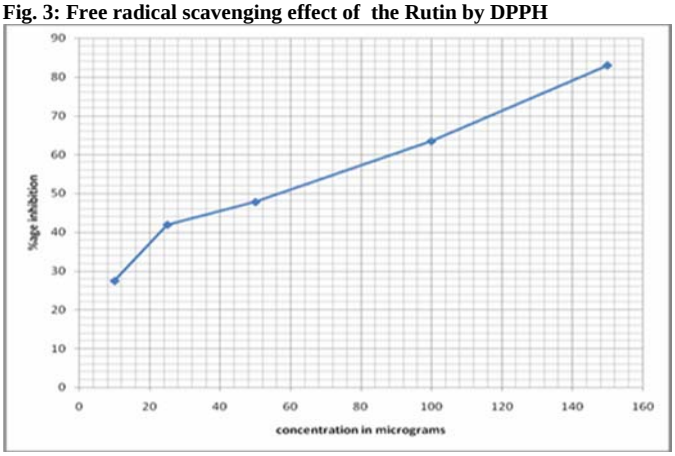


Fig. 4: Nitric oxide scavenging effect of ethyl acetate fractions of the methanol extract of the *C.arvensis*

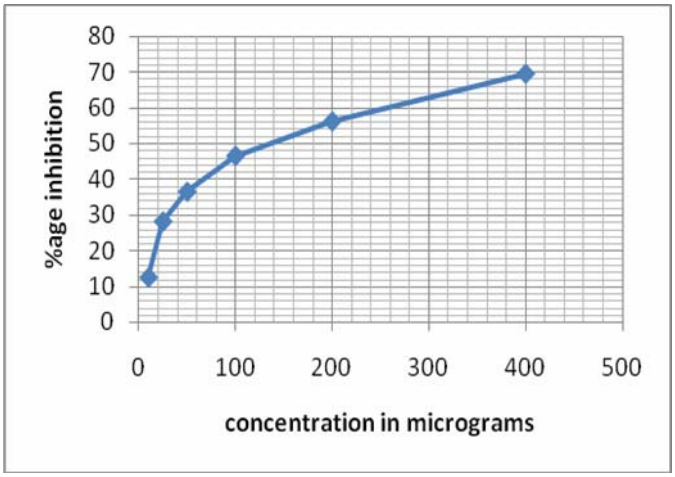


Fig.5: Nitric oxide scavenging effect of methanolic extract of aerial parts of *C.arvensis*

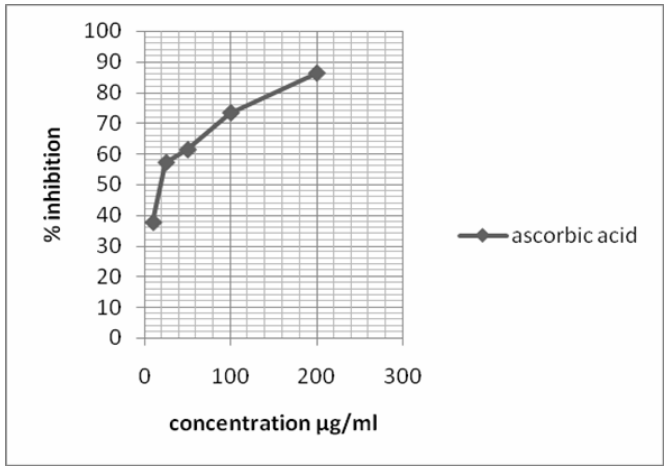


Fig. 6 : Nitric oxide scavenging effect of the ascorbic acid

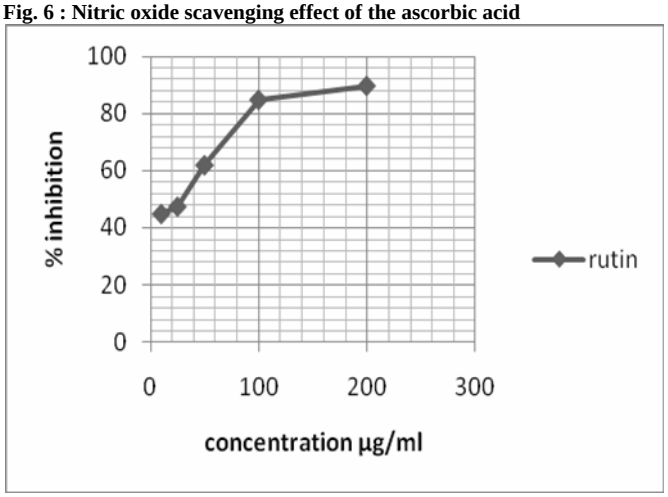


Fig. 7: Nitric oxide scavenging effect of the Rutin

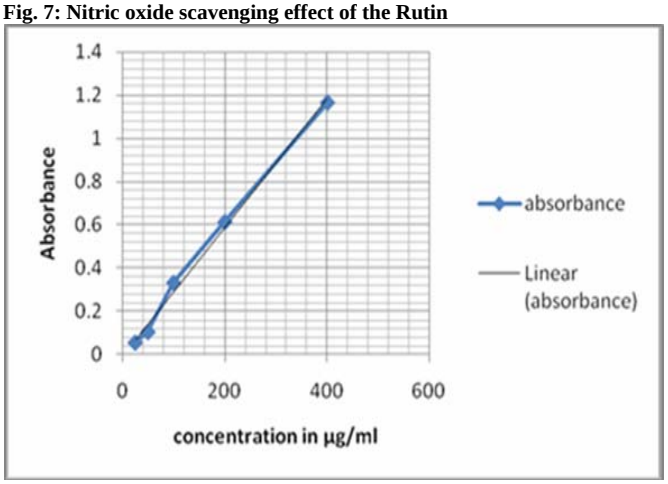


Fig. 8: Total reducing power of the methanol extract of *C. arvensis*

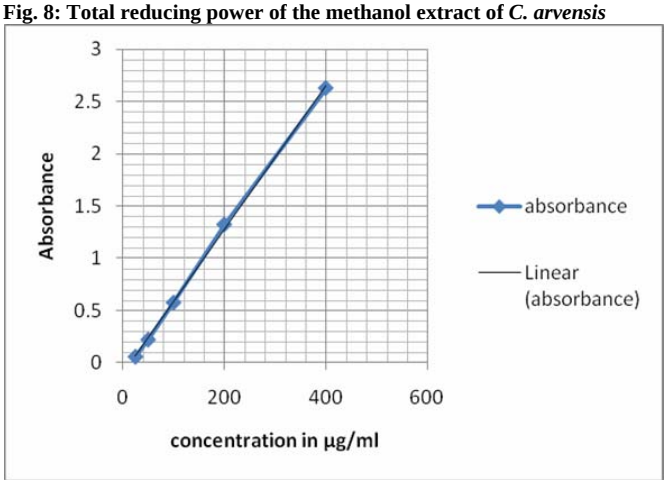


Fig. 9: Total reducing power of ethyl acetate fraction

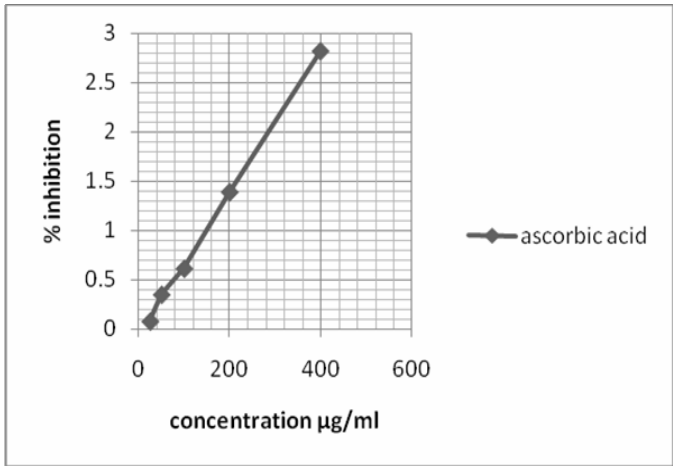


Fig. 10: Total reducing power of the Ascorbic acid

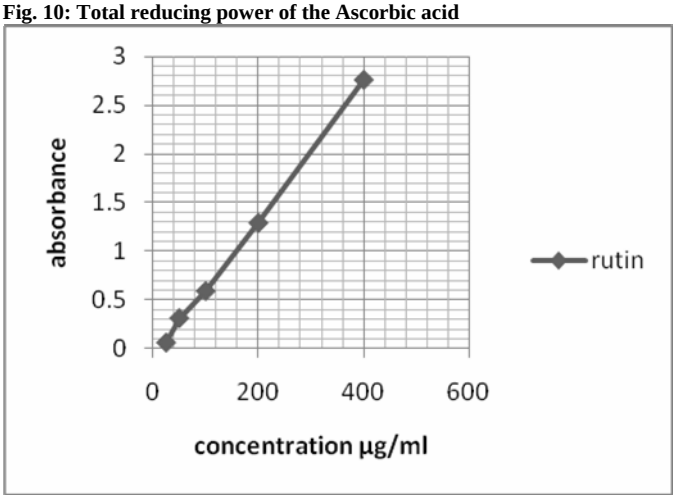


Fig. 11: Total reducing power of the Rutin

Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities. [33] Although nitric oxide is involved in host defence, over production of these this radical contributes to the pathogenesis of some inflammatory diseases. [34] Moreover in the pathological conditions, nitric oxide reacts with superoxide anion and form potentially cytotoxic molecules, peroxynitrite. Nitric oxide inhibitors have been shown to have beneficial effects on some aspect of inflammation and tissue damage seen in inflammatory diseases. [26] *C. arvensis* significantly inhibited nitric oxide in a dose dependent manner (Table 2) with the IC_{50} being 130.12 ± 2.46 and 57.5 ± 4.45 µg/ml for methanolic and its ethyl acetate fraction respectively as compared with the standards Ascorbic acid and Rutin having the IC_{50} values of 21.06 ± 0.953 and 29.93 ± 0.324 µg/ml respectively.

Fig. 8 shows the reductive capability of the methanolic extract is less prominent than ethyl acetate fraction with reference to Ascorbic acid and Rutin (standards). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing activities are generally associated with the presence of reductones, which have been shown to exhibit antioxidant action by breaking the chain reactions by donating a hydrogen atom. Reductones are also reported to react with

certain precursors of peroxide, thus preventing peroxide formation. [35] For the measurement of the reductive ability, we investigated the ferric (Fe^{3+}) – ferrous (Fe^{2+}) transformation in the presence of extracts using the method of Oyaizu. In this method, antioxidant compounds form a coloured complex with potassium ferricyanide, trichloroacetic acid and ferric chloride that was measured at 700 nm. Increase in absorbance of the reaction mixture indicates the increase in the reducing power of the sample. The antioxidant activity has been attributed to various mechanisms, among which are the prevention of chain initiation, the binding of transition metal ion catalysts, decomposition of peroxides, the prevention of continued hydrogen abstraction, the reductive capacity and free radical scavenging. Both the Ethyl acetate and methanolic extract were showing the antioxidant potential but the antioxidant potential of ethyl acetate fraction is far higher than the methanolic extract and is comparable to the standards Rutin and L-Ascorbic acid. Aerials parts of *C. arvensis* are reported to contain flavonoids. Thus UV analysis of the ethyl acetate fraction also revealed the presence of flavonoids. These flavonoids may be responsible for the antioxidant potential of the plant hence; the plant *C. arvensis* have antioxidant potential, which is due to the presence of flavonoids.

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