International Journal of Phytomedicine 9 (2017) 351-363
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

In vitro antioxidant activity of *Polygonum glabrum*

Raja S*and Ramya I

***Corresponding author:**

Raja S

Department of pharmaceutical chemistry
GITAM Institute of Pharmacy
GITAM University, Andhra pradesh,
India.

Abstract

Assessment of antioxidant activity was imperative in the screening of medicinal plants for potential health benefits. In present study methanol extract of *Polygonum glabrum* (polygonaceae) was screened for its in vitro antioxidant activity using biologically relevant methodologies which scavenge radicals such as 1,1 diphenyl 2 picryl hydrazyl (DPPH), nitric oxide, hydrogen peroxide, hydroxyl, superoxide anion and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS). Total reducing ability by conversion of ferric (III) to ferrous (II) and molybdenum (VI) to molybdenum (V), metal ion chelating capacity and anti lipid per oxidation activities were also examined. The antioxidant ability of *Polygonum glabrum* whole plant extract was found to be in a dose dependent manner. The IC_{50} values for scavenging of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radicals were 13.18 μg/ml and 20.46 μg/ml. For scavenging of nitric oxide, hydrogen peroxide, hydroxyl and superoxide anion radicals, the IC_{50} values were found to be 80.22 μg/ml, 33.06 μg/ml, 52.26μg/ml and 36.98 μg/ml respectively. Further, addition of 120μg/ml of plant extract to the reaction mixture produced 50% lipid per oxidation inhibition activity. Commercial antioxidants such as vitamin E, quercetin, butylated hydroxytoluene and ascorbic acid were used as reference compounds. The strong antioxidant activity of *Polygonum glabrum* may be credited to the presence of triterpenes [beta-hydroxyfriedalanol], phenols [3-hydroxy-5-methoxystilbene], flavonoids [pinocembrin and pinocembrin-5-methylether], steroids [sitosterol - (6-O-palmitoyl)- 3-O-β-D glucopyranoside and sitosterol-3-O-β-D glucopyranoside], sesqueterpenes [2,3-dihydroxy isodrimeninol] and pigments etc in methanol extract.

Keywords: *Polygonum glabrum*, methanol extract, hydroxyl radical, lipid peroxidation, sesqueterpenes.

Introduction

Conceptually, free radicals are unpaired, unstable, highly reactive ions, atoms or molecules. Free radicals are acquired from four elements including oxygen, nitrogen, sulphur and chlorine [1]. Over the years, several studies state that a negative correlation exists between free radicals and antioxidants. Free radicals initiate and propagate chain reactions inflicting oxidative stress while antioxidants terminate chain reaction through free radical scavenging [2]. Overproduction of reactive species such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and nitric oxide ($NO^{\bullet}$) often results in oxidative stress [3]. To encounter the threat caused by oxidative stress in the body, a network of enzymatic (superoxide dismutase (SOD), catalase, glutathione, NADH peroxidase) and non enzymatic antioxidants (glutathione, vitamin C, α-tocopherol) act to ensure that the steady state concentration of free radicals are within limits [4]. However, under ischemic condition the inbuilt antioxidant system fails by compromising cell health and viability leading to chronic conditions such as cardiovascular disease, cancer, liver damage, diabetes, asthma etc [5].

Contemporary treatment with synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propylgallate may be effective but are viewed as a threat to human health owing to their undesired side effects. Since plants have resourceful enzymatic and non enzymatic antioxidant defense systems in various compartments of their cell they become the natural choice. Indeed most of the beneficial health effects of flavones, isoflavones, phenols, anthocyanins, terpenes etc occurring in fruits and vegetables are attributed to their antioxidant and chelating abilities [6]. Naturally occurring antioxidants (vitamin A, citrin, hydroxytyrosol, vitamin E and ascorbic acid) are highly proposed by researchers as their credential in prevention of neurological disorders and immune deficiency diseases are widely reported.

Polygonum glabrum is a semi aquatic perennial plant. Traditionally, the root stock is used for the treatment of jaundice and piles. A decoction of the leaves and seeds are used as cardiotonic, astringent and anthelmentic [7]. Peels from stem are

DOI:10.5138/09750185.2049

used for treating rheumatism. Pharmacologically, the plant has been effectively screened for several studies. The methanol extract of leaves of *Polygonum glabrum* showed optimum cytotoxic activity at LC_{50} value 0.74 ± 0.045µg/ml [8]. Oral administration of methanol extract produced significant nephro protection in the rats at a dose of 200mg/kg and 400mg/kg. The ethanol extract showed potential hypolipidaemic effects in carbon tetrachloride induced hepatic injury [9]. A significant reduction in the yeast elevated temperature was noted in an experiment that supported the anti pyretic effect. The methanol extract showed maximum activity against pathogens such as *Staphylococcus aureus, Micrococcus luteus, Candida tropicalis* etc [10]. Substantial literature survey suggests that genus *Polygonum* are rich source of flavonoids. Madhukar et al isolated (2)-2-methoxy-2-butenolide-3-cinnamate along with known compounds like β-hydroxyfriedalanol, 3-hydroxy-5- methoxystilbene, pinocembrin, sitosterol-(6 -O-palmitoyl)-3-O-β-Dglucopyranoside, pinocembrin-5-methyl ether and sitosterol-3-O-βDglucopyranoside [11]. Flavonoids like avicularin, rhamnetin, diosmetin, cyanidin 3 5-diglucoside and delphinidin 3, 5, diglucoside were isolated from the methanol extract of leaves and flowers of *Polygonum glabrum* [12]. Straight phase and reverse phase chromatography of methanol extracts of leaves of *Polygonum glabrum* led to the isolation of four new sesqueterpene diesters [13]. From the above works it was evident that *Polygonum glabrum* consists of assorted compounds in methanol extract. The present study was aimed to further investigate the antioxidant activity of methanol extract of whole plant of *Polygonum glabrum* by use of different in vitro assays.

Materials and methods

Chemicals and reagents

All reagents used were of analytical grade. Chemicals ferric chloride, linoleic acid, 2,2- diphenyl-1-picryl-hydrazyl(DPPH), ethylenediamine tetracetic acid (EDTA), potassium persulfate, 2,2′azinobis(3-ethylbenzothiozoline-6-sulfonic acid) disodium salt (ABTS), ferrous chloride, ammonium thiocyanate, hydrogen peroxide, ferrous ammonium sulfate and hydroxylamine hydrochloride were obtained from Aman Scientific Products, Vijayawada.

Collection of plant material

Polygonum glabrum was collected from Tirupati (Andhra Pradesh) and further identified, confirmed & authenticated by Dr. Madavchetty, Professor, Botany department, Sri Venkateswara University, Tirupati. The plant was preserved in the herbarium of GITAM Institute of Pharmacy, GITAM University, for future reference (Voucher specimen No -1916). The whole plant was washed, air-dried, homogenized to fine powder and stored under ambient conditions.

Preparation of extract

The air dried and powdered plant material (500gm) was extracted with methanol in a soxhlet apparatus. The extraction was done continuosly till a clear solvent was observed in the thimble. The methanol extract was evaporated using a rotary vacuum evaporator. Then the extract was concentrated in a water bath. The percentage yield of the extract was calculated.

In vitro antioxidant assay

DPPH radical scavenging assay

The free radical scavenging activity of *polygonum glabrum* extracted with methanol was measured by 1, 1 diphenyl 2 picryl hydrazyl (DPPH) method [14]. A stock solution was prepared by dissolving methanol extract in distilled water. From this working solution of different concentration (5, 10, 20, 40, 80 µg/ml) were prepared. A 0.1 mM solution of DPPH dissolved in ethanol was prepared. 1ml of working solution was added to 3 ml of plant extracts of different concentration. The mixture was shaken vigorously and allowed to stand at room temp for 20 minutes. Absorbance was measured at 517 nm by using spectrophotometer. Quercetin was used as reference standard compound and serial dilutions of standard compound was also prepared.

Nitric oxide radical inhibition assay

The nitric oxide radical scavenging activity of *polygonum glabrum* extracted with methanol was measured according to method of Parul et al [15]. Initially a stock solution with plant extract was prepared. From this working solution of 5, 10, 20, 40, 80 and 160 µg/ml was prepared. Two ml of sodium nitroprusside (10mM) in phosphate buffered saline (0.5ml) was mixed with different concentrations of methanol extract of plant and incubated at 30 C for 2 hours. After the incubation period, 1ml of Griess reagent (1% sulphanilamide, 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 2% orthophosphoric acid), Phosphate buffer (pH-7.4) was added. The mixture was then incubated at room temperature for further 30 min and its absorbance was measured at 550 nm. Rutin was served as standard.

Hydrogen peroxide scavenging capacity

The ability of *polygonum glabrum* methanol extracts to scavenge hydrogen peroxide was determined by method of Gayathri et al [16]. From the stock solution 4 ml of methanol extract in various dilutions (12.5-100 µg/ml) was made as working solution. To this 0.6 ml of 4mM hydrogen peroxide solution prepared in phosphate buffer (0.1M, pH 7.4) was added. It was incubated for 10 minutes. Absorbance of hydrogen peroxide at 230 nm was determined

against a blank solution. The experiments were done in triplicates. Vitamin E was used as reference.

Hydroxyl radical scavenging activity

The ability of *polygonum glabrum* methanol extracts to scavenge hydroxyl radical was determined. Working solutions of various concentrations (10-160 μg/ml) were prepared with methanol extract of *polygonum glabrum* [16]. To 500 μl of methanol extract at various concentrations, 100 μl of 2-deoxy2- ribose and 200 μl of 1.04 mM ethylene diamine tetra acetic acid (EDTA) was added. Further 200μM ferric chloride (1:1, v/v) and 100 μl of 1.0 mM hydrogen peroxide were added. Finally 100μl of 1.0 mM vitamin C was added. All samples were incubated at 37 C. After one hour 1ml of 1% thiobarbituric acid (TBA) and 1.0 ml 2.8% trichloroacetic acid (TCA) were added to the reaction mixture and incubated at 100ºC for 20 min. The absorbance was measured at 532 nm against a blank. Vitamin E at various concentrations was used as a positive control.

Superoxide anion radical scavenging activity

Measurement of superoxide anion radical scavenging activity of *polygonum glabrum* methanol extract was carried out by method of Nishimiki et al [17]. A working solution of serial dilutions (5, 10, 20, 40, 80, 160 μg/ml) were prepared from methanol extract. To each dilution 1ml of nitroblue tetrazolium (NBT) solution and 1 ml NADH solution were added. The reaction was initiated by adding 100 μl of phenazine methosulphate (PMS) solution. The reaction mixture was incubated at 25 C for 5 min and the absorbance at 560 nm was measured against blank sample. Curcumin was used as reference compound.

Total antioxidant activity (Phosphomolybdic acid method)

The free radical scavenging activity of *polygonum glabrum* extracted with methanol was measured by Phosphomolybdic acid method [18]. From the stock solution of methanol extract, working solutions at various concentrations like 100, 200, 400, 800 and 1600 μg/ml were prepared. 0.4 ml from each dilution was mixed with 4 ml of reagent solution which comprises of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The samples were incubated at 90 C for 90 min. The samples were taken out, kept in room temperature and absorbance of the mixture was measured at 695 nm against a blank. Ascorbic acid was taken as reference.

ABTS radical scavenging assay

Total antioxidant activity of *polygonum glabrum* was assessed by measuring the reduction of the ABTS radical cation. An aqueous solution of ABTS (7 mmol/L) and potassium persulfate (2.4 mmol/L) were taken in equal proportion. They were allowed to react for 12 h at room temperature in the dark [19]. 2.5 ml of this solution was mixed with 1 ml of methanol extract of *polygonum glabrum* (5-160 μg/ml) and the absorbance was taken at 734 nm using spectrophotometer. Butylated hydroxy toluene was taken as reference.

Metal ion chelating activity

Working solution of methanol extract (100-1600μg/ml) was prepared. 1ml of test solution was mixed with 0.1 ml of 2 mM $Fecl_2$ and 0.2 ml of 5 mM ferrozine. The tubes were vortexed and kept for 10 min. The absorbance was measured at 562 nm. Distilled water served as control. The ability of plant extract to chelate ferrous ion was calculated [20]. EDTA was used as reference.

Reducing power activity

The reducing power of the methanol extract of polygonum glabrum was determined according to the method of Jayanthi et al [21]. One ml of working solution of plant extract containing (100-1600 μg/ml) was added to 2.5 ml of 0.2 M phosphate buffer pH 6 and 2.5 ml of potassium ferricyanide (1% w/v). The mixture was incubated for 20 min at 50 C. Further 2.5 ml of 10% trichloroacetic acid was added and centrifuged for 10minutes. The upper layer of the solution was mixed with 2.5ml distilled water and Ferric chloride (0.5 ml, 0.1%). The absorbance was measured at 700 nm after 30 min. Butylated hydroxy toluene was used as reference. Increased reducing power was indicated by increased absorbance of the reaction mixture.

Xanthine oxidase inhibitory activity

The xanthine oxidase (XO) inhibitory activities of the methanol extract of *Polygonum glabrum* was determined with xanthine as the substrate [22]. The reaction mixture was obtained by mixing 1.0 ml of serial dilution of methanol extract of *Polygonum glabrum* (10-160 μg/ml) prepared in 12% v/v dimethyl sulfoxide and 1.9 ml phosphate buffer (pH 7.5). Later 0.1 ml of xanthine oxidase enzyme solution (0.045units/ml) and 1ml of 100 μM xanthine was added to assay mixture. The reaction mixture was incubated for 15 min at 25 C. The enzyme reaction was stopped by adding 1ml of 1M HCl and the absorbance was measured at 295 nm against blank solution. Allopurinol was used as standard.

Lipid per oxidation assay

This assay was used to determine thiobarbituric acid reactive substances. Rat liver microsomal fraction and methanol extract of

polygonum glabrum (10–160 μg/ml) in various concentrations were prepared by the method of Bouchet et al [23]. Five hundred μl of liver microsomal fraction, 300 μl of working solution of plant extract and 100 μl of $FeCl_3$ (1mM) were mixed. 100 μl vitaminC (1mM) was added finally. Samples were incubated at 37 C for 1 h and lipid per oxidation was measured using the reaction with thiobarbituric acid (TBA). The absorbance was measured at 532 nm. All reactions were done in triplicate. Vitamin E was used as a standard.

Results

Percentage yield

The percentage yield of methanol extract of *Polygonum glabrum* was found to be 7.6 gm (Table 1).

Table 1: Percentage yield of methanol extract of *Polygonum glabrum*

Extract name	Yield (gm)
Methanol extract	7.6

In vitro antioxidant assay

DPPH radical scavenging activity

DPPH is 2, 2-diphenylpicrylhydrazyl, a stable free radical which in the presence of a antioxidant gets reduced to 2,2-diphenylpicrylhydrazine. This reduction was observed by a colour change from purple to yellow. The decolourisation is correlated to decrease in absorbance of the solution. The scavenging of DPPH radical by methanol extract of *Polygonum glabrum* was monitored and illustrated in Figure 1. The IC_{50} value (IC_{50} 13.18 μg/mL) of methanol extract of *Polygonum glabrum* was found to be more than that of standard, Quercetin (IC_{50} value of 9.5 μg/mL).

Nitric oxide radical inhibition assay

Sodium nitroprusside has the ability to produce nitric oxide in phosphate buffer saline. The pathways that lead to the discharge of nitric oxide may be either through photolytic decomposition or reaction with thiol group in griess reagent. The generated nitric oxide undergoes addition reaction with oxygen to produce stable nitrates and nitrites. Methanol extract of *Polygonum glabrum* decreased the concentration of nitric oxide by antioxidant ability. The scavenging activity with the IC_{50} values 80.22 μg/mL and 48.06 μg/mL for methanol extract of *Polygonum glabrum* and rutin, respectively is mentioned in Figure 2.

Hydrogen peroxide scavenging capacity

In experiments, hydrogen peroxide was frequently used as a source of oxygen derived free radicals. Hydrogen peroxide was not a stable compound. Under natural conditions it slowly disintegrates into water and oxygen. Maximum scavenging activity was observed at 100μg/ml (Figure 3). The IC_{50} value of methanol extract of *Polygonum glabrum* and standard (ascorbate) was found to be 33.05 μg/ml and 19.4 μg/ml

Hydroxyl radical scavenging assay

The potential of different concentrations of methanol extract of *Polygonum glabrum* to scavenge hydroxyl radicals produced by Fenton reaction was measured. By the addition of iron salts to ascorbic acid and EDTA, hydroxyl radicals are generated. Degradation of monosaccharide by condensation with thiobarbituric acid was quantified. The concentrations of 50% inhibition were found to be 52.2 μg/mL and 34.2 μg/mL for methanol extract of *Polygonum glabrum* and standard compound vitamin-E, respectively. The IC_{50} value (52.2 μg/mL) of *Polygonum glabrum* extract was found to be more than the standard.

Superoxide anion scavenging activity

The formation of superoxide radicals when phenazine methosulfate reacts with nicotinamide adenine dinucleotide was studied. Nitro blue tetrazolium gains electrons from superoxide anion radicals and gets reduced to blue formazan. The decrease in the absorbance with the methanol extract of polygonum glabrum indicated the consumption of superoxide anion in the reaction mixture. As mentioned in Figure 5, the plant extract as well as curcumin showed the scavenging activity with IC_{50} values, 36.98 μg/mL and 18.05 μg/mL, respectively.

Total antioxidant activity (phosphomolybdic acid method)

The assay was based on reduction of phosphomolybdic acid to phosphomolybdenum by plant extracts. Since molybdenum was a transition metal it exists in many oxidation states. The molybdate anion exists in +6 oxidation state. The formation of a green phospho molybdenum complex was had a maximum absorption at 695 nm. Maximum scavenging activity was observed at 1600μg/ml (Figure 6). The IC_{50} of the methanol extract of *Polygonum glabrum* and standard (ascorbate) was found to be 416.5 μg/ml and 225.4 μg/ml.

ABTS radical cation decolourization assay

Polygonum glabrum was evaluated for its ability to scavenge ABTS radical cation by incubating the reaction mixture with various concentrations of extract. The inhibition was found to be concentration dependent. Under similar experimental condition the

standard BHT exhibited effective antioxidant activity with IC_{50} value of 11.2 μg/ml. IC_{50} value of *Polygonum glabrum* was found to be 20.4 μg/ml.

Metal ion chelating ability assay

The ferrous ions chelating activity of methanol extract of *Polygonum glabrum* and standards were investigated. The extract showed 9.2%Fe^{++} ion chelating ability at 100 μg/ml and the standard EDTA showed 14.3% at the same concentration. The IC_{50} value of the extract was also good when compared with the IC_{50}value of the reference standard EDTA.

Reducing power ability

In this method, a colored complex formed between plant antioxidant and reagents (potassium ferricyanide, trichloro acetic acid and ferric chloride) was measured at 700 nm. The methanol extract of *Polygonum glabrum* showed potent reducing ability as compared to standard BHT. The reducing power increased with increasing amount of the concentrations.

Xanthine oxidase assay

The xanthine oxidase inhibitory activity of *Polygonum glabrum* methanol extract was studied. In this study different dilutions of methanol extracts and reference compound were tested and a dose response curve was plotted as shown in Figure 10. The IC_{50} values of methanol extract of *Polygonum glabrum* and reference compound were found to be 44.6 μg/ml and 19.4 μg/ml respectively.

Lipid per oxidation assay

The effect of methanol extract of *Polygonum glabrum* and vitamin E on the in vitro inhibition of lipid per oxidation was showed in Figure 11. Lipid per oxidation in rat liver microsomes was initiated by addition of ferrous ascorbate. The extract of *Polygonum glabrum* showed inhibition of per oxidation effect in all concentrations, which showed 50% inhibition effect at 130 μg/ml. The extract inhibition value was found to be more than the standard, vitamin E (IC_{50} value 123.6 μg/ml).

Discussion

In current study, multiple assays were adapted to evaluate antioxidants activity of methanol extract of *Polygonum glabrum.* DPPH radical assay is one of the most feasible models at laboratory. The results of present stuy suggested that *Polygonum glabrum* is an efficient scavenger of DPPH radicals. Reduced DPPH loses its colour from purple to yellow. The degree of discoloration was coupled with a decrease in the absorbance. In 1990 Bors et al reported that hydroxyl groups attached to B ring of a flavonoid molecule could act as reducing agent by donating hydrogen atom [24]. Since flavonoids like delphinidin-3,5-diglucoside, cyanidin-3,5-diglucoside and quercetin are reported to be present in methanol extract of *Polygonum glabrum* they might inactivate the DPPH free radical by providing a hydrogen atom. Present study proved that the extract studied had good nitric oxide scavenging activity. Few studies proved that flavonoids inhibited nitric oxide release as well as efficiently acted as nitric oxide scavangers [25]. Being the precursor, sodium nitro prusside releases nitric oxide which gets oxidized to peroxynitrites and nitrates in the solution. A gradual decrease in absorbance was recorded which was directly related to the concentration of nitrites. The plunge in absorbance may be due to the plant extract which competes with oxygen to react with nitric oxide leading to decrease of nitric oxide availability.

Hydrogen peroxide naturally gets decomposed to oxygen and water. Nevertheless, in presence of certain metal ions it may react to form cell sensitive hydroxyl radicals. Ozyurek et al reported that phenolic compounds are better scavengers of hydrogen peroxide since they can donate electrons to H_2O_2, thus neutralizing it to water [26]. Recently ezhilan et.al reported the presence of six different phenol compounds in whole plant methanol extract of *Polygonum glabrum* [27]. Since phenols have the ability to donate a hydrogen atom, it resulted in quenching of absorbance. As the extract concentration increases, more H_2O_2 was scavenged and less H_2O_2 remains. From the results, it appeared that the H_2O_2 scavenging activity of the plant extract is in par with that of the standard. The significance of Fenton reagent has been long recognized among others in textile industries and refineries to remove toxic waste. The efficiency of the Fenton reaction depends mainly on concentration of hydrogen peroxide, ferrous ions, pH and reaction time [28]. Hydroxyl radicals produced in the presence of a reducing agent like ascorbic acid attacks hydrogen atom of deoxyribose. The degraded sugar molecule reacts with thiobarbituric acid to form thiobarbituric acid reactive substances. The presence of EDTA was recommended since it increased the formation of TBARS to four-fold higher than in its absence. In 2000, Cheng and Breen reported the ability of flavonoids to suppress Fenton reaction. They found that quercetin and luteolin could suppress the catalytic wave of the iron ATP/Hydrogen peroxide system [29]. The decrease in the absorbance at 560nm by a flavonoid and phenol rich fraction of *Polygonum glabrum* implicates the exploitation of hydroxyl radical in the reaction mixture.

Besides monoterpenes, sesquiterpenes are the second important groups of active constituents in essential oils of plants. Karimian et al studied the antioxidant capacity of *Tagetus minuta* essential oil by measuring reactive oxygen species [30]. In 1992, Jaccobson amd Muddathir isolated some sesqueterpenes (2 , 3β-diangeloyloxyisodrimeninol, 2 -angeloyloxy-38-2'-methylpent-2'-enoyloxyisodrimeninol) from *Polygonum glabrum.* This

sesqueterpenes may be beneficial in scavenging superoxides. Discoloration of formazan and decreased absorbance indicates the consumption of superoxide anion by plant terpenes in the reaction mixture. The present study proved that the extract studied has efficient superoxide scavenging activity. Chemically, phosphate ions liberated from sodium phosphate forms a complex with ammonium molybdate in a solution of sulfuric acid. Previous works reported that sugar sources such as sucrose, glucose and fructose act as preferred electron donors for molybdate reduction [31]. Since phytochemical screening of methanol extracts of *Polygonum glabrum* showed positive for presence of sugars they may aid in the reduction of molybdenum (VI) to molybdenum (V) of ammonium molybdate. The reduced substrate reacted with sodium phosphate and formed a blue colour complex called phosphomolybdenum. This complex was measured spectrophotometrically. As shown in figure 6, the reducing power of the plant extract was found to be good.

The scavenging effect of methanol extract of *Polygonum glabrum* and BHT was observed to be a linear increase in ABTS radical scavenging activity with increasing concentration. In 1998 Schottner et al reported that, the antioxidant activity of non caloric phenolic plant compounds called lignans which are present in cell wall of plants [32]. Doss et al identified the presence of dibromoschizandrin, a lignan in leaves of *Polygonum glabrum* [33]. Through ABTS method, methanol extract recorded good antiradical activity by reduction of cation leading to decolourisation of reaction mixture. This chain of reactions may be attributed to the presence of phenols and lignans which neutralised the blue coloured ABTS radical cation. This affirms the potency of the extract in dose dependent ABTS radical scavenging activity. The present investigation also demonstrated the chelating effects. Transition metals such as copper and iron catalyse the conversion of hydrogen peroxide to toxic free radicals. Interactions of hydroxyl or carbonyl groups of flavonoids with metal ions can lead to chelate formation. Interestingly, the metal-flavonoid complexes were found to have a much stronger free radical scavenging properties than the free flavonoids [34]. Flavonoids like pinocembrin and pinocembrin-5-methyl ether were isolated from methanol extract of *Polygonum glabrum* may work by chelating ferrous ions. Hence availability of metal ions to generate a complex with ferrozine was greatly reduced. This study approved the chelating ability of *Polygonum glabrum*.

Further, in vitro antioxidant activity of methanol extract of *Polygonum glabrum* was determined by exploitation of reducing power technique. Many phytoconstituents could act as electron donors or strong reducing agents. In the presence of plant extract, reduced potassium ferrocyanide (Fe2+) from potassium ferricyanide was formed. The reduced cation then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. By increasing the sample concentration range from 100 to 500µg/ml, the reducing power also increased in the dose dependent manner. From the results, it seems that the reducing power of the plant extract was good when compared with that of the standard. A combination of ferrous sulphate and ascorbic acid proves a good promoter system for inducing oxidative stress. The ferrous ascorbate complex generates reactive hydroxyl radical. Hydroxyl radical attacks the unsaturated fatty acids of liver microsomes causing lipid per oxidation [35]. This result in generation of carbonyl fragments called MDA (malonodialdehyde) which react with thiobarbituric acid to form a pink compound asorbed at 532 nm. The methanol extract of *Polygonum glabrum* and Vitamin E exhibited a steady scavenging impact of hydroxyl group at different concentrations. The marked inhibition of lipid per oxidation by the methanol extract may be due to the presence of reductants like phenols and steroids in the extract.

Conclusion

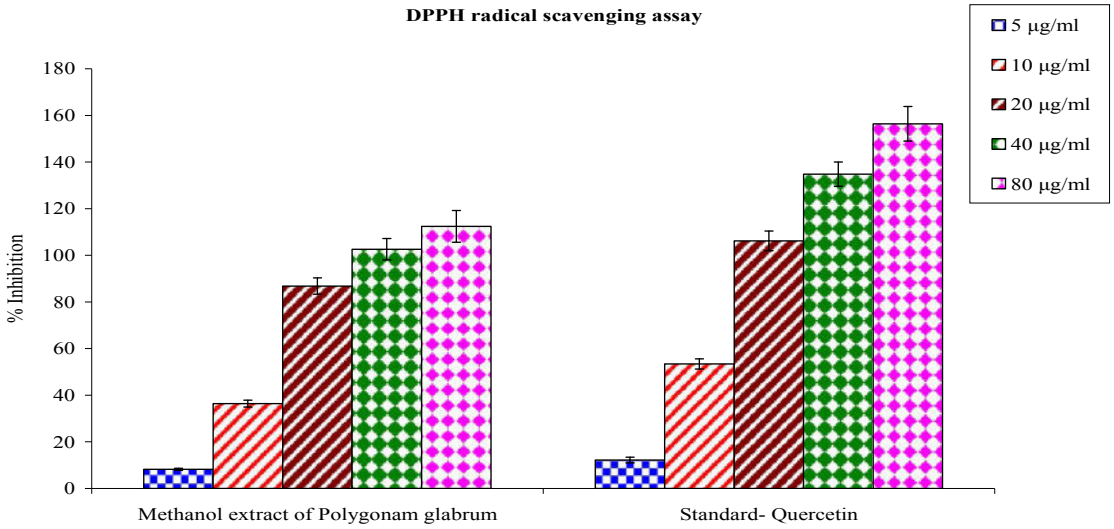

Figure 1: Scavenging effect of methanol extract of *Polygonum glabrum* and standard quercetin on DPPH radical.

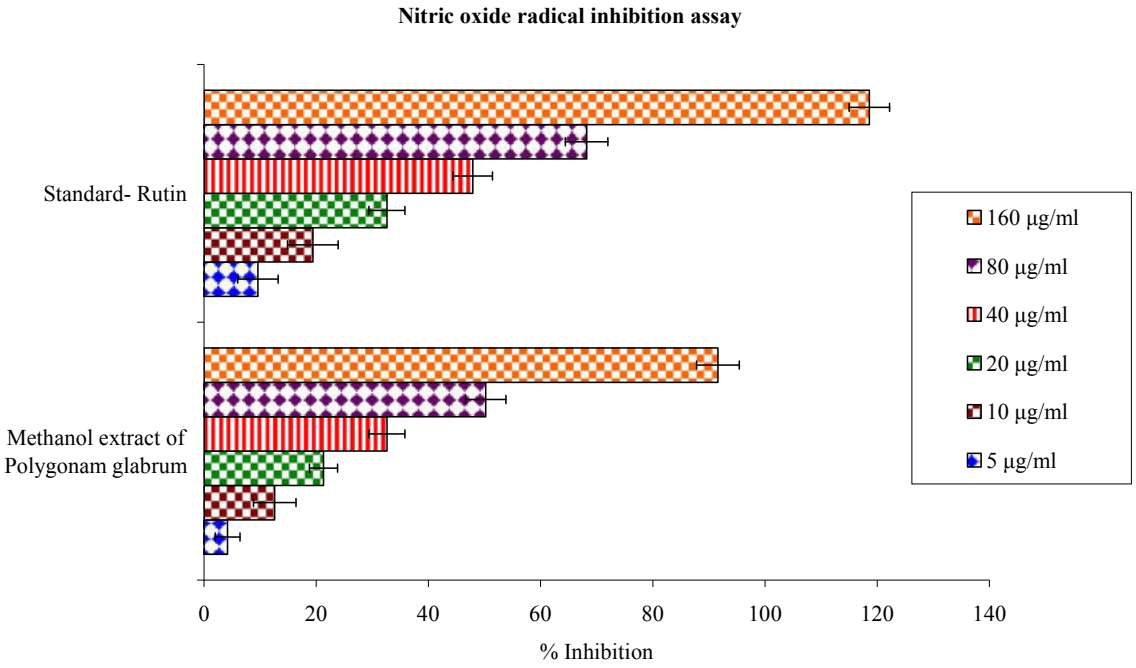

Figure 2: Scavenging effect of methanol extract of *Polygonum glabrum* and standard rutin on Nitric oxide radical.

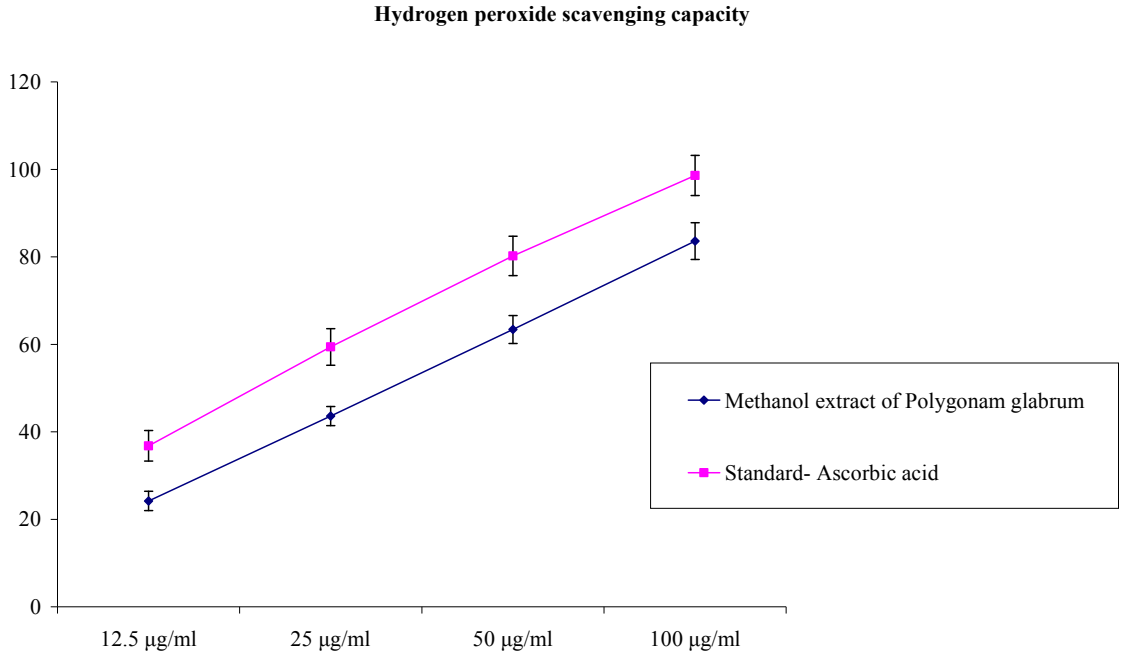

Figure 3: Effect of methanol extract of *Polygonum glabrum* and ascorbic acid on hydrogen peroxide radical assay.

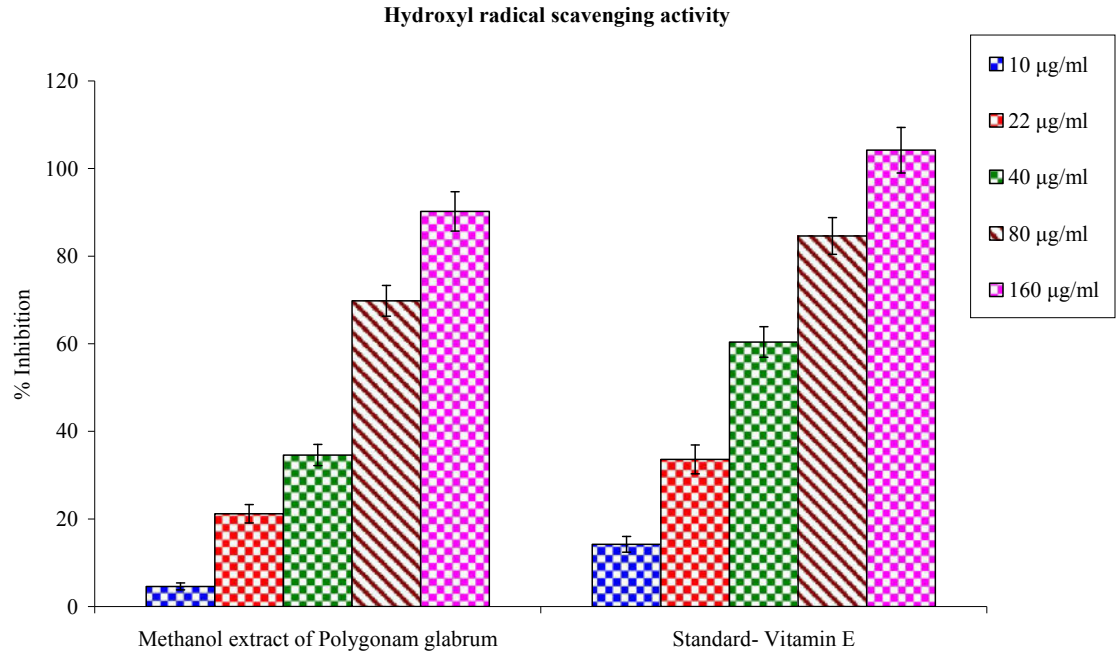

Figure 4: Effect of methanol extract of *Polygonum glabrum* and vitamin E by hydroxyl scavenging method.

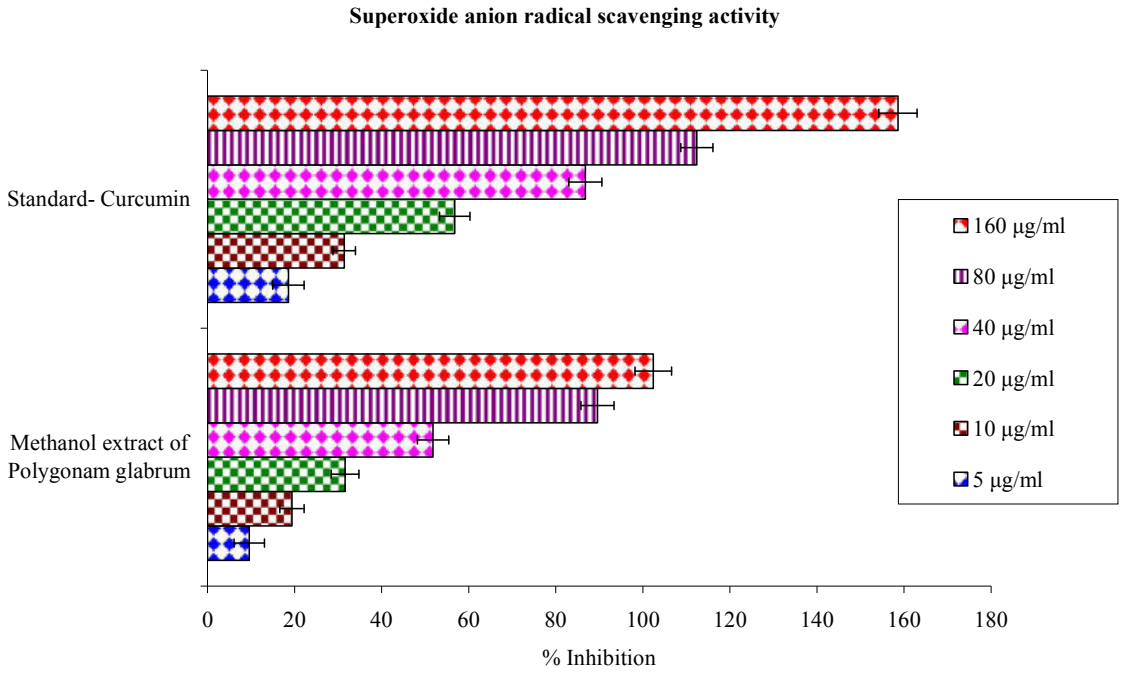

Figure 5: Effect of methanol extract of *Polygonum glabrum* and curcumin on scavenging of superoxide anion radical formation.

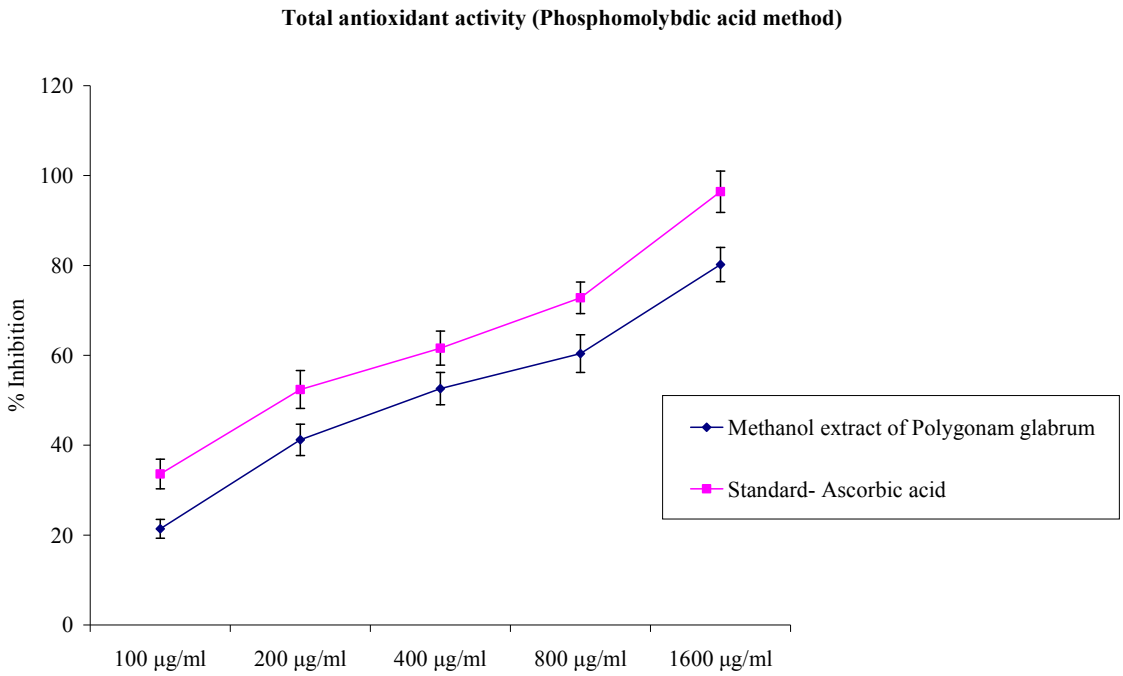

Figure 6: Effect of methanol extract of *Polygonum glabrum* and ascorbic acid by phosphomolybdate method assay.

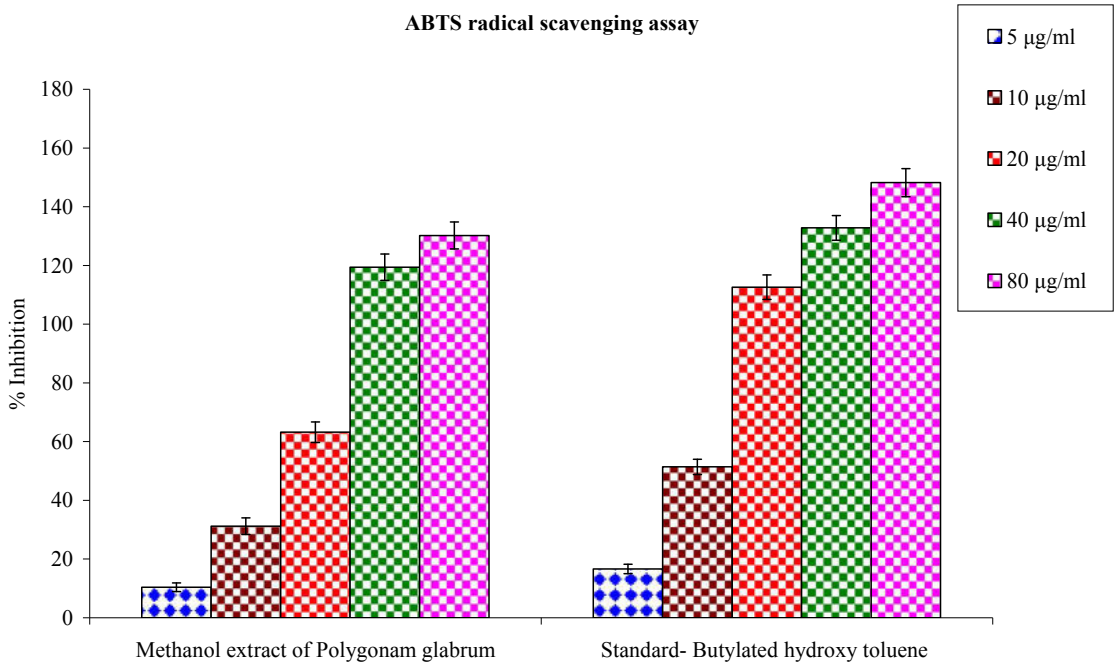

Figure 7: Effect of methanol extract of *Polygonum glabrum* and butylated hydroxy toluene by ABTS radical scavenging assay

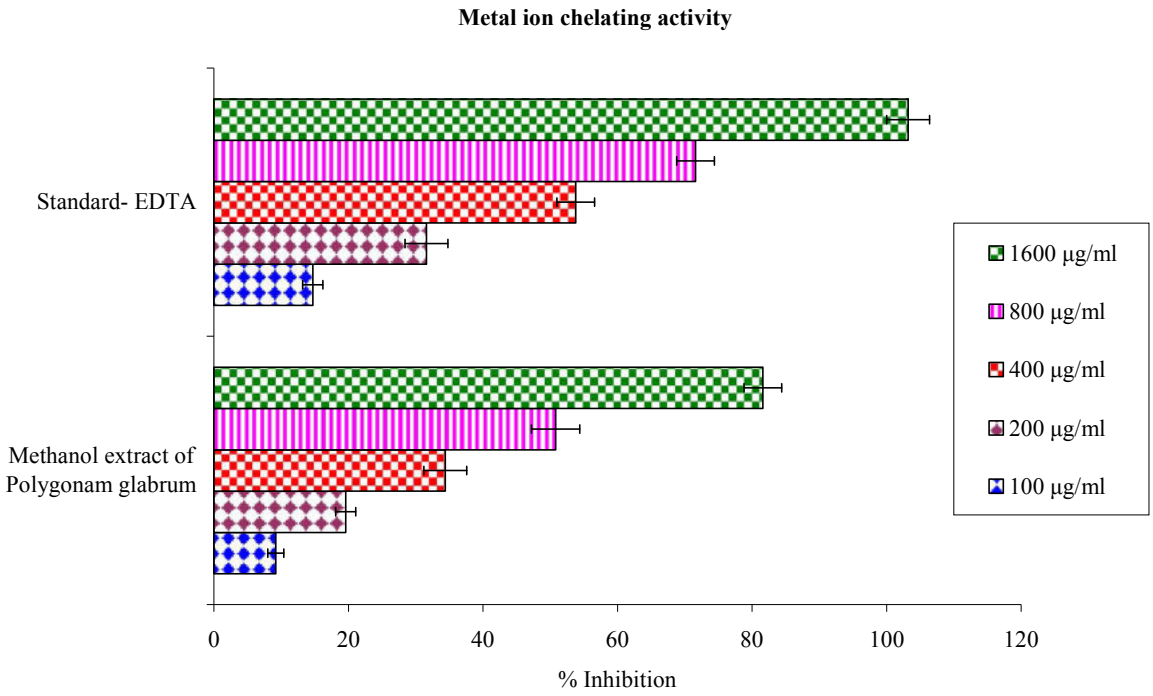

Figure 8: Effect of methanol extract of *Polygonum glabrum* and EDTA by metal ion chelation assay.

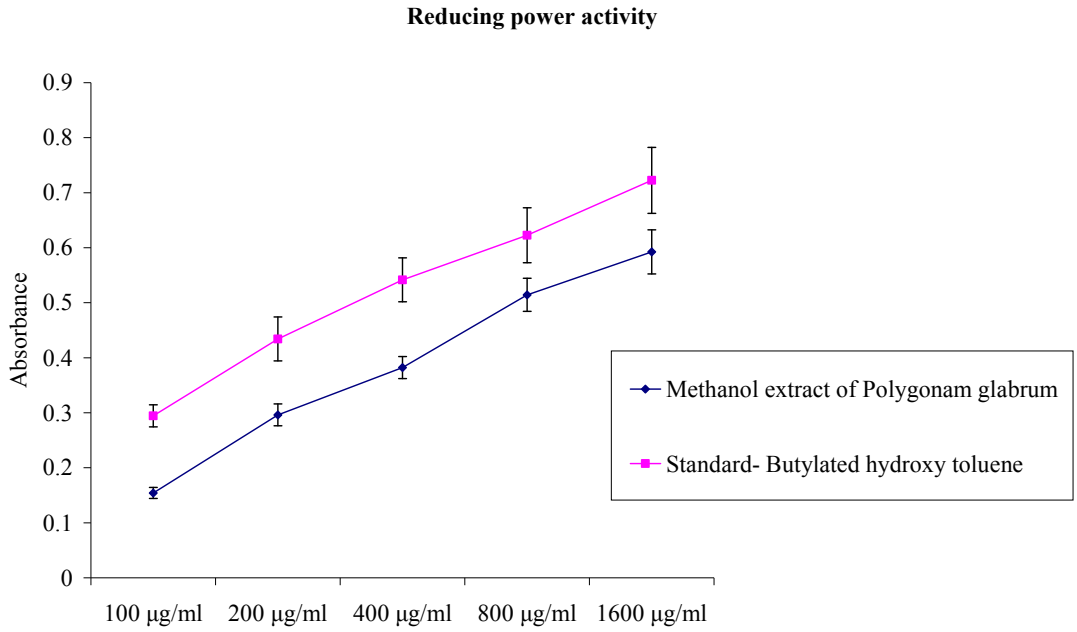

Figure 9: Effect of methanol extract of *Polygonum glabrum* and butylated hydroxyl toluene on reducing power activity.

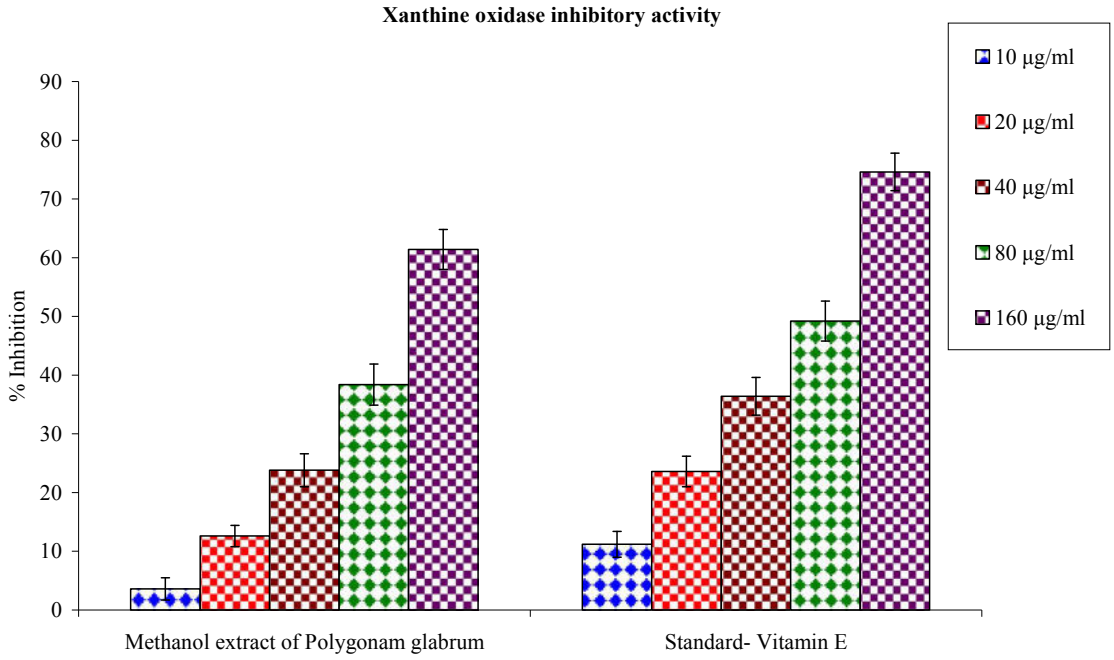

Figure 10: Effect of methanol extract of *Polygonum glabrum* and allopurinol by xanthine oxidase assay.

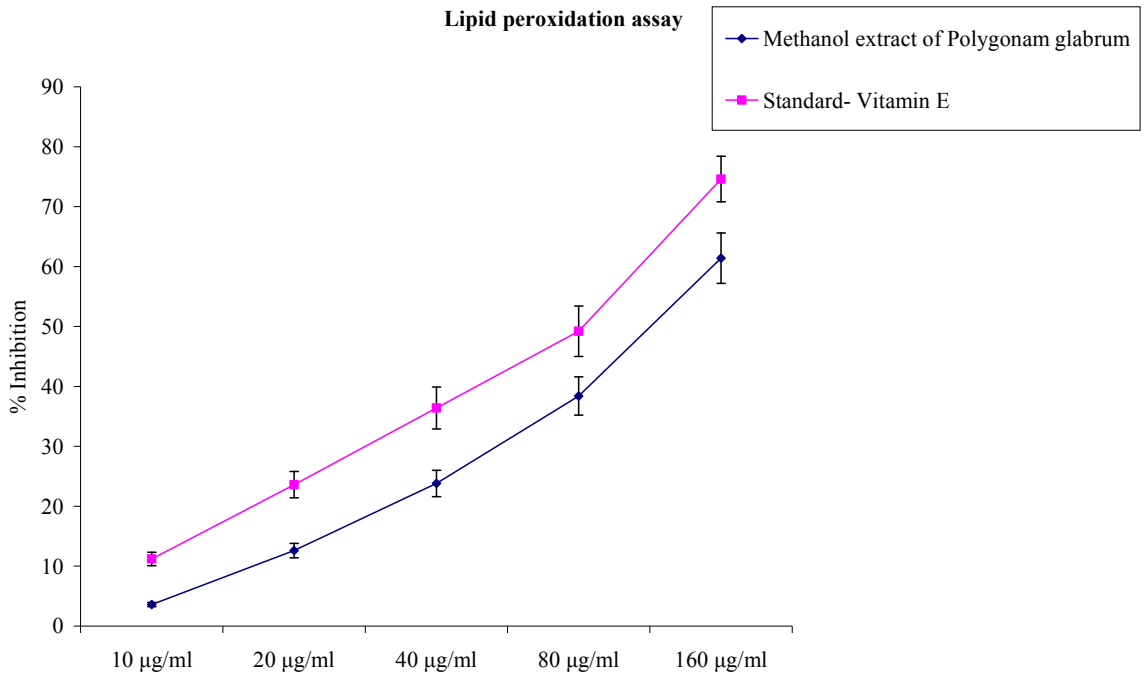

Figure 11: Effect of methanol extract of *Polygonum glabrum* and vitamin E on lipid per oxidation of liver microsome induced by Fe2+/ascorbate.

According to the results obtained from the present study, methanol extract of *Polygonum glabrum* was found to be an effective antioxidant in various in vitro assays. The synergistic action of each phytoconstituent irrespective of their concentration might lead to

augmentation of antioxidant effect. Further studies on invivo antioxidant activity of methanol extract of *Polygonum glabrum* and isolation of the active compounds are in progress.

Abbreviations

ABTS: 2, 2'- azino-bis (3-ethylbenzothiazoline- 6-sulphonic acid); BHT: Butylated hydroxytoluene; BHA:Butylated hydroxyanisole; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; EDTA: Ethylenediamine tetracetic acid; MDA: malonodialdehyde NADH: Nicotinamide adenine dinucleotide; NBT: Nitro blue tetrazolium; PMS: Phenazine methosulphate; ROS: Reactive oxygen species; TBA: thiobarbituric acid;.

Acknowledgements

The authors are thankful to the management of GITAM University, Visakhapatnam, Andhra Pradesh, India, for providing necessary facilities to carry out the research work.

Author's contribution

The complete research work was suggested and designed by S. Raja (First Author). The extraction process and anti oxidant activity were carried out by Ramya . I (Second author). The draft manuscript was prepared by Ramya . I. and the final manuscript was edited by S. Raja (First Author). Authors read and approved the final manuscript.

Conflict Of Interest

No

References

[1]. Freeman BA, Campo JD. Biology of disease: free radicals and tissue injury. Lab Invest. 1982; 47(5): 412–426.

[2]. Sengupta A, Ghosh M, Bhattacharyya DK. In vitro antioxidant assay of medium chain fatty acid rich rice bran oil in comparison to native rice bran oil. J Food Sci Technol. 2015; 52(8):5188–5195.

[3]. Mojarab M, Emami SA, Hassanzadeh-Khayyat M. Antioxidant activity of methanol extracts of different species of Artemisia from Iran. Pharmacologyonline. 2009; 2:797–807

[4]. Michiels C, Raes M, Toussaint O, Remacle J. Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. Free Radic Biol Med. 1994; 17: 235–248.

[5]. Small DM, Coombes JS, Bennett N, Johnson DW, Gobe GC.: Oxidative stress, anti-oxidant therapies and chronic kidney disease. Nephrology (Carlton). 2012; 17(4): 311–321.

[6]. Alagumanivasagam G, Pasupathy R, Kottaimuthu A, Manavalan R. A Review on in-vitro antioxidant methods. Int J Pharm & Chem Sci. 2012; 1(2): 662-674.

[7]. Shankar LH, Mishra PK. Study of aquatic medicinal plants of Hazaribagh district of Jharkhand,India. Int Res J pharm. 2012; 3(4): 405-409.

[8]. Khan MF, Islam Rabbi SN, Fahima Aktar MD, Kawsar H. In vitro Cytotoxic, membrane stabilizing and thrombolytic activities of *Polygonum glabrum Willd.* Bangladesh pharm. j. 2014; 17(2): 202-204

[9]. Babitha S, David B, Otilia JFB. Investigation on antioxidant and hepatoprotective activity of ethanolic leaf extract of *Polygonum glabrum Wild* on carbon tetrachloride- induced hepatotoxicity in rats. Spatula DD. 2012; 2(4): 199-205.

[10]. Palani R, Karunakaran D, Rajesh V, Mathivanan K ,Jayaraman P. Analysis of antioxidant, antimicrobial activity and phytochemical potential of *Cleistanthus collinus Roxb.,Polygonum glabrum Wild. and Melia azedarch Linn.* Asian J. Med. Pharm. Sci. 2014; 2(2): 149-159.

[11]. Said MS, Chinchansure AA, Nawale L, Durge A, Wadhwani A, Kulkarni SS, Sarkar D & Joshi SP. A new butenolide cinnamate and other biological active chemical constituents from *Polygonum glabrum*. Nat. Prod. Res. 2015;29(22): 2080-2086,

[12]. Mohammed IS. Phytochemical studies of flavonoids from *Polygonum glabrum L.* of Sudan. MSc thesis, Chemistry, University of Khartoum; 1996.

[13]. Jacobsson U, Muddathir AK. Four biologically active Sesquiterpenes of the drimane type isolated from *Polygonum glabrum*. Phytochemistry. 1992; 31(12):4207–4211.

[14]. Tailor CS, Goyal A. Antioxidant Activity by DPPH Radical Scavenging Method of *Ageratum conyzoides* Linn. Leaves. Am J Ethnomed. 2014; 1 (4): 244-249.

[15]. parul R, kundu SK, pijush saha P. In vitro nitric oxide scavenging activity of methanol extracts of three Bangladeshi medicinal plants. The pharm innov j. 2013; 1(12) : 83 -88.

[16].Gayathri G, nair BR, babu V. Scavenging of free radicals and total phenols of methanol extract of *Azima tetracantha lam*. Int j pharm sci. 2014; 6(9): 347-351.

[17]. Nishimiki M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun 1972; 46:849-53.

[18]. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of

antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E Anal. Biochem. 1999; 269(2):337-41.

[19]. Re R, Pellegrini N, Proteggente A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999; 26:1231–7.

[20]. Adjimani JP, Asare P. Antioxidant and free radical scavenging activity of iron chelators. Toxicology Reports. 2015; 2:721–728.

[21]. Jayanthi P, Lalitha P. Reducing power of the solvent extracts of *eichhornia crassipes* (Mart.) solms. Int j pharm pharma sci.2011; 3(3): 126-128.

[22]. Chiang HC, Lo YJ, Lu FJ. Xanthine oxidase inhibitors from the leaves of *Alsophila spinulosa* (Hook) Tryon. J Enzyme Inhib. 1994; 8:61–71.

[23]. Bouchet N, Barrier L, Fauconneau B. Radical scavenging activity and antioxidant properties of tannins from *Guiera senegalensis* (Combretaceae). Phytother Res. 1998; 12:159-62.

[24]. Bors W, Heller W, Michel C, Saran M. Flavonoids as antioxidants: determination of radical-scavenging efficiencies. Methods Enzymol. 1990;186: 343-355.

[25]. Van Acker SA, Tromp MN, Haenen GR, van der Vijgh WJ, Bast A. Flavonoids as scavengers of nitric oxide radical. Biochem Biophys Res Commun. 1995; 214 (3):755-9.

[26]. Ozyurek M B, Bektasoglu, Guclu K, Gungor N, Apak R. A novel hydrogen peroxide scavenging assay of phenolics and flavonoids using cupric reducing antioxidant capacity (CUPRAC) methodology. J. Food Compos. Anal. 2010;23 (7): 689–698.

[27]. Ezhilan B, Neelamegam R. GC-MS Determination of bioactive compounds of *Polygonum glabrum* (Wild). Journal of Phytology. 2011; 3(9): 23-25.

[28]. Barbusi ski K. Fenton reaction-controversy concerning the chemistry. Ecol Chem Eng. 2009;16(3):348-358.

[29]. Cheng IF, Breen K. On the ability of four flavonoids, baicilein, luteolin, naringenin, and quercetin, to suppress the Fenton reaction of the iron-ATP complex. Biometals. 2000; 13(1):77-83.

[30]. Karimian P, Kavoosi G, Amirghofran Z. Anti-oxidative and anti-inflammatory effects of Tagetes minuta essential oil in activated macrophages. Asian Pac J Trop Biomed 2014;4(3):219-27.

[31]. Ahmad SA, Shukor MY, Shamaan NA, Mac Cormack WP, Syed MA. Molybdate reduction to molybdenum blue by an antarctic bacterium. Biomed res int. 2013; 2013: 871941.

[32]. Schottner M, Gansser D, Spiteller G. Interaction of lignan with human sex hormone binding globulin (SHBG). J Biosci. 1997; 52:834-843.

[33]. Doss A, Parivuguna V. Gc Ms Analysis Of *Polygonum Glabrum* leaf petroleum ether extract. Journal of Modern Drug Discovery and Drug Delivery Research. 2015; 3(4): 1-3.

[34]. Dusan Malesev D, kunti V. Review investigation of metal–flavonoid chelates and the determination of flavonoids via metal–flavonoid complexing reactions. J. Serb. Chem. Soc. 2007; 72 (10) 921–939.

[35]. Braughler JM, Duncan LA, Chase RL. The involvement of iron in lipid peroxidation. Importance of ferric to ferrous ratios in initiation. 1986; 261(22):10282-10289.