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EVALUATION OF *POLYGONUM GLABRUM* WILLD. LEAF EXTRACTS FOR ANTIOXIDANT PROPERTY

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ABSTRACT: *Polygonum glabrum*, commonly called dense flower knotweed, is a perennial plant found in most parts of India. The leaves of plants *Polygonum glabrum* Willd. Belonging to the family, Polygonaceae are a rich source of flavonoids and tannins but not yet scientifically investigated for antioxidant activity. The present study aimed to offer a novel scientific background to the leaves of *Polygonum glabrum* by evaluating it for antioxidant activity using *in-vitro* and *in-vivo* methods. The ethyl acetate and ethanolic extracts of *Polygonum glabrum* Willd. leaves showed significant antioxidant activity by using *in-vitro* models, which may be due to the presence of phytoconstituents such as polyphenol, tannins and flavonoids. Based on the *in-vitro* results, potent extracts were further evaluated for antioxidant studies by using *in-vivo* models. Acute toxicity studies were carried out as per OECD guidelines, and the doses were fixed at 200 and 400 mg/kg b.w. The ethyl acetate and ethanolic extracts at the dose of 400 mg/kg showed the degree of protection by measuring antioxidant enzymes CAT, SOD, non-enzymatic antioxidant GSH and level of lipid peroxidation. These results suggest that *Polygonum glabrum* Willd. has a potent antioxidant activity and could be further explored to isolate a novel natural antioxidant molecule.

INTRODUCTION: The adverse effects of oxidative stress on human health have become serious issues due to the modern lifestyle associated with an unhealthy diet, exposure to different combinations of chemicals from various sources such as pesticides, heavy metals, food additives, and environmental pollution [1].

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Under stress, our bodies produce more reactive oxygen species (ROS) whose imbalance can contribute to the increasing burden of chronic diseases, as is suggested by several experimental and human studies [2]. Fortunately, various beneficial compounds known as antioxidants control free radical formation naturally.

The World Health Organization (WHO) has estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components. A lack of antioxidants, which can quench the reactive free radicals, facilitates the development of

degenerative diseases. One solution to this problem is to supplement the diet with antioxidant compounds that are contained in natural plant sources which have long been used as safe, effective and sustainable sources of natural antioxidants or free radical scavengers, particularly phenolic compounds, such as phenolic acids, favonoids, tannins, stilbenes and anthocyanins. These natural plant antioxidants can therefore serve as a type of preventive medicine [3, 4].

Polygonum glabrum which is commonly called as dense flower knotweed, is a semi-aquatic perennial plant belonging to the family Polygonaceae [5]. *Polygonum glabrum* Willd. is a small perennial herb mainly in the eastern area at an altitude of up to 1900 m. The stem is stout, simple or slightly branched or procumbent and usually reddish below, then erect, the young stem usually green, polished with a dark reddish-brown ring at each node. Leaves are simple, petiolate, stipulate, narrow lanceolate in shape. The flower is pink, in paniculate slender racemes; peduncles usually glabrous. Nutlets is broadly ovoid or suborbicular, compressed, biconvex, black and shining [6]. The leaves contain flavonoids-quercetin, rhamnetin, quercitrin, avicularin, and rutin. Flowers contain pigments, delphinidin-3,5-diglucoside and cyanidin-3,5-diglucoside and Quercetin [7].

Studies have proved that many of the natural products possess chemicals like polyphenolic compounds and flavonoids *viz.* Quercetin [8], kaempferol, quercetin, myricitrin, myricitrin, rutin can act as antioxidants; from literature review it has been found that *Polygonum glabrum* Willd. contains polyphenolic compound and flavonoids such as avicularin, cynidine-3,5-diglucoside, delphinidin-3,5-diglucoside quercetin, quercetrin, ramnetin, rutin which are known to act as antioxidant [9]. Plant *Polygonum glabrum* Willd. traditionally used as Piles, Jaundice, Pneumonia, Colic pain, Febrifuge, Anthelmintic, Antiviral, and Antibacterial. This study aims to scientifically validate and establish the antioxidant property of the leaves of *Polygonum glabrum* Willd.

MATERIALS AND METHODS:

Plant Material: The leaves of *Polygonum glabrum* were collected and authenticated by Dr. B. D. Huddar, Head, Department of Botany, Shri Kadasiddheshwar arts college and H.S. Kotambari Science institute, Hubballi.

Preparation of Extracts: The *Polygonum glabrum* Willd. leaves were shade dried at room temperature, pulverized to a coarse powder, and successively extracted with petroleum ether (40-60), chloroform, ethyl acetate, and ethanol in a Soxhlet extractor with increasing order of polarity. The extracts were concentrated using a rotary flash evaporator and were dried in a desiccator over sodium sulfite. After drying, the respective extracts were weighed and the percentage yield was determined.

Phytochemical Analysis: The qualitative chemical investigations of all the extracts were carried out to check the presence of various phytoconstituents.

Determination of Antioxidant Activity: Antioxidant activity was measured using *in-vitro* methods and *in-vivo* animal studies.

***In-vitro* Measurement:**

Determination of Total Phenolic Content: The total phenolic compounds in the extracts were determined with Folin-Ciocalteu reagent according to Slinkard and Singleton [10] using gallic acid as a standard phenolic compound.

The gallic acid calibration curve was constructed with a linearity range of 10-100 µg/ml Concentration of plant extract were also prepared in distilled water, and 0.5 ml of each sample were introduced into test tubes and mixed with 2.5ml of a 10 fold dilute Folin-Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 min at room temperature before the absorbance was taken against blank at 760 nm. All tests were carried out in triplicate, and the results are given as gallic acid equivalents (GAE) per g of dry extract.

Free Radical Scavenging Capacity on DPPH Radical: The DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay is one of the most commonly employed methods because, in general terms, it is simple, efficient, and inexpensive. The hydrogen atom donating ability of the plant extractives was determined by the decolorization of methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces violet/purple color in methanol

solution and fades to shades of yellow color in the presence of antioxidants. The samples were prepared in different concentrations for the present study, *i.e.* 10-100µg/ml in AR grade methanol. About 2 ml of various concentrations of each extract were added to 2 ml solution of 0.1 mM DPPH ml in AR grade methanol. After 20 min of incubation at room temperature, the resulting solution's absorbance was measured at 517nm using BHT as standard. The experiment was performed in duplicates. A decrease in absorbance indicated the antioxidant activity. Radical scavenging activity was expressed as percentage inhibition of DPPH and was calculated as follows [11].

$$RSC\ (\%) = 100 \times A\ control - A\ sample\ / A\ control$$

RSC = Radical Scavenging Capacity, A control = Absorbance of blank, and A sample= Absorbance of the sample. From the obtained RSC values, the IC_{50} were calculated, which represents the concentration of the scavenging compound that caused 50% neutralization

Super Oxide Scavenging Activity by Alkaline DMSO Method: In this method, superoxide radical is generated by the addition of sodium hydroxide to air saturated dimethyl sulfoxide. The generated superoxide remains stable in solution, which reduces nitro blue tetrazolium (NBT) into formazan dye at room temperature, and that can be measured at 560 nm.

Briefly, to the reaction mixture containing 0.1ml of NBT (1 mg/ml solution in DMSO) and 0.3ml of the extracts of different concentrations and standard in DMSO, 1 ml of alkaline DMSO (1ml DMSO containing 5 mM NaOH in 0.1ml water) was added to give a final volume of 1.4ml. The absorbance was measured at 560 nm. The experiment was performed in triplicate [12].

***In-vivo* Measurement:**

Acute Toxicity Study: Approval of the Institutional Animals Ethical Committee was obtained prior to the experimentation on animals. The acute toxicity tests for ethyl acetate and ethanolic extracts of *Polygonum glabrum* Willd. were performed on female Albino mice weighing between 25-30 gms following OECD Guidelines (no. 423). The animals were fasted overnight prior to experiment [13]. As per the OECD guideline no. 423 fixed-dose method procedure; the maximum nonlethal dose was found to be 2000 mg/kg body weight; hence, 1/10th and 1/5th of the dose was taken as effective dose (200 mg/kg, 400 mg/kg body weight) of the extract, to evaluate antioxidant activity.

Evaluation of *In-vivo* Antioxidant Activity: Albino rats (Wistar) of either sex weighing 150-180 gm were selected for *in-vivo* antioxidant activity. Thirty-six rats were randomly divided into six groups, each consisting of 6 animals, including Group 1: Untreated control rats that received distilled water for 21 days. - Group 2: Serve as paracetamol control and receive paracetamol (1 g/kg, p.o) for first seven days. Group 3: Silymarin, serve as the positive control and received paracetamol (1g/kg, p.o.) for first seven days and silymarin (100 mg/kg, p.o.) from 4th day to 12th day. Group 4 and 5: Animals were treated with different dose of ethyl acetate extract of *Polygonum glabrum* Willd. and received paracetamol (1g/kg) for first 7 days and ethyl acetate extract (200 and 400 mg/kg, p.o.) from 4th to 12 day.

Group 6 and 7: Animals were treated with different doses of ethanolic extract of *Polygonum glabrum* Willd. and received paracetamol (1g/kg) for the first 7 days and ethanolic axtract (200 and 400 mg/kg, p.o.) from 4th to 12 days. All the drugs were prepared in 1 % CMC for oral administration. The animals will be sacrificed on day 13th with an excess of light ether anesthesia. The liver will be isolated, rinsed in water, weighed and homogenized with phosphate buffer. After centrifugation, the clear supernatant was used for the estimation of Malondialdehyde (MDA), reduced glutathione (GSH), Superoxide dismutase (SOD) and catalase (CAT) by standard methods.

Analytical Methods for Estimation of Oxidative Stress:

1. Estimation of Reduced Glutathione (GSH): Reduced glutathione was determined by the method of Ellman.1.0 g of scrapped tissue of the liver is homogenized with 10 ml of 10% TCA in ice-cold condition and centrifuged at 3000 rpm for 10 minutes. The supernatant was separated. 3ml of 0.2 M phosphate buffer of (pH 8.0) added to 1.0 ml of supernatant along with 0.5 ml of Ellman's reagent,

and the absorbance was taken immediately at 412 nm. The amount of glutathione was calculated using the absorption 13,600M^{-1} cm^{-1} [14].

Calculation:

$$Co = A / \varepsilon \times D$$

Where, Co = original concentration, A = absorbance at 412 nm, ε = extinction coefficient = 13,600/M/cm. D = dilution factor.

2. Estimation of Lipid Peroxidation (MDA): Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS), using malondialdehyde (MDA) as standard by the method of Buege and Aust. 0.1 ml of the tissue homogenate was added with 2.0 ml of the TCA-TBA- HCl reagent (15%w/v TCA, 0.375% w/v TBA and 0.25 N HCl).

The contents were boiled for 15 minutes, cooled, and centrifuged at 10000 rpm for 10 min and the precipitate was removed. The absorbance was read at 535 nm, and the malondialdehyde concentration of the sample was calculated using an extinction coefficient of 1.56 x 105$M^{-1}cm^{-1}$ [15].

Calculation:

$$Co = A / \varepsilon \times D$$

Where, Co = original concentration, A = absorbance at 412 nm, ε = extinction coefficient = 1.56 x 105M-1cm^{-1}, D = dilution factor.

3. Estimation of Superoxide Dismutase (SOD): The estimation was done as per Kakkar *et al*. The scrapped tissue of the liver was homogenized by using a Potter-Ellvehjem glass homogenizer for 30 sec either in ice-cold 0.9% saline (5%). Briefly, to 0.4 ml of the homogenate was added 1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml of 186 µM of phenazine methasulphate (PMS), 0.3 ml of 300 µM nitro blue tetrazolium (NBT). The reaction was started by the addition of 0.2 ml of NADH (780 µM). It was incubated at 30°C for 60 sec. The reaction was stopped by the addition of 0.1 ml glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 ml of n-butanol. The mixture was allowed to stand for 10 min, centrifuged, and n-butanol layer was separated. Colour intensity of the chromogen was measured against n-butanol at 560 nm using a spectrophotometer. A system devoid of enzyme activity was defined as enzyme concentration required decreasing the rate of reaction by 50% in one min under the assay conditions. The results are expressed as units (U) of SOD activity/mg protein [16].

4. Estimation of Catalase (CAT): Catalase (CAT) was assayed colorimetrically as described by Sinha. The scrapped tissue of the liver was weighed, and 30% homogenate was prepared with 0.2 M phosphate buffer pH 8.0. After centrifugation, the clear supernatant was used for the assay of enzyme activity. The reaction mixture (1.5 ml, vol.) contained 1.0 ml of 0.01 M (pH 7.0) phosphate buffer, 0.1ml of tissue homogenate (supernatant) and 0.4 ml of 2 M H_2O_2. The reaction was stopped by adding 2.0 ml of dichromate-acetic acid reagent (5%) potassium dichromate and glacial acetic acid mixed in 1:3 ratios. Colour intensity was measured colorimetrically at 620 nm and expressed as µmoles of H_2O_2 consumed /min/mg protein [17].

RESULTS AND DISCUSSION:

TABLE 1: QUALITATIVE PHYTOCHEMICAL SCREENING OF VARIOUS SOLVENT EXTRACTS OF *POLYGONUM GLABRUM* WILLD. LEAF

Phyto-Constituents	Successive Extracts			
	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract
Carbohydrates	-	-	-	-
Glycosides	-	-	+	+
Steroids	+	+	-	-
Triterpenoids	+	+	-	-
Tannins & Phenolic Group	-	-	+	+
Alkaloids	-	-	-	-
Flavonoids	-	-	+	+
Protein & Amino acid	-	-		-
Saponin	-	-	-	+

+ = Present, = Absent

Determination of Total Phenolic Content of *Polygonum glabrum* Willd. in Different Extracts:

TABLE 2: ABSORBANCE OF STANDARD COMPOUND (GALLIC ACID)

Conc. of Standard (µg/mL)	Absorbance λ_{max}=760 nm
10	0.0715
20	0.191
30	0.304
40	0.419
50	0.525
60	0.521
70	0.671
80	0.776
90	0.900
100	0.988

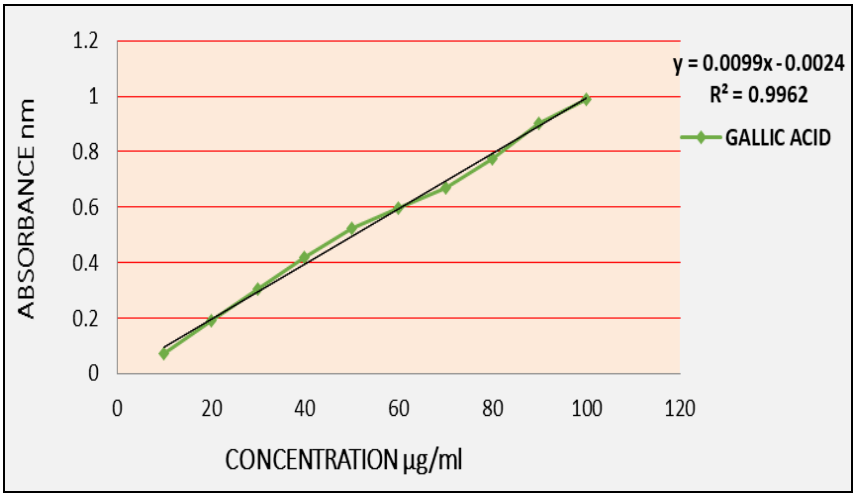

FIG. 1: STANDARD CURVE OF GALLIC ACID

TABLE 3: TOTAL PHENOLIC CONTENT OF *POLYGONUM GLABRUM* WILLD. IN DIFFERENT SOLVENT EXTRACTS

Plant Extracts	Concentration (µg/ml)	Mean (mg/g)
Petroleum Ether	100	11.11
Chloroform	100	15.88
Ethyl acetate	100	63.00
Ethanol	100	51.44

Each Value in the Table was obtained by Calculating the Average of Three Experiments:

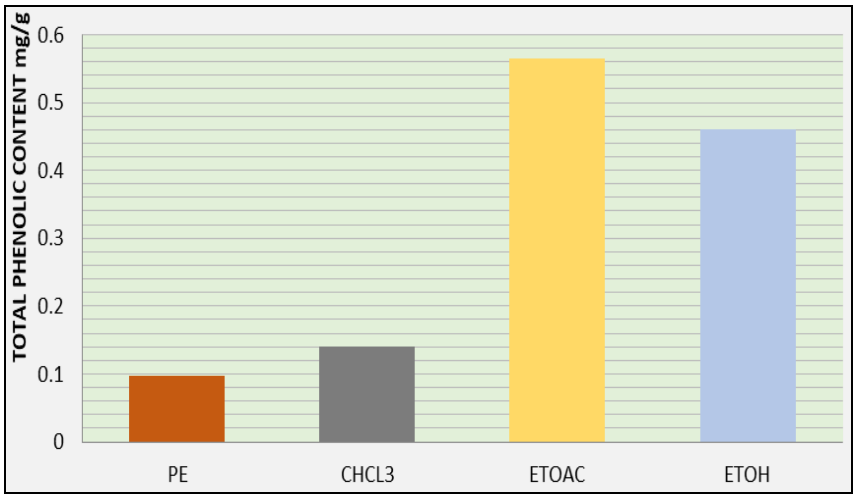

FIG. 2: TOTAL PHENOLIC CONTENT OF DIFFERENT SOLVENT EXTRACTS

DPPH Radical Scavenging Activity:

TABLE 4: MEASUREMENT OF ABSORBANCE AT 517NM AND INHIBITION EFFECT (%) OF DIFFERENT EXTRACTS WITH STANDARD BHT FROM CONCENTRATION RANGE OF 10-100 µg

Conc. Of Extract/ Standard (µg)	(%) Inhib. Effect BHT (Standard)	(%) Inhib. Effect Pet. Ether extract	(%) Inhib. Effect Chloroform extract	(%) Inhib. Effect Ethyl acetate extract	(%) Inhib. Effect Ethanol extract
10	40.33	20.68	21.73	43.43	43.21
20	45.41	25.55	27.17	50.93	48.57
30	49.75	29.6	32.64	59.68	54.64
40	54.34	33.26	35.86	65.31	60.35
50	57.97	36.10	41.66	71.11	63.21
60	62.56	39.75	47.82	75.56	70.35
70	66.66	42.19	53.62	82.5	76.42
80	71.01	45.23	57.97	88.75	82.50
90	78.50	48.47	61.95	91.25	87.85
100	81.88	51.72	65.94	92.5	90.89
IC_{50} value	31.31	93.46	65.76	15.45	22.35

(Given values are average of triplicate i.e. n=3), Inhib.-Inhibition. Key: BHT - Butylated Hydroxy Toluene. IC_{50} -Inhibition Concentration (the concentration was producing 50% of maximal Inhibition).

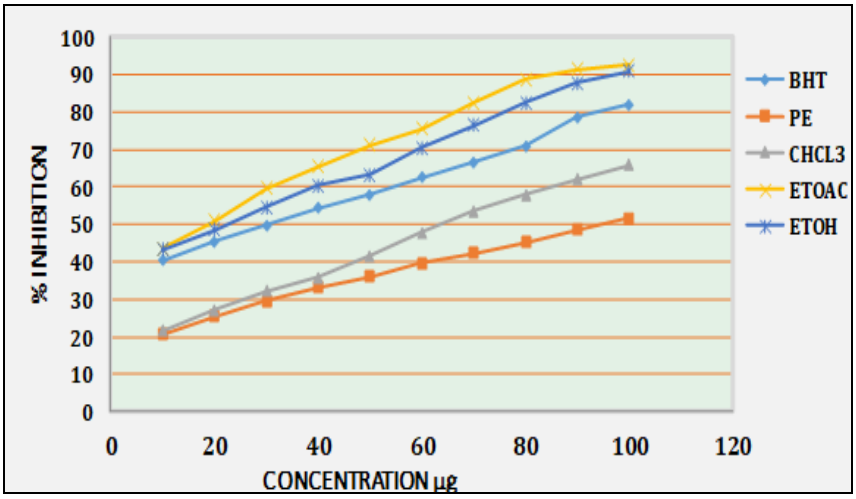

FIG. 3: FREE RADICAL SCAVENGING ACTIVITY OF DIFFERENT SOLVENT EXTRACT ON DPPH RADICAL

TABLE 5: SUPER OXIDE RADICAL SCAVENGING ACTIVITY BY BHT, DIFFERENT SOLVENT EXTRACTS

Conc. of Extract / Standard (µg)	(%) Inhib. Effect BHT (Standard)	(%) Inhib. Effect Pet. Ether extract	(%) Inhib. Effect Chloroform extract	(%) Inhib. Effect Ethyl acetate extract	(%) Inhib. Effect Ethanolic extract
10	36.84	17.39	16.00	32.60	33.33
20	45.45	24.00	25.00	44.56	42.68
30	57.14	29.62	32.25	54.41	52.00
40	65.71	35.29	38.23	63.95	59.00
50	75.00	44.11	47.50	70.19	65.21
60	84.00	50.00	53.33	76.69	70.37
70	92.20	54.76	60.37	82.18	76.69
80	95.83	57.77	65.25	87.34	80.89
90	96.51	60.41	71.23	89.00	84.01
100	97.01	63.46	73.75	91.48	86.04
IC_{50} value	20.97	66.90	57.69	24.54	29.36

(Given values are average of triplicate i.e. n=3), Inhib. - Inhibition. BHT - Butylated Hydroxy Toluene. IC_{50}- Inhibition Concentration (the concentration 50% of maximum inhibition).

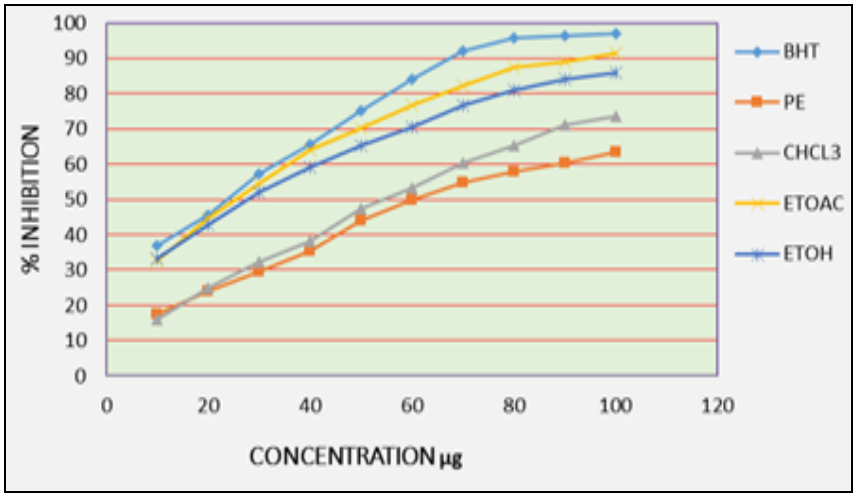

FIG. 4: SUPEROXIDE RADICAL SCAVENGING ACTIVITY OF DIFFERENT SOLVENT EXTRACTS

TABLE 6: EFFECTS OF ETHYL ACETATE AND ETHANOLIC EXTRACTS OF POLYGONUM GLABRUM WILLD. ON ANTIOXIDANT ENZYMES

Treatment	Dose (mg/kg p.o)	Antioxidant level			
		LOP nmol/mg wet tissue	GSH nmol/mg wet tissue	SOD U/mg protein	CAT U/mg protein
Normal control	1ml/kg	2.45± 0.11	6.45± 0.15	12.91 ± 0.24	47.07± 0.41
Paracetamol (PCM)	1000 mg/kg	6.4± 0.18#	2.55± 0.27#	4.58 ± 0.13#	25.45± 1.80#
PCM + Silymarin	1000 + 100 mg/kg	3.13± 0.15***	5.40±0.40***	11.50± 0.12***	39.06 ±0.42***
PCM + ETOAC	1000 + 200 mg/kg	5.6± 0.33*	4.50± 0.73*	6.33 ± 0.46**	30.08± 0.42*
PCM + ETOAC	1000 + 400 mg/kg	3.98± 0.11***	5.22±0.57***	10.15± 0.98***	31.84± 1.11***
PCM+ ETOH	1000 + 200 mg/kg	5.8± 0.16ns	3.00± 0.30ns	6.07± 0.70*	24.34± 0.32ns
PCM+ ETOH	1000 + 400 mg/kg	5.26± 0.24**	4.64± 0.35**	9.66± 0.15***	30.94± 0.87**

Data are expressed as Mean ± SEM, n = 6 animals in each groups; #p<0.001 when compared with normal; *p<0.05, **p<0.01, ***p<0.001, ns (non-significance) when compared with PCM. PCM-Paracetamol ETOAC- Ethyl acetate ETOH- Ethanol.

Phytochemical Investigations: Qualitative chemical investigations of various extracts revealed the presence of terpenoids, steroids, flavonoids, carbohydrates, glycosides, saponin, and tannins. The results are tabulated in **Table 1.** Chromatographic studies did further confirmation of above phytoconstituents. Total phenolic content in petroleum ether, chloroform, ethyl acetate and ethanolic extract was found to be 11.11, 15.88, 63.00, 51.44 mg/g, respectively equivalent to gallic acid in *Polygonum glabrum* Willd. leaves. The results are as tabulated in **Table 2.** Antioxidant activity of various solvent extracts of *Polygonum glabrum* Willd. was performed using different in-vitro models and Butylated Hydroxy Toluene (BHT) as standard. The results are as tabulated in **Tables 3, 4, 5**. Antioxidant activity of various solvent extracts was performed by using free radical scavenging on DPPH Radical, and it was observed that extracts scavenged free radicals in a concentration-dependent manner. The maximum % inhibition on DPPH was 51.72 (Pet ether), 65.94 (Chloroform), 92.50 (Ethyl acetate), 90.89 (Ethyl alcohol), and 81.88 (BHT) at a concentration of 100 µg. IC_{50} Values of pet ether, Chloroform, Ethyl acetate, Ethyl alcohol, and BHT was 93.46 µg, 65.76 µg, 15.45µg, 22.35 µg and 31.31 µg respectively. The maximum % hydroxyl radical scavenging activity was 63.46 (Pet ether), 73.75 (Chloroform), 91.48 (Ethyl acetate), 86.04 (Ethyl alcohol), and 97.01 (BHT) at a concentration of 100 µg. IC_{50} Values of pet ether, Chloroform, Ethyl acetate, Ethyl alcohol, and BHT were 66.90 µg, 57.69 µg, 24.54 µg, 29.36 µg and 20.97µg respectively. The Ethyl acetate extract showed the maximum free radical scavenging capacity as compared to other extracts.

The antioxidant activity in ethyl acetate and ethyl alcohol extract may be due to the synergistic effect of the flavonoids, tannins, and percentage of phenolic compounds present in the extract, which are known to possess a potent antioxidant activity. Since ethyl acetate and ethanolic extracts of *Polygonum glabrum* Willd. leaf were found to be active in the *in-vitro* screening; these extracts were

further investigated for *in-vivo* antioxidant activity using paracetamol-induced hepatotoxicity in which pathological conditions associated with the generation of free radicals are involved.

***In-vivo* Models:**

Pharmacological Screening: Acute toxicity study: Acute toxicity studies were performed by OECD guidelines, method no: 423, and the dose was fixed as 200 and 400 mg/kg b.w. of ethyl acetate and ethanol extracts.

***In-vivo* Antioxidant Model:** The two different doses (200 and 400 mg/kg bw) of ethyl acetate and ethanolic extracts of leaves of *Polygonum glabrum* Willd. were evaluated for Antioxidant activity in Wistar Albino rats by Paracetamol (PCM) induced hepatotoxicity. Lipid peroxidation has been postulated to the destructive process in liver injury due to PCM administration [18]. In the present study, an elevation in MDA levels in liver of animals treated with PCM was observed. The increased MDA in the liver suggests provoked lipid peroxidation, leading to tissue damage and failure of antioxidant defense mechanism to prevent the formation of excessive free radicals. Treatment with 400 mg/kg b.w/ ethyl acetate and ethanolic extracts play a role in reducing the free radicals, resulting in the subsequent decrease in the membrane damage and MDA level. Hence it may be possible that the hepatoprotective mechanism by ethyl acetate and the ethanolic extract is due to its antioxidant effect.

The non-enzymic antioxidant, glutathione is one of the most abundant naturally occurring tripeptides present in the liver. Its functions are mainly concerned with the removal of free radical species such as hydrogen peroxide, superoxide radical, alkoxy radical, and maintenance of membrane protein thiols and as substrates for glutathione peroxidase and GSH. In the present study, the decreased level of GSH has been associated with enhanced lipid peroxidation in paracetamol-treated rats. Administration of ethyl acetate and ethanolic extract significantly increased the glutathione level in a dose-dependent manner. A decrease in enzyme activity of SOD is the sensitive index in hepatocellular damage, and it's the most sensitive enzymatic index in liver injury. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system [19]. It scavenges the superoxide anion to form hydrogen peroxide, thus diminishing this radical's toxic effect caused by this radical. In higher dose *i.e.,* 400 mg/kg ethyl acetate and ethanolic extracts of leaves of *Polygonum glabrum* Willd. showed a significant increase in hepatic SOD activity and thus reduced free radical-induced oxidative damage in the liver.

CAT is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver. Since H_2O_2 acts as a substrate for a specific reaction that generates highly hydroxyl radicals, the primary role of catalase in cellular antioxidant defense mechanisms is to reduce the accumulation of H_2O_2 [20].

CAT decomposed hydrogen peroxides and protected the tissue from highly reactive hydroxyl radicals. Therefore, the reduction in the activity of these enzymes may result in several deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Administration of higher dose (400mg/kg) Ethyl acetate and Ethanolic extracts of *Polygonum glabrum* Willd. increases the level of CAT in paracetamol-induced liver damage in rats to prevent excessive free radical accumulation and protect the liver from highly reactive hydroxyl radicals.

CONCLUSIONS: Experimental evidence obtained from the present study showed ethyl acetate and ethanolic extract (400 mg/kg b.w.) of leaves of *Polygonum glabrum* Willd. Shows a promising antioxidant effect which may be due to the presence of flavonoids and other components present in the leaf. It can be concluded that leaves of *Polygonum glabrum* Willd. can be a great source of antioxidants. However, further studies are needed to isolate a potent natural antioxidant molecule to suppress the effects induced by free radicals and explore the mechanism responsible for its antioxidant effect.

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