

**IN VITRO ANTIOXIDANT AND PRELIMINARY HEPATOPROTECTIVE
ACTIVITY OF *OROXYLUM INDICUM* VENT LEAF EXTRACTS**

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Summary

Present study was aimed to evaluate *in vitro* antioxidant activity and hepatoprotective activity of different extracts [petroleum ether (PEE), ethanol (EE), water (WTE) and chloroform (CE) extracts] of *Oroxylum indicum* Vent. Leaves in different system viz. radical scavenging activity by DPPH reduction, nitric oxide radical scavenging activity in sodium nitroprusside / Griess reagent system, superoxide radical scavenging, hydroxyl radical scavenging method and reducing power assay. Ethanol extract was found to be good solvent for extraction and having good antioxidant activity in all the assays. Hepatotoxicity was induced by CCl₄ which leads to significant increased in the level of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin whereas there was a significant decrease in the activity of total protein. Administration of different extracts of *Oroxylum indicum* (300mg/kg) leads to significant alteration in biochemical parameters towards normal. In conclusion the *Oroxylum indicum* leaf extract showed good antioxidant and hepatoprotective activity.

keywords: *Oroxylum indicum*, Antioxidant, Carbon tetrachloride, Hepatoprotective

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1. Introduction

Oroxylum indicum Vent. Family Bignoniaceae, commonly known as Indian caper, is a climbing shrub found through out India and has been used as a 'Rasayana' drug in the traditional Ayurvedic system of medicine. The plants are used for curing stomach disorders, diarrhea, dysentery and rheumatic swelling (1). The root bark is used in fever, bronchitis, intestinal worms, asthma, inflammation, anal troubles etc. The fruit and seeds are used as expectorant, purgative and bitter tonic (2). In Hindu the root, bark, stem and leaf are prescribed for snake bite(3). The leaves of the plant are also reported for its analgesic (4) and antimicrobial (5) activity. The plant contain flavonoids such as chrysin (5, 7-dihydroxyflavone), oroxylin A (5, 7-dihydroxy-6-methoxyflavone), baicalein (5, 6, 7-trihydroxyflavone) and baicalein glycoside, benzoic acid and fatty acids. Ethanol -CH₂Cl₂-soluble fraction prepared from twigs and leaves of this plant have reported for their anti-mutagenic activity (6).

Reactive oxygen species are implicated in some diseases such as inflammation, cancer, ageing, anemia, liver disease, degenerative diseases and atherosclerosis (7). Free radicals have been implicated to a major extent in pathology of liver disorders. Oxidative damage through free radical generation is one of the mechanisms involved in the hepatotoxic effect of carbon tetrachloride and paracetamol (8).

A great number of aromatic, spicy, medicinal and other plants contain chemical compounds exhibiting antioxidant property. Scientific information on chemical constituent and antioxidant properties of various plants less widely used in the medicine is still rather scarce. Hence assessment of chemical constituent and such properties remain an interesting and useful task particularly for finding new source of natural antioxidant, functional food and nutraceuticals including polyphenolic such as flavonoid, tannin, proanthocyanidin (9). The objective of the present study was to investigate the *in vitro* antioxidant activity and hepatoprotective activity of petroleum ether, ethanol, water and chloroform extract of *O.indicum* leaves.

2. Material and Methods

2.1 Collection and extraction of the Plant

Oroxylum indicum leaves were collected from Wardha District, Maharashtra, India in the month of August 2007. The plant was identified and authenticated by Dr. Chitle, Nagpur University, Nagpur. A voucher specimen (No.9179) was deposited with the, "Post graduate Teaching Department of Botany, Nagpur.

The shade dried and powdered leaves of *Oroxylum indicum* Vent, were subjected to successive extraction in a Soxhlet apparatus with petroleum ether (60-80°), ethanol, chloroform and finally macerated with water so as to get respective extracts. All extracts were individually filtered, through Whatmann filter paper # 42 and evaporated to dryness at 50°C in oven. The extracts were then stored in desiccators till further use. Percentage yield of the respective extracts was found to be 7.8%, 5.7%, 15.1 and 19% respectively.

2.2 Chemicals

1, 1-diphenyl-2-picryl hydrazide (DPPH), bovine serum albumin, butylated hydroxy toluene (BHT) were purchased from Sigma Ltd. Folin Ciocalteu reagent; Tannic acid were purchased from Merck Ind. Ltd. All solvents for extraction, sodium lauryl sulphate, triethanolamin, ferric chloride, aluminum chloride and carbon tetrachloride were purchased from Loba Chemical. α -tocopherol, ascorbic acid, rutin, sulphanyl amide, naphthyl ethylene diamine dihydrochloride, HPLC grade acetonitrile, methanol, water were purchased from Sd Fine Chemicals. All the other chemicals used were of AR grade.

2.3 In Vitro antioxidant activity

2.3.1 DPPH radical scavenging activity (10)

The free radical scavenging activity of petroleum ether, ethanol, chloroform and water extracts of *Oroxylum indicum* leaves and ascorbic acid was measured in terms of hydrogen donating ability using the stable radical DPPH. About 0.3 mM solution of DPPH in 100% ethanol was prepared and 1 ml of this solution was added to 3 ml of the extract at different concentrations (10–100 mcg/ml). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. The IC₅₀ value of the crude extract was compared with that of ascorbic acid, which was used as the standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

2.3.2 Nitric oxide radical scavenging activity (11)

The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and extract or standard solution (0.5 ml) was incubated at 25 °C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride (1%) was added, mixed and allowed to stand for 30 min. A pink coloured chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solution. The IC₅₀ value is the concentration of sample required to inhibit 50% of nitric oxide radical. All determinations were performed in triplicates.

2.3.3 Super oxide radical scavenging (12)

About 1 ml of nitroblue tetrazolium (NBT) solution (156 µM NBT in 100 mM phosphate buffer, pH 7.4) 1 ml NADH solution (468 µM in 100 mM phosphate buffer, pH 7.4) and 0.1 ml of sample solution of *Oroxylum indicum* (1.25 µg to 10 µg) in water were mixed. The reaction started by adding 100 µl of phenazine methosulphate (PMS) solution (60 µM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 min, and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. Curcumin was used as a positive control.

2.3.4 Reducing power (13)

The reducing power is determined by the Fe³⁺ – Fe²⁺ transformation in the presence of extracts. One milliliter of the plant extract (0–1000 mcg/ml), 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide were incubated at 50 °C for 30 min and 2.5 ml of 10% trichloro acetic acid was added to the mixture and centrifuged for 10 min at 3000 rpm. About 2.5 ml of the supernatant was diluted with 2.5 ml water and is shaken with 0.5 ml of freshly prepared 0.1% ferric chloride. The absorbance was measured at 700 nm. Butylated hydroxy toluene (BHT) was used as the standard. All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

2.3.5 Hydroxyl radical scavenging activity (14)

All solutions were prepared freshly. 1.0 ml of the reaction mixture contained 100 µl of 28 mM 2-deoxy-2-ribose (dissolved in phosphate buffer, pH 7.4), 500 µl solution of various concentrations of the *Oroxylum indicum* (10 to 100 µg), 200 µl of 200 µM FeCl₃ and 1.04 mM EDTA (1:1 v/v), 100 µl H₂O₂ (1.0 mM) and 100 µl ascorbic acid (1.0 mM). After an incubation period of 1 hour at 37°C the extent of deoxyribose degradation was measured by the TBA reaction. Measure the absorbance at about 532 nm against the blank solution. Vitamin E was used as a positive control.

2.4 Acute oral toxicity test:

Acute oral toxicity test was carried out as per the protocol given in the OECD guidelines (15) and a dose of 300 mg/kg was selected for the animal activity.

2.5 Experimental animals:

Adult albino rats (Wistar strain), of either sex weighing 150-180 g were used in the entire study. The animals were housed in the polypropylene cages with 12 hours dark-light cycle, at appropriate temperature and humidity conditions. Animals were fed with a balanced diet and water *ad libitum*.

All animal experiments were approved by the Institutional Animal Ethical Committee (Registration No.535 / 02 / a / CPCSEA / Jan 2002) of 'Institute of Pharmaceutical Education and Research, Wardha.

2.5.1 Hepatoprotective activity

Hepatotoxicity was induced by administration of CCl₄ (0.7 ml/kg, i.p) (16) for five days. On the sixth day enzymatic levels were noted. After intoxication with CCl₄, drug extracts and standard Silymarin were administered for five days and on the 11th day various biochemical parameters were recorded.

The rats were divided in seven groups with each group contains six animals (n=6).

Group I: Animals served as control.

Group II: Animals received CCl₄ for five days (0.7ml/kg, i.p).

Group III: Animals received CCl₄ and Silymarin (100 mg/kg, p.o) as standard hepatoprotective drug.

Group IV: Animals received CCl₄ and ethanol extract (300 mg/ kg, p.o).

Group V: Animals received CCl₄ and water extracts (300 mg/kg, p.o).

Group VI: Animals received CCl₄ and chloroform extracts (300 mg /kg, p.o).

Group VII: Animals received CCl₄ and Petroleum ether extracts (300 mg/ kg, p.o).

2.6 Biochemical analysis

At the end of experiment period (on day 11) blood samples was withdrawn by orbital sinus under ether anesthesia and were centrifuged by using table top centrifuge at 2000 rpm for 30 minute so as to separate the serum. Quantitative determination of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total protein and total bilirubin were carried out using standard diagnostic kit obtained from Merck Ltd. Mumbai.

2.7 Statistical analysis

Results were expressed as mean±S.D. For determining the significant intergroup differences each parameter was analyzed by one way ANOVA followed by Dunnett 't' test.

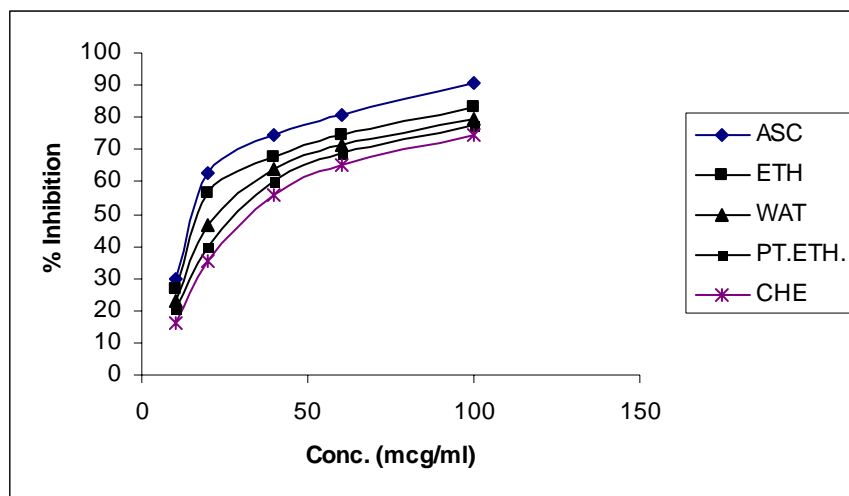
3. Results and Discussion

Preliminary phytochemical analysis showed the presence of steroid, fats, carbohydrate, proteins, alkaloids, phenolic like saponin, flavonoid, tannins. Flavonoids and phenolic compounds have been reported to possess antioxidant activity. So the extracts were evaluated for *in vitro* antioxidant activity.

3.1.DPPH radical scavenging activity

DPPH is nitrogen centered free radical that shows strong absorbance at 517 nm. Deep violet coloured methanolic DPPH solution changes to yellow colour in presence of DPPH radical scavengers. DPPH radical accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Extent of DPPH radical scavenged was determined by the decrease in intensity of violet colour in the form of IC₅₀ values. The antioxidant activity was compared with ascorbic acid (ASC) as standard. The IC₅₀ values of ASC, EE, WTE, PEE and CE were found to be 16.6, 18.8, 21.36, 25.25 and 28.08 µg/ml respectively (Fig 1). The EE has lower IC₅₀ value among the extracts and hence maximum antioxidant activity. The antioxidant activity of other extracts was in the order ASC > EE > WTE > PEE > CE (Fig 1).

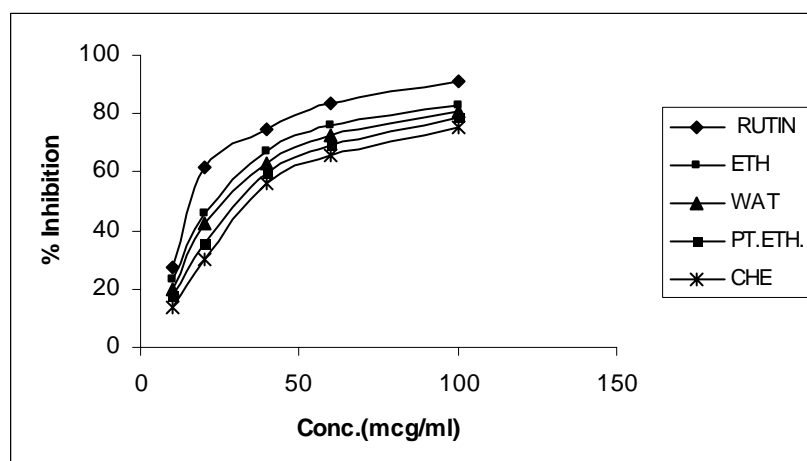
Fig 1- DPPH radical scavenging activity of different extracts of *Oroxylum indicum* leaves



3.2. Nitric oxide radical scavenging activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of Griess Illosvoy reaction. Extent of nitric oxide radical scavenged was determined by the decrease in intensity of pink coloured chromophore in the form of IC_{50} values. The absorbance of pink coloured chromophore was determined at 540 nm. The antioxidant activity was compared with rutin as standard. The IC_{50} values of rutin, EE, WTE, PEE and CE were found to be 18.38, 21.64, 23.58, 28.27 and 33.13 $\mu\text{g/ml}$ respectively (Fig 2). The EE has lower IC_{50} value among the extracts and hence maximum antioxidant activity. The antioxidant activity of other extracts were in the order rutin > EE > WTE > PEE > CE (Fig 2).

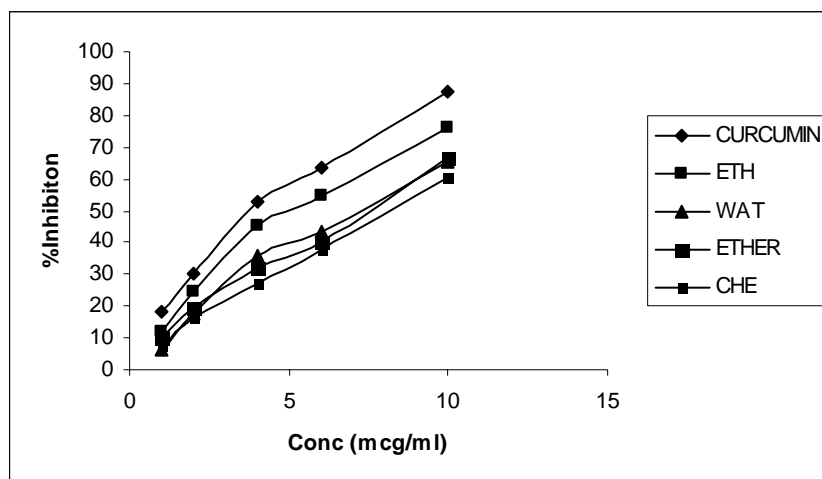
Fig 2: Nitric oxide radical scavenging activity of different extracts of *Oroxylum indicum* leaves .



3.4. Superoxide radical scavenging

Extent of superoxide radical scavenged was determined by the decrease in intensity of pink coloured chromophore in the form of IC_{50} values. Lower IC_{50} value represents higher antioxidant activity. The antioxidant activity was compared with curcumin as positive control. The IC_{50} values of curcumin, EE, WTE, PEE and CE were found to be 3.32, 4.44, 6.95, 7.4 and 8.12 $\mu\text{g/ml}$ respectively (Fig. 3). The EE has lower IC_{50} value among the extracts and hence maximum antioxidant activity. The antioxidant activity of other extracts were in the order curcumin > EE > WTE > PEE > CE. (Fig 3).

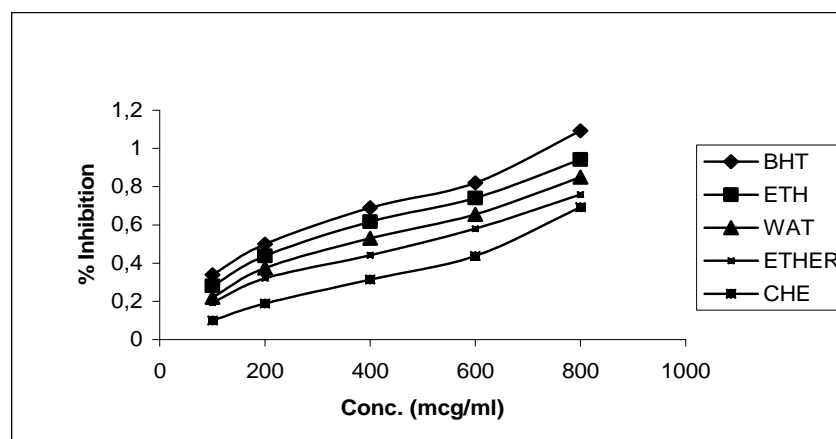
Fig 3. Superoxide radical scavenging activity of different extracts of *Oroxylum indicum* leaves .



3.5. Reducing power

In this method, antioxidant compounds form a colored complex with potassium ferricyanide, trichloro acetic 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. Butylated hydroxyl toluene (BHT) was used as standard. There was concentration dependent increase in the absorbance of reaction mixture for all the extracts and standard. EE has shown the maximum absorbance and hence maximum reducing power among the extracts (Fig. 4). The reducing power of other extracts were in the order BHT > EE > WTE > PEE > CE (Fig. 4).

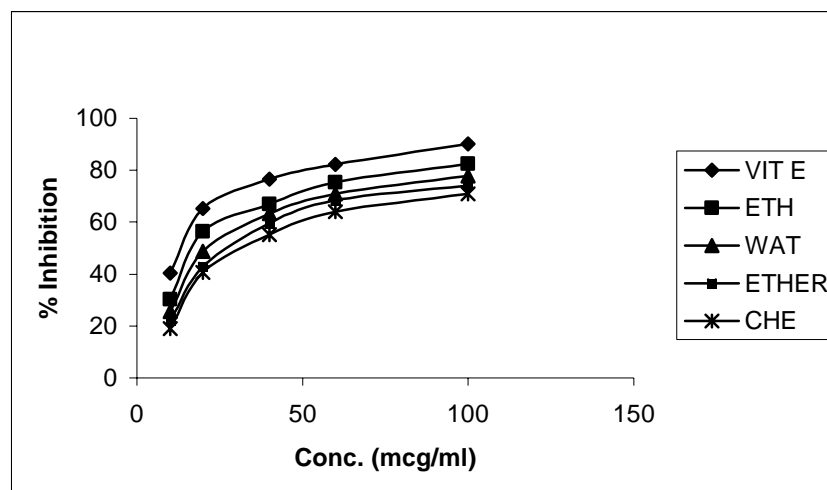
Fig 4. Reducing power assay of different extracts of *Oroxylum indicum* leaves .



3.6. Hydroxyl radical scavenging activity

When EDTA chelated iron- (III) ions are incubated with reducing agent and H₂O₂ in the assay, OH radicals are generated in free solution that attack the deoxyribose substrate and fragmenting it into thiobarbituric acid reactive substances (TBARS). The generated TBARS reflect the extent of generation of OH. Extent of hydroxyl radical scavenged was determined by the decrease in intensity of pink coloured chromophore in the form of IC₅₀ values. The absorbance of pink coloured chromophore was determined at 532 nm. Lower IC₅₀ value represents higher antioxidant activity. The antioxidant activity was compared with Vitamin E as positive control. The IC₅₀ values of Vitamin E, EE, WTE, PEE and CE were found to be 12.34, 16.44, 20.53, 23.31 and 24.57 μg/ml respectively (Fig. 5). The EE has lower IC₅₀ value among the extracts and hence maximum antioxidant activity. The antioxidant activity of other extracts was in the order Vitamin E > EE > WTE > PEE > CE (Fig. 5).

Fig 5. Hydroxyl radical scavenging activity of different extracts of *Oroxylum indicum* leaves .



3.7. Hepatoprotective activity of the extracts

The toxicity produced by CCl₄ is due to the reaction of free radicals ($\cdot\text{CCl}_3$ or $\text{CCl}_3\text{COO}\cdot$) with lipids and proteins and with various tissue constituents. The free radical causes the peroxidation of the poly-enoic lipids of the endoplasmic reticulum and generation of secondary free radicals derived from these lipids, a chain reaction. This destructive lipid peroxidation leads to breakdown of membrane structure and function; as a result there is elevation of enzyme levels in plasma (16).

A significant increased in the activity of SGOT, SGPT, ALP, total bilirubin content and a significant decreased in the level of total protein in CCl₄ treated rats showed induction of hepatotoxicity. All the extract showed significant protection against hepatotoxicity by normalizing the level of enzymes. Ethanol extract of *Oroxylum indicum* leaves had shown better protection amongst PEE, WTE and CE. Thus hepatoprotective action of these extracts is likely to be due to its ability to scavenge free radicals and induce microsomal enzymes there by inhibition of the lipid per oxidation induced by CCl₄ (Table 1).

Table 1: Effects of Oroxyllum indicum leaves extracts on liver function in rats treated with CCl₄

Groups	SGOT (mg/dl)	SGPT(mg/dl)	ALP(U/L)	Total Protein (U/L)	Total bilirubin (mg/dl)
Gr.I	201.5 ± 2.12	62.17±4.49	183.59±6.40	8.20 ±0.40	0.83±0.06
Gr.II	338.2 ± 4.52**	126.50±6.30**	500.20±3.11**	5.30 ±0.28**	2.54±0.15**
Gr.III	246.5± 4.95**	73.56±4.97**	220.80±9.60**	6.65 ±0.35**	0.90±0.10**
Gr.IV	251.5 ± 9.19**	77.80±3.50**	241.30±4.60**	5.90±0.14**	1.00±0.04**
Gr.V	272 ± 9.89**	84.06±2.93**	259.50±3.50**	4.70 ±0.28**	1.04±0.07**
Gr.VI	286.5 ± 3.42**	86.25±6.01**	284.00±4.20**	3.50 ±0.14**	1.18±0.04**
Gr.VII	299.5 ± 6.43**	88.15±4.10**	291.50±3.50**	3.20 ±0.28**	1.31±0.10**

Results are expressed as mean±S.D, ANOVA followed by Dunnett 't' test. *P<0.05 and **P<0.01. Group I: control, Group II: Animals received CCl₄ for five days, Group III: Animals received CCl₄ and Silymarin, Group IV: ethanol extract, Group V: water extracts, Group VI: chloroform extracts, Group VII: Petroleum ether extracts.

In conclusion the results of this study demonstrated that using several *in vitro* models *Oroxylum indicum* was found to have antioxidant and hepatoprotective activity. This activity was found due to presence of polar phenolic compound flavonoid, tannin etc. Overall *Oroxylum .indicum* could be considered as a model herbal drug for experimental studies including free radical induced disorders like cancer, diabetes, atherosclerosis etc. Further studies are required to establish its *in vivo* antioxidant activity using different animal models.

Acknowledgment

The authors are grateful to All India Council for Technical Education New Delhi for providing financial assistance.

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