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**Rajesh S.Telekone**
*Padm.Dr. D.Y. Patil College of Pharmacy, Akurdi, Pune 411044, India*

**Mohib Khan**
*Oriental College of Pharmacy, Sanpada, Mumbai 400705, India*

**Correspondence:**
**Rajesh S.Telekone**
Padm.Dr. D.Y. Patil College of Pharmacy, Akurdi, Pune 411044, India
**E-mail:**
rajeshtelekone@yahoo.co.in

# Antiinflammatory and antioxidant activity of extracts and isolated compounds from *Derris brevipes* Benth (Baker)

*Rajesh S.Telekone*, Mohib Khan*

## Abstract

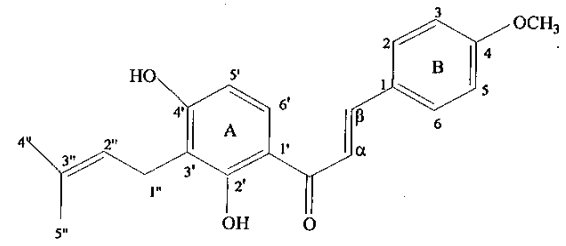
The role of medicinal plants in promoting the ability of human health to cope with the unpleasant and difficult situations is well documented from ancient times till date all over the world. The purpose of this study is to define antioxidant and anti-inflammatory activity of chloroform, methanol extract and isolated flavonoid compounds 2',4'-dihydroxy-4-methoxy-3'-prenyl chalcone (compound 1) and Leutolin (compound 2) from the methanol extract of *Derris brevipes* Benth (Baker). This study displayed potential total antioxidant capacity, reducing power, efficacy in scavenging singlet oxygen and nitric oxide radicals of extracts and isolated compounds. The study also revealed anti-inflammatory capability of extracts and compounds. Out of the two extracts, methanol extract showed higher antiinflammatory activity and of the two compounds compound 2 outscored compound 1 in antiinflammatory efficacy. As an anti-inflammatory test molecule the efficacy of compound 2 was nearly equal to that of the standard ibuprofen. $IC_{50}$ values for antioxidant activities were also less for methanol extract and compound 2 as compared to chloroform extract and compound 1. These results confirm the role of isolated flavonoids as promising free radical scavengers, potent antioxidants and anti-inflammatory agents.

**Keywords:** Antioxidant, Anti-inflammatory, Free radical, Flavonoid, $IC_{50}$ value.

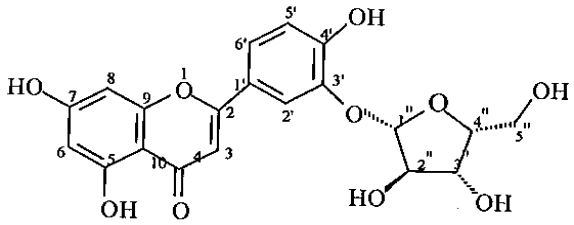
## Introduction

The genus Derris belongs to the tribe Tephrosieae, subfamily Papilionnoidae of the family Leguminosae[1] and comprises of approximately 80 species distributed mainly in the tropical and subtropical regions of the world. Derris is popular ichthyotoxic[2], insecticide[3], pesticides[4], antiviral[5] and have antibacterial[6] activity. Derris is also used as analgesic[7], antipyretic[8], for arthistic symptoms[9], antidysenteric[10], antidiuretic[10], antispasmodic[11] counter-irritant[11], for purification of blood[7], for treatment of leprosy[8], sore throat[12] and rheumatism[13]. Literature review revealed that Derris species mainly contain flavanoids[14] rotenoids, coumarins[15], triterpinoids[16], sterols[14] and glycosides[9]. Additionally, the rotenoids for example deguelin also displayed antitumor activity, by inhibiting pulmonary adenoma formation and growth with no detectable toxicity.[17] Derris species from India are known for medicinal value and different parts of species have been used in folk medicine for bronchitis[18], cough, rheumatoid arthritis, diabetes and anti-fertility[19]. *Derris brevipes* is a climbing shrub, distributed widely in India. Literature review revealed that chemical investigation of the aerial part of *Derris brevipes* has not been carried out. In an attempt to discover naturally occurring

bioactive compounds from the chloroform and methanol extract of the aerial parts of this plant antiinflammatory and antioxidant activity were performed. Both chloroform and methanol extract showed antiinflammatory and antioxidant activity. This prompted us to investigate the bioactive constituents from this part of the plant to provide the theoretical basis for comprehensive utilization of this plant. Chemical investigation on the methanol extract of the aerial parts of Derris brevipes yielded 2', 4'-dihydroxy-4-methoxy-3'-prenyl chalcone (Compound 1) and Leutolin-3'-O-xilofuranose (Compound 2). Herein is described the evaluation of antiinflammatory and antioxidant activity of chloroform, methanol extract, compound 1 and compound 2.




2', 4'-dihydroxy-4-methoxy-3'-prenyl chalcone




Leutolin-3'-O-xilofuranose

**Figure 1:** Structure of Compound 1 (2', 4'-dihydroxy-4-methoxy-3'-prenyl chalcone) and 2 (Leutolin-3'-O-xilofuranose)

## Materials and Methods

### Plant material and preparation of plant extract

The leaves and stems of *Derris brevipes* (Benth.) baker were collected from Amboli Ghat, Konkan coast of Maharashtra, India, and was authenticated by The Dean, College of Forestry, Balasaheb sawant kokan krishividyapeeth, Dapholi, Maharashtra. Voucher specimen has been deposited in the library of College of Forestry. The air-dried leaves and stems (1.5 kg) of *Derris brevipes* were powdered in a wiley mill and extracted with chloroform. The extract was concentrated under vacuum to get the corresponding residue of 12.5 gm. The plant material left after extraction with chloroform was consecutively extracted with methanol.

### Preliminary phytochemical investigation

Both chloroform and methanol extracts of *Derris brevipes* (benth.) baker were subjected to qualitative tests for the identification of various active constituents present.[20] Standard qualitative methods as described previously were carried out in an order to investigate the presence of phytochemicals like alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins, tannins, terpenoids, anthraquinones, and triterpenoids in both the extracts. Results are presented in the Table 1.

### Isolation of active constituents (Flavonoids) by column chromatography from methanol extract and their structural elucidation

Methanol extract was column chromatographed using a silica gel column having diameter of 50 mm and length of 1 meter. Silica gel used for packing the column was having mesh size range between 130-270.The column was eluted initially with solvent chloroform followed by combination of chloroform and methanol. Polarity of solvent system was increased by gradually adding methanol in chloroform. Five parts of methanol were added to 95 parts of chloroform similarly polarity was increased by adding methanol in multiples of 5 to chloroform and reducing quantity of chloroform in proportion. Eluents of all solvent combinations were analysed on TLC and eluents which showed similar TLC patterns were mixed. Further isolation and purification of isolated compounds was done by preparative thin layer chromatography.Structural elucidation of isolated compounds was done by performing Infrared spectroscopy, Proton (1H) and (13C) Carbon nuclear magnetic resonance spectroscopy, 2D nuclear magnetic resonance spectroscopy and Mass spectrophotometry. The structure of isolated compounds 2', 4'-dihydroxy-4-methoxy-3'-prenyl chalcone (compound 1) and Leutolin-3'-O-xilofuranose (compound 2) are shown in Fgure 1.

### Antiinflammatory activity by carageenen induced rat paw oedema method

#### *Methodology*

The chloroform extract, methanol extract and isolated compounds were evaluated for their invivo anti-inflammatory activity by carrageenan induced rat paw

oedema method. The animals used were wister albino rats of either sex weighing (150 to 200 gm), were divided into ten groups of six animals each. First group served as negative control and received vehicle only (Dimethylsulphoxide) which was used to dissolve extracts and isolated compounds, second group served as positive control and received ibuprofen 10 mg/kg as per body weight. Third and fourth groups received 250 mg/kg and 500 mg/kg body weight of chloroform extract solution respectively. Fifth and the sixth group received 250 mg/kg and 500 mg/kg body weight of methanol extract, seventh and eighth group recieved low dose and medium dose of compound 1 respectively nineth and tenth group recieved low dose and medium dose compound 2 respectively as per calculations by the formula. The rats were fasted for 24 hrs prior to the experiment. The solutions were given orally with the help of an oral feeding tube before two hours, and then a sub-cutaneous injection of 0.1ml of 1% solution of carrageenan was administered in the left paw to all groups. The paw volume was measured with the help of Hugo Basile Plethysmometer. The paw volume was measured at 1st, 2nd, 3rd, 4th, 5th and 6th hrs respectively. Finally, the average paw swellings of groups treated with extract, isolated compounds and standard drug were compared with negative control group treated with vehicle only. The % inhibition of oedema was calculated.

$$\% \text{ Inhibition of oedema} = [1 - Vt/Vc] \times 100$$

Where, Vt = mean relative change in paw volume in test group.
Vc = mean relative change in paw volume in control group.

Values of % Inhibition of inflammation of test groups and standard are presented in the table 2 and 3.

Sample preparation:
*Chloroform and methanol extract*

For the extracts 250mg/ kg 500mg/kg body weight were low and high dose respectively.

Isolated compounds:

For the molecules 10 mg/kg and 20 mg/kg body weight were low and medium dose respectively. All the solutions were prepared in Dimethyl sulphoxide.

**Antioxidant activity**

***Total antioxidant activity assay***

Antioxidant capacities of both extracts and isolated compounds was determined by an improved ABTS•+ radical cation decolorisation assay in comparison to trolox standard.21 The ABTS•+ radical cation was pregenerated by mixing 7 mM ABTS stock solution and 2.45 mM potassium persulfate followed by overnight incubation in dark at room temperature. Then 10 µl sample solution was mixed with 3 ml ABTS•+ solution and the absorbance was measured at 734 nm. All experiments were repeated 6 times and results were then analysed in order to create relevent graphs. The Trolox equivalent antioxidant capacity (TEAC) was determined by plotting the percentage inhibition of absorbance as a function of concentration of standard and sample (Table 4 and 5).

***FRAP assay***

FRAP assay depends upon the conversion ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reductant at low pH. Fe(II)-TPTZ has an intensive blue color and can be monitored at 593 nm. The literature review revealed that FRAP is a sensitive method for the measurement of total antioxidant power of the fresh biological fluids and plant products. To FRAP solution (3.6 ml) added distilled water (0.4 ml) and incubated at 37˚C for 5 min. Then this solution was mixed with certain concentration of the plant extract (4 ml) and incubated at 37˚C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentrations of $FeSO_4$, $7H_2O$ (0.1, 0.4, 0.8, 1, 1.12, 1.5 mM) were used and the absorbance values were measured as for sample solutions. Linearity of FRAP (dose–response line) for standard solutions is shown in Figure 9. The results of the FRAP assay are reported in Table 6. The antioxidant activities were expressed as the concentrations of antioxidant having a ferric reducing ability equivalent to that of 1 mM of Ferrous sulphate.

***Nitrous oxide radical Scavenging assay***

Nitrous oxide (NO) in oxygen-containing aqueous solution has a short half-life due to its rapid oxidation. It has been reported that NO in aqueous solution containing oxygen is oxidized primarily to nitrite ($NO^{2-}$) with little or no formation of nitrate ($NO^{3-}$).[22] So, the NO formation is assessed by measuring $NO^{2-}$. The assay relies on a diazotization reaction. The reaction utilizes sulfanilamide

and N-1-napthylethylenediamine dihydrochloride (NED) under acidic conditions. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent.[23] Scavengers of NO compete with oxygen leading to reduced production of nitrite ions.[24] Sodium nitroprusside (5 mM in PBS at pH 7.4) 100 µl solutions was mixed with 750 µl of different concentrations of sodium nitrite (10 – 70 mM in water) or the equal volume of extract, isolated compounds and incubated at 25ºC for 150 min. After incubation 200 µl of Griess reagent, containing 1% (w v-1) sulphanilamide, 0.1% (w v-1) NED and 2.5% (v v-1) phosphoric acid, was added and the absorbance of the coloured compound formed due to diazotization of nitrite with sulfanilamide and subsequent coupling with NED was read at 546 nm (Table 7 and 8).

### *Antioxidant activity by Thiobarbituric acid method*

Assay of Thiobarbaturic acid reactive substances (TBARS) measure malondialdehyde (MDA) present in the sample, as well as MDA generated from lipid hydroperoxides by the hydrolytic conditions of the reaction. MDA is one of several low-molecular-weight end products formed via the decomposition of certain primary and secondary lipid peroxidation products and only certain lipid peroxidation products generate MDA. Induction of Fe+2/Ascorbate system inhibition of Lipid Peroxidation was determined by thiobarbituric acid method. 0.1 ml of mice liver homogenate, 0.1 ml of Potassium chloride (30 mM), 0.1 ml of Ammonium Ferrous sulphate, 0.1 ml of ascorbic acid (0.6 mM), 0.1 ml of various concentrations of Plant extract solution, 0.1 ml of isolated compounds solution and 0.1 ml of standard vitamin E solution were mixed and Incubated at 37ºC for 1 hour. To the above solution 0.2 ml of Sodium dodecyl sulphate, 1.5 ml of 0.8 % Thiobarbaturic acid and 1.5 ml of 20% acetic acid were added, volume was made upto 4 ml with distilled water and heated in oil bath at 95ºC for 1 hour. After 1 hour 1ml of distilled water and 5 ml of 15 : 1 % v/v of n-Butanol and Pyridine solution were added when the test tubes reached normal temperature and shaken vigorously, centrifuged at 4000 rpm for 15 min and the organic layer was taken separately and the absorbance read at 532 nm using a UV Visible spectrophotometer.

### *Free radical scavenging activity by DPPH method*

DPPH (1, 1- diphenyl- 2- picryl- hydrazyl) is stable free radical and methanolic solution of it is used to evaluate the antioxidant activity of several natural compounds. Antioxidants on interaction with DPPH, either transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character, and convert it to 1,1- diphenyl- 2- picryl- hydrazine and the degree of discoloration indicates the scavenging activity of the drug. The change in the absorbance produced at 517 nm, has been used as a measure of antioxidant activity. 1 ml each of individual concentrations of extract, compound, and standard solutions were taken in different test tubes. To this 5 ml of methanolic solution of DPPH was added, shaken well and mixture was incubated at 37ºC for 20 min. Absorbance of all the samples and standard were measured against blank at 517 nm. Absorbance of DPPH alone was taken as control. Percent radical scavenging activity can be calculated by using following formula.

$$\% \text{ Anti-radical activity} = \frac{\text{Control Abs - Sample Abs}}{\text{Control Abs}} \times 100$$

### *Superoxide radical scavenging assay*

Superoxide anions were generated from the dissolved oxygen by PMS/NADH coupling reaction that reduces the NBT (yellow dye) to blue colored product called formazan. Drugs possessing super oxide scavenging activity thereby decrease the reduction of NBT, which is a measure of superoxide anion scavenging activity. The reaction mixture consists of 1 ml solution of different concentration of extracts to which 1ml of Nitro blue tetrazolium (NBT) solution and 1 ml Nicotinamide adenine dinucleotide (NADH) solution were added. The reaction was started by adding 100 µL of phenazine methosulfate (PMS) solution to the reaction mixture. The reaction mixture was incubated at 25ºC for 5 min. and the absorbance at 560 nm was measured against blank. Decreased absorbance of reaction mixture indicated increased superoxide anion scavenging activity.

## Result and Discussion

The natural antioxidants have the capacity to work as singlet and triplet oxygen scavengers, peroxide decomposers, various enzyme inhibitors and synergists. The more the amount of total antioxidants present in the plant the more will its derived crude extracts show ROS scavenging potentials. In ABTS•+ scavenging assay both the extracts showed good total antioxidant capacities, methanol extract and compound 2 showed a better activity. The basic principle of the assay was the reduction of

ABTS•+, a blue chromophore produced by the reaction between ABTS and potassium persulfate, to ABTS on a concentration dependant manner upon addition of any antioxidant. DPPH scavenging activity, is a complementary assay for accessing the total antioxidant capacity and is displayed by all the four test samples. Linearity of FRAP (dose response line) for standard solutions is shown in Figure no.9. The results of FRAP are reported on Table 6. The antioxidant activities were expressed as the concentrations of antioxidant having a ferric reducing ability equivalent to that of 1mM of $FeSO_4$. The NO scavenging activity of two extracts and isolated compounds was estimated in a diazotization reaction. The calibration curve for sodium nitrite (10-70 mM) was used to calculate the NO scavenging activity of samples, which was expressed as equivalent to mM of sodium nitrite. Among the two extracts, the methanol extract showed the maximum NO scavenging activity, which was equivalent to 4.75 mM of sodium nitrite. The NO scavenging activity of compound 1 and compound 2 were equivalent to 0.7 and 1.44 mM of sodium nitrite respectively. In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease in absorbance at 560 nm with antioxidants thus indicate the consumption of superoxide anions in the reaction mixture. Addition of extracts and compounds in the above reaction showed decrease in absorbance indicating their capability for scavenging superoxide anions. In order to determine if the extracts were capable of reducing in vitro oxidative stress, the traditional lipid peroxidation assay that determines the production of malondialdehyde and related lipid peroxides in mice liver homogenate was carried out. Thiobarbituric acid reactive substances are produced as by-products of lipid peroxidation- induced by the ferrous sulfate:ascorbate system. The $IC_{50}$ values for inhibitory effect of extracts and compounds against ferrous sulfate and ascorbic acid-induced lipid peroxidation is shown in table 6. Carrageenan-induced rat paw oedema is used widely as a working model of inflammation in the search for new anti-inflammatory drug. The anti inflammatory activity of chloroform, methanol extract and isolated compounds of *Derris brevipes* was evaluated by carrageenan-induced rat paw oedema method Table no. 2 and 3 shows the effect of extracts, compounds and standard drug as compared to carrageenan control at different hours in carrageenan-induced rat paw oedema model. The anti-inflammatory activity was found to be dose dependent for both the extracts and compounds. The extracts and compounds were tested at two different dose levels. The results show that the methanol extract with a dose of 500 mg/kg body weight showed 63.66% inhibition of inflammation at 1st hour and compound 2 at its medium dose is having antiinflammatory activity equivaent to the standard Ibuprofen.

**Table 1:** Result for Preliminary phytochemical evaluation of extract of ariel parts

| S. No | Phytochemical constituents | Chloroform extract | Methanol extract |
|---|---|---|---|
| 1 | Alkaloids | - | - |
| 2 | Sterols | | - |
| 3 | Glycosides | | - |
| 4 | Fixed oils and fats | | - |
| 5 | Phenolic compounds | | |
| 6 | Tannins | - | - |
| 7 | Gum and mucilage | - | - |
| 8 | Flavonoids | - | |
| 9 | Carbohydrates | - | - |
| 10 | Proteins & amino acids | | - |

| | | | |
|---|---|---|---|
| 11 | Saponins | - | - |

**Table 2:** % Inhibition of inflammation for Ibuprofen, Chloroform and Methanol extract

| S. No | Drug | Dose | % Inhibition of inflammation | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 hr ± SEM | 2 hr ± SEM | 3 hr ± SEM | 4 hr ± SEM | 5 hr ± SEM | 6 hr ± SEM |
| 2 | Ibuprofen | 20mg/kg | 68.08±1.987 | 63.34±5.356 | 65.84± 3.113 | 60.63± 5.055 | 53.23± 7.813 | 57.07±5.113 |
| 3 | Chloroform extract (low dose) | 250mg/kg | 52.35±3.811 | 30.36±1.882 | 28.88±1.8775 | 9.5± 2.613 | 12.24±2.114 | 16.34±1.443 |
| 4 | Chloroform extract (High dose) | 500 mg/kg | 61.91±5.77 | 40.13±6.621 | 49.77± 4.310 | 36.62±7.179 | 42.24±5.689 | 42.23±5.633 |
| 5 | Methanol extract (low dose) | 250 mg/kg | 46.41± 3.337 | 44.03±4.040 | 36.22±4.983 | 22.96±4.800 | 28.27±2.503 | 27.16±1.225 |
| 6 | Methanol extract (High dose) | 500 mg/kg | 63.66±5.946 | 48.46±4.870 | 44.42± 4.689 | 46.07±7.246 | 41.32±5.180 | 52.99±3.991 |

**Table 3:** % Inhibition of inflammation for Ibuprofen, compound 1 (2',4'-dihydroxy-4-methoxy-3'-prenyl chalcone) and compound 2 (Leutolin)

| S. No | Drug | Dose | % Inhibition of inflammation | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 hr± SEM | 2 hr ± SEM | 3 hr ± SEM | 4 hr ± SEM | 5 hr ± SEM | 6 hr ± SEM |
| 2 | Ibuprofen | 20mg/kg | 68.08 ± 1.987 | 63.34 ± 5.356 | 65.84 ± 3.113 | 60.63 ± 5.055 | 53.23 ± 7.813 | 57.07 ± 5.113 |
| 3 | Compound 1 low dose | 10 mg/kg | 57.69 ± 3.483 | 51.57 ± 4.661 | 53.74 ± 6.521 | 48.69 ± 3.723 | 44.61 ± 5.703 | 47.82 ± 1.503 |
| 4 | Compound 1 Medium dose | 20 mg/kg | 62.31 ± 4.223 | 56.17 ± 2.367 | 58.37 ± 6.713 | 51.84 ± 4.957 | 48.31 ± 2.608 | 52.68 ± 4.213 |
| 5 | Compound 2 low dose | 10 mg/kg | 61.67 ± 5.613 | 55.12 ± 2.576 | 57.38 ± 4.213 | 51.08 ± 3.283 | 47.11 ± 6.213 | 51.57 ± 2.687 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 6 | Compound 2 Medium dose | 20 mg/kg | 65.18 ± 3.317 | 59.63 ± 4.301 | 61.57 ± 4.783 | 56.13 ± 4.723 | 51.5 ± 3.593 | 56.01 ± 2.645 |

**Table 4:** Trolox equivalent antioxidant capacity for ABTS assay of extracts

| **Extract** | **Concentration (µg/ml)** | **TEAC value (µg/ml)** | **Extract** | **Concentration (µg/ml)** | **TEAC value (µg/ml)** |
|---|---|---|---|---|---|
| Chloroform extract | 250 | 1.67 | Methanol extract | 250 | 2.48 |
| | 500 | 1.71 | | 500 | 2.64 |
| | 750 | 2.26 | | 750 | 3.13 |
| | 1000 | 3.05 | | 1000 | 3.74 |
| | 1250 | 3.52 | | 1250 | 4.0 |

**Table 5:** Trolox equivalent antioxidant capacity for ABTS assay of compounds

| **Compound** | **Conc.(µg/ml)** | **TEAC value (µg/ml)** | **Compound** | **Conc.(µg/ml)** | **TEAC value (µg/ml)** |
|---|---|---|---|---|---|
| Compound 1 | 50 | 1.03 | Compound 2 | 50 | 1.09 |
| | 100 | 1.41 | | 100 | 2.17 |
| | 150 | 1.76 | | 150 | 2.56 |
| | 200 | 2.63 | | 200 | 3.26 |
| | 250 | 3.66 | | 250 | 3.95 |

**Table 6:** Antioxidant activity of extracts and compounds using FRAP assay

| **Extract** | **Concentration of antioxidant activity sample equivalent to 1mM of $FeSO_4$.µg/ml (Mean ± SD)** |
|---|---|
| Chloroform extract | 1921 ± 1.961 |
| Methanol extract | 1788 ± 1.63 |
| Compound 1 | 842 ± 2.75 |
| Compound 2 | 778 ± 1.19 |

**Table 7:** Nitrous oxide radical scavenging assay for extracts

| Extract | Concentration (µg/ml) | sodium nitrite equivalent value (mM) | Extract | Concentration (µg/ml) | sodium nitrite equivalent value(mM) |
|---|---|---|---|---|---|
| Chloroform extract | 250 | .07 | Methanol extract | 250 | .60 |
| | 500 | 0.72 | | 500 | 1.64 |
| | 750 | 1.39 | | 750 | 2.65 |
| | 1000 | 2.0 | | 1000 | 3.73 |
| | 1250 | 3.01 | | 1250 | 4.75 |

**Table 8:** Nitrous oxide radical scavenging assay for compounds

| Compound | Conc.(µg/ml) | sodium nitrite equivalent value | Compound | Conc.(µg/ml) | sodium nitrite equivalent value |
|---|---|---|---|---|---|
| Compound 1 | 50 | 0.149 | Compound 2 | 50 | 0.102 |
| | 100 | 0.293 | | 100 | 0.372 |
| | 150 | 0.402 | | 150 | 0.926 |
| | 200 | 0.720 | | 200 | 1.44 |
| | 250 | 1.07 | | 250 | 1.94 |

**Table 9:** $IC_{50}$ values for antioxidant activity

| Activity | Extract/compound/standard | $IC_{50}$(*) value µg/ml |
|---|---|---|
| Thiobarbituric acid method | Chloroform extract | 1558 |
| | Methanol extract | 1340 |
| | Compound 1 | 167.5 |
| | Compound 2 | 143 |
| | Vitamin E | 42 |

| | | |
|---|---|---|
| DPPH radical scavenging assay | Chloroform extract<br>Methanol extract<br>Compound 1<br>Compound 2<br>Ascorbic acid | 175<br>167.5<br>335<br>210<br>17 |
| Superoxide radical scavenging assay | Chloroform extract<br>Methanol extract<br>Compound 1<br>Compound 2<br>Ascorbic acid | 210.15<br>173.61<br>81<br>49<br>12 |

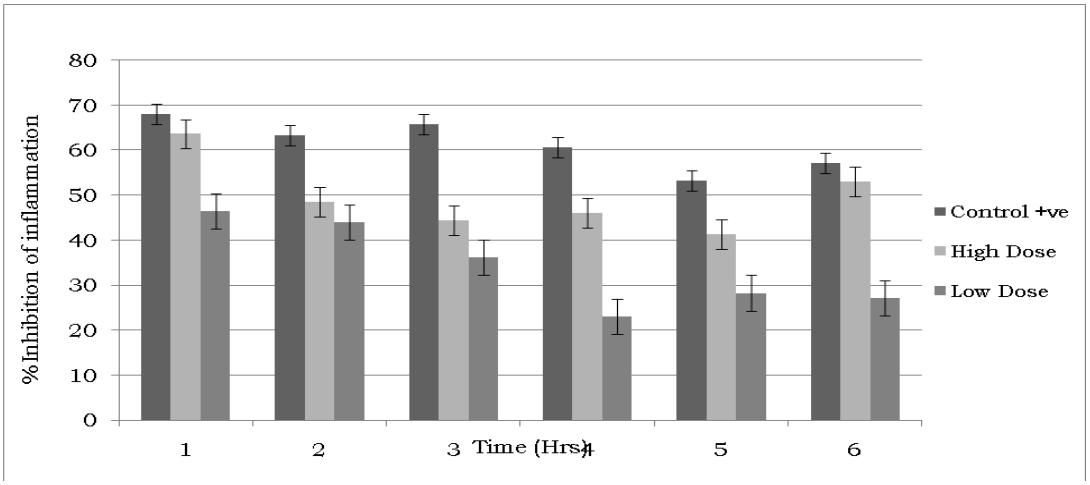



**Figure 2:** % Inhibition of Inflammation by Positive control (Ibuprofen), Low and High dose of chloroform extract

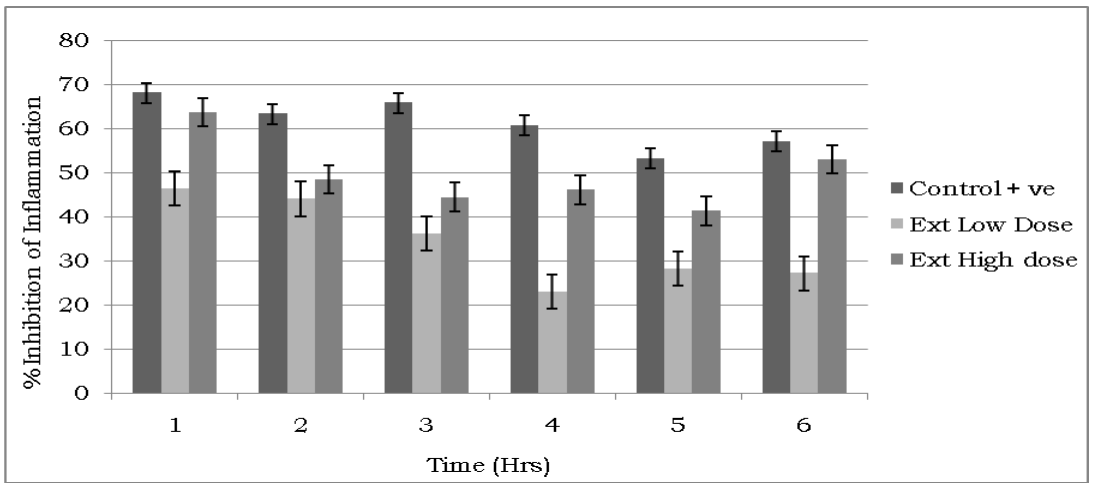



**Figure 3:** % Inhibition of Inflammation by Positive control (Ibuprofen), Low and High dose of methanol extract

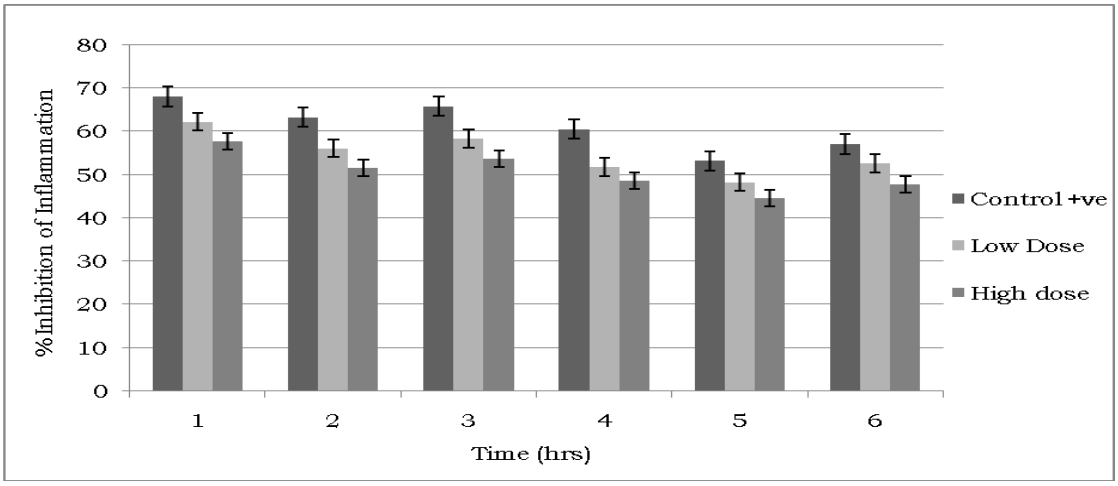



**Figure 4:** % Inhibition of Inflammation by Positive control (Ibuprofen), Low and Medium dose of Compound 1

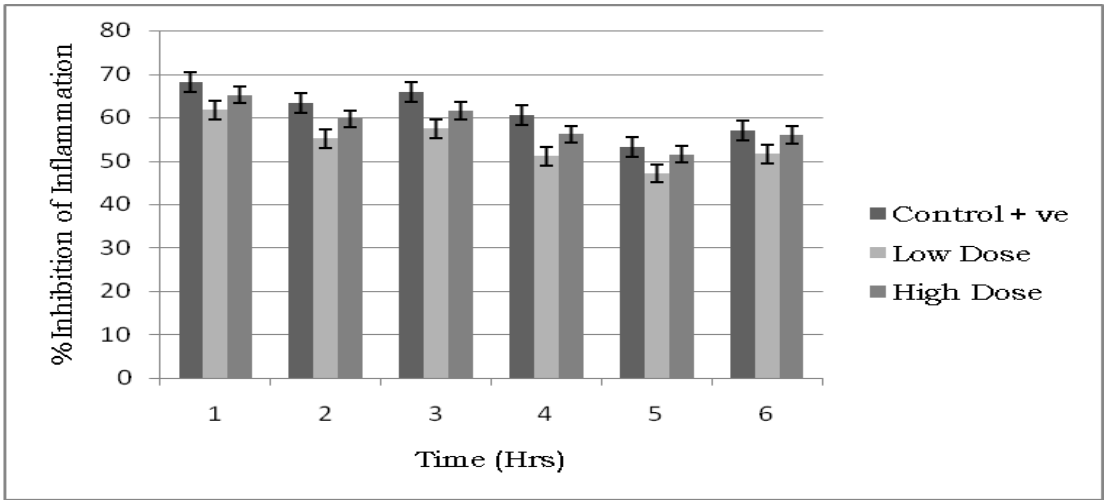



**Figure 5:** % Inhibition of Inflammation by Positive control (Ibuprofen), Low and Medium dose of Compound 2

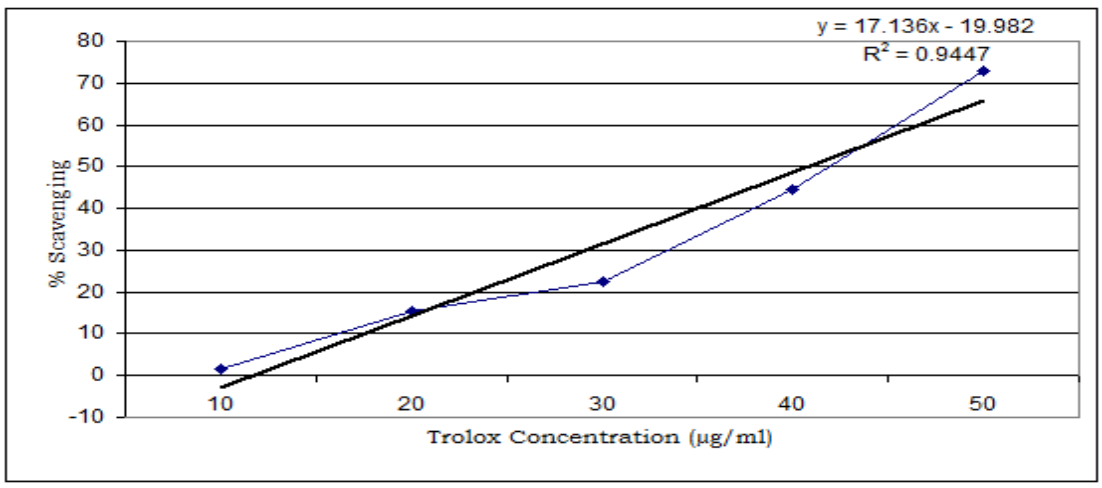



**Figure 6 :** Trolox standard calibration curve for ABTS method.

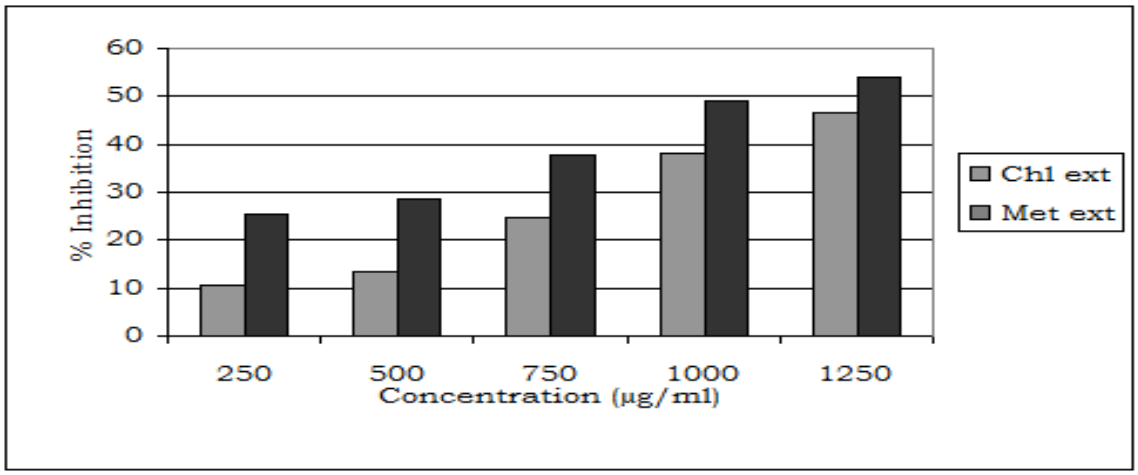



**Figure 7:** % Inhibition of chloroform and methanol extract for ABTS method

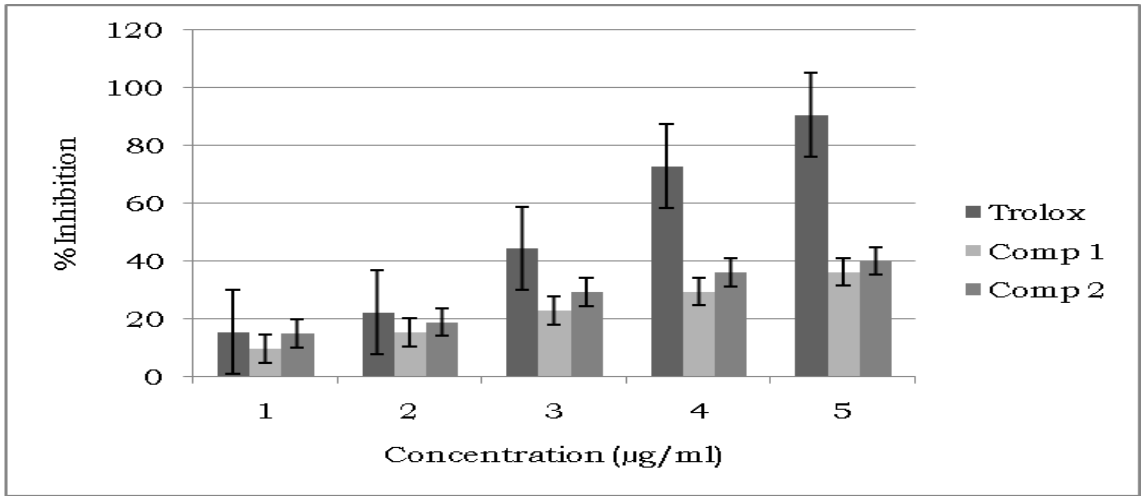



**Figure 8:** % Inhibition of Compound 1 and Compound 2 for ABTS method

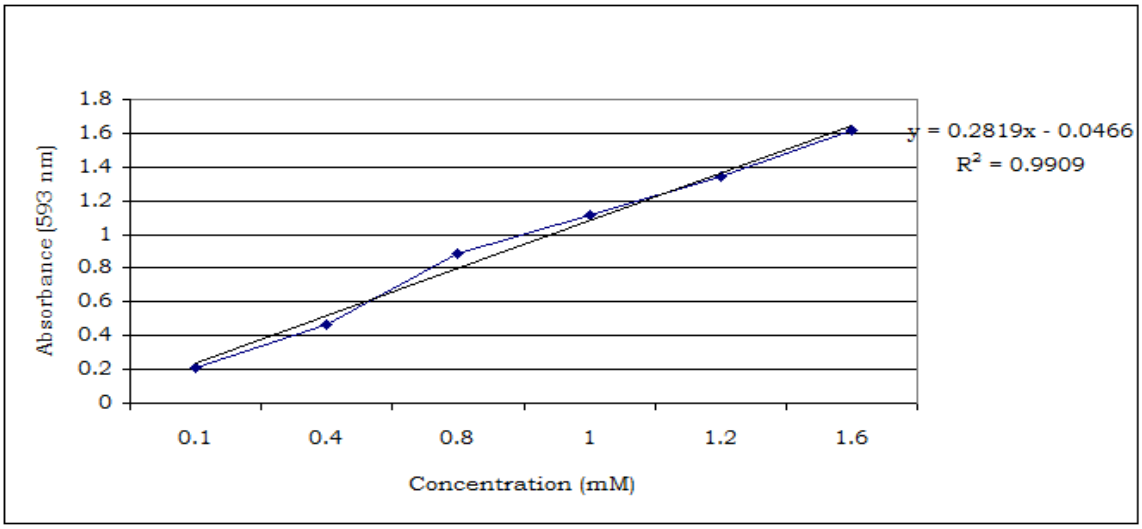



**Figure 9:** Standard curve of ferrous sulphate in FRAP assay

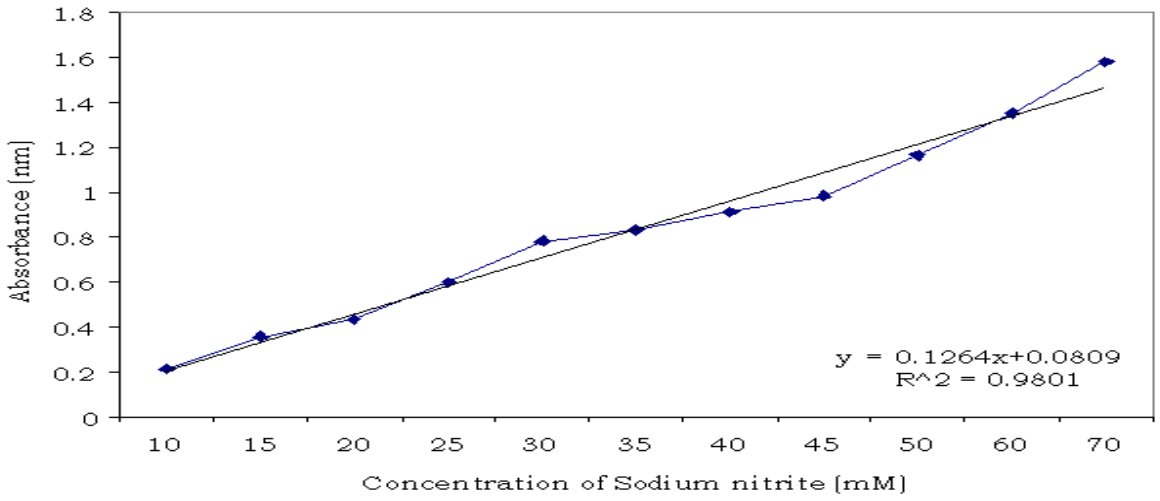



**Figure 10:** Standard curve of sodium nitrite in nitrous oxide radical scavenging assay

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