INTRODUCTION
Aristolochia bracteata is a perennial herb. This plant belongs to the family Aristolochiaceae. This species which had been shown to be nephrotoxic, mutagenic and carcinogenic due to the cytotoxicity of the Aristolochic acid constituents. The leaves of the plant which are used by native tribal and the villagers. It is commonly called as worm killer in English and adaaduthendapaalai in Tamil. It is used in traditional medicines as a gastric stimulant and in the treatment of cancer, lung inflammation, dysentery and snakebites. Methanolic extract of plant parts of Aristolochia bracteata were the source of physiological active compounds. The use of A. bracteata as an anti malarial plant is not recommended in its crude form. The whole plant was used as a purgative, anti pyretic and anti inflammatory. The plant contain Aristolochic acid has many medicinal properties in various disease condition. The phytochemical screening revealed the presence of alkaloids, triterpenoids, steroids and sterols, flavanoids, tannins and phenolic compounds and carboxylic acids. The root part was used to treat syphilis, gonorrhea and also used during labours to increase uterine contraction. The plant produced diverse range of bioactive molecules making them a rich source of different types of medicine. The important property of the plant that inhibits the microorganism and is also important for the human health. This plant is the source of natural antioxidant and some of the compounds have significant antioxidant property. It also has insecticidal properties. The plant has shown anti plasmodial, antibacterial, insecticidal, anti inflammatory and analgesic activity.

MATERIALS AND METHODS

1. PLANT MATERIAL COLLECTION:
The plant part (root, stem and leaf) of Aristolochia bracteata was collected from Palani, Tamilnadu, India and the plant material air dried, powdered & stored in deep freezer at -20°C.

2. SOLVENT EXTRACTION (HARBONE 1988)
The powdered plant material 10 gm was extracted with 100ml of methanol in a shaker for 72 hrs. The extract was concentrated to remove the solvent & filter through whatman N01 filter paper. The clean extract was used for preliminary screening for bioactive compounds

3. PRELIMINARY SCREENING
The presence of bio active compounds was screened for alkaloids, flavanoids, terpenoids, saponins, glycosides and tannins.

4. CONFIRMATION OF ALKOLOIDS BY UV AND TLC ANALYSIS
The presence of alkaloids was confirmed by UV and TLC analysis.

4.1. UV ANALYSIS
The sample was run on a chromatogram paper and was confirmed by viewing under UV light.

4.2. TLC ANALYSIS
Presence of alkaloids was analyzed using TLC by spraying the TLC plate with Dragendorff reagent.

5. HPTLC ANALYSIS FOR ALKALOID PROFILE (Dalmia Research Centre, Coimbatore)

5.1 SAMPLES GIVEN

<table>
<thead>
<tr>
<th>Aristolochia bracteata</th>
<th>methanolic extract of stem-E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>methanol extract of root-F</td>
</tr>
</tbody>
</table>

5.2 PROCEDURE
Orange brown coloured zones were shown in all sample tracks at day light mode, it was observed from the given extracts were used as test solutions for HPTLC profile analysis.

5.3. SAMPLE AND REFERENCE STANDARD APPLICATION
0.5µl of each test solutions and 5µl of standard solution were loaded as 6mm band length in the 9 x 10 Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

5.4. SPOT DEVELOPMENT
The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (Alkaloid) and the plate was developed in the respective mobile phase up to 90mm.

5.5. PHOTO- DOCUMENTATION
The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254nm and UV366nm.

5.6. DERIVATIZATION
The developed plate was sprayed with respective spray reagent (Alkaloid) and dried at 120°C in Hot air oven. The plate was photo-documented in Daylight and UV366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber.

5.7. SCANNING
Before derivatization, the plate was fixed in scanner stage and scanning was done at 366nm. The Peak table, Peak display and Peak densitogram were noted.

5.8. ANALYSIS DETAILS

MOBILE PHASE
N-Butanol-acetic acid-water (4: 4: 1)

SPRAY REAGENT
Dragendorff reagent followed with10% ethanolic sulphuric acid

6. ANTIOXIDANT ACTIVITY:
The assay of superoxide dismutase was done according to the procedure of Das et al. (16). In this method 1.4ml aliquots of the reaction mixture (comprising 1.11ml of 50 mM phosphate buffer of pH 7.4, 0.0075 ml of 20mM L-Methionine,0.04ml of 1% (v/v) Triton x-100,0.075 ml of 10mM Hydroxylamine hydrochloride and 0.1 ml of 50mM EDTA) was added to 100µl of the sample extract and incubated at 30°C for 5 minutes. 80µl of 50mM riboflavin was then added and the tubes were exposed for 10 min to 200 W-Philips fluorescent lamps. After the exposure time, 1ml of Greiss reagent (mixture of equal volume of 1% sulphamylamide in 5% phosphoric acid) was added and the absorbance of the colour formed was measured at 540 nm. One unit of enzyme activity was measured as the amount of SOD capable of inhibiting 50% of nitrite formation under assay conditions.

6.1a. ASSAY OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY
The enzyme superoxide dismutase was assayed using the method of Wetzler (17). The enzyme extract (0.5 ml) was
added to the reaction mixture containing 1ml of 0.01 M phosphate buffer (pH-7.0), 0.5 ml of (0.2 M Hydrogen peroxide, 0.4 mL water and incubated for different time period. The reaction was terminated by the addition of 2ml of acid reagent (dichloromethane-acetic acid mixture) which was prepared by mixing 5% potassium dichromate with glacial acetic acid (1:3 by volume). To control the enzyme the assay was added after addition of acid reagent. All the tubes were heated for 10 minutes and the absorbance was read at 610nm. Catalase activity was expressed in terms of μmoles of hydrogen peroxide consumed/min/mg protein.

6. i.e. ASSAY OF PEROXIDASE ACTIVITY:
The assay was carried out by the method of Addy and Goodman (18). The reaction mixture consisted of 3ml of buffered pyrogallol (0.005 M pyrogallol in 0.1M phosphate buffer (pH7.0) and 0.5ml of 1%hydrogen peroxide. To this added 0.1ml enzymes extracted and O.D change was measured at 430nm for every 30 seconds for 2 minutes. The peroxidase activity was calculated using an extinction coefficient of oxidized pyrogallol (4.5 liters/mmol).

6. i.d. ASSAY OF ASCORBATE OXIDASE ACTIVITY:
Assay of ascorbate oxidase activity was carried out according to the procedure of Vines and Oberbacher (20). The sample was homogenized (1:5 w/v) with phosphate buffer (0.1 M pH 6.5) and centrifuged at 3000g for 15 mins at 5°C. The supernatant obtained was used as enzyme source. To 3.0ml of the substrate (8.8mg ascorbic acid in 300 ml phosphate buffer, PH 3.6)0.1ml of the enzymes extract was added and the absorbance change at 265nm was measured for every 30-second for a period of 5 minutes. One enzyme unit is equivalent to 0.01 O.D. changes per minute.

6. i.e. ASSAY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY:
The enzyme was assayed by the method of Balinsky and Bernstein (21). The solution containing 0.1ml each of 0.1M Tris-HCl BUFFER pH 8.2, 0.2 M NADP and 0.1M MgCl was taken in a cuvette along with 0.5ml of water and suitable aliquots of enzyme extract. The reaction was initiated by the addition of 0.1ml of 6mM glucose-6-phosphate and O.D. increase was measured at 340nm. The activity of the enzymes is expressed in terms of units/mg protein, in which one unit is equal to the amount of the enzymes that brought about an increase in O.D. of 0.01/min.

ii) NON ENZYMATIC ASSAYS

6. ii.a SCAVENGING ACTIVITY OF DPPH
Scavenging activity of anthocyanins against DPPH radicals was assessed according to the method of Keyes, Sanches-Moreno, and Saura-Calixto (1998) with some modifications. Briefly, 0.1 mM DPPH-methanol solution was mixed with 1ml of 0.1mM DPPH methanol solution. After the solution was incubated for 30 min at 25°C in dark, the decrease in the absorbance at 517 nm was measured. Control contained methanol instead of DPPH solution in the experiment, while blanks contained methanol instead of antioxidant solution. The scavenging activity of anthocyanins was assessed according to the following equation:

DPPH-scavenging activity (%) = (1 - (absorbance of the sample-scavenging of blank))/ absorbance of the control × 100

6. ii.b. DETE RMINATION OF SUPEROXIDE RADICAL SCAVANGING ACTIVITY
Superoxide radicals were generated by the method of Ginnopolites and Ries (1977), described in Larrauri, Sanchez-Moreno, and Saura-Calixto (1998) with some modifications. The reaction mixture, without sample, was used as a control. The scavenging activity was calculated as follows:

Scavenging activity (%) = (1 - (absorbance of the sample-scavenging of blank))/ absorbance of the control × 100

6. ii.e. DETE RMINATION OF REDUCING POWER
The reducing power was determined according to the method of Oyaizu (1986). A 0.25ml aliquot of various concentrations of anthocyanins was mixed with 2.5 ml of 200mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50°C for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v) were added; the mixture was centrifuged at 650g for 10 min. A 5ml aliquot of the upper layer was mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride at 700nm was measured. A higher absorbance indicates a higher reducing power.

6. ii.d. HYDROXYL RADICAL SAC VANGING ACTIVITY
The hydroxyl radical scavenging activity was determined according to the methods described by Singh, Misra, and Jyoti Kaur (2005). 0.1 ml of different extracts of anthocyanins samples extract was taken in different test tubes. 1.0 ml of iron-EDTA solution (0.1% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of DMSO (0.85% v/v in 0.1 M Phosphate buffer, pH 7.4) were added to these tubes, and the reaction was initiated by adding 0.5 ml of 0.22% ascorbic acid. Test tubes were capped tightly and heated on a water bath at 80-90°C for 15 min. The reaction was terminated by the addition of 1ml of ice cold TCA (17.5 %w/v), 3 ml of Nash reagent (75 g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetic acetone were mixed and measured at 340nm) and all of the tubs and left at room temperature for 15 min for the color development. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against the reagent blank. The percentage of hydroxyl radical scavenging activity is calculated by using the formula:

% hydroxyl radical scavenging activity = 1 - (absorbance of sample-absorbance of blank) × 100

6. ii.e. TOTAL PHENOLIC ASSAY
Total phenolic compounds in antioxidant samples were quantified by using Folinicciocalteau’s method. 50 µl of Folinciocalteau’s reagent (50% V/V) was added to 50 µl of sample extract. It was incubated for five minutes. After incubation 50 µl of 20 % (W/V) sodium carbonate and water was added to a final volume of 200 µl. Blank was prepared by replacing the reagent by water to correct for interfering compounds. After 30 minutes incubation the absorbance was measured using spectrophotometer at 760nm.

6. ii.f. QUANTIFICATION OF ASCORBIC ACID
This assay was carried out by the method of Sadassivam and Manickam (1997). 0.1 ml of bромinated sample extract was added with 2.9 ml of distilled water. Then 1 ml of 2% DPPH reagent and 1-2 drops of Thourera was added with sample. After incubation at 37°C for 3 hours, the range-red osazone crystals that were formed were dissolved by the addition of 7 ml of 80% Sulphuric acid. Again incubated for 5 minutes. After incubation absorbance was measured at 450 nm. Vitamin C concentration was expressed in terms of mg/g of sample.

7. RESULT AND DISCUSSION

7.1. PRELIMINARY SCREENING FOR BIO-ACTIVE COMPOUNDS
On preliminary screening the methanol extracts of Aristolochia bracteata (leaf, root, and stem) showed the presence of alkaloids, flavonoids, steroids, triterpenoids, phenols, cardio glycosides and anthraquinones like compounds.

7.1. A. PRESENCE OF ALKALOIDS
Two ml of extract was treated with the following to test the presence or absence of alkaloids.

DRAGNEDROFF’S REAGENT
The presence of alkaloid was confirmed by indicating orange colour precipitate

MAYER’S REAGENT
The presence of alkaloid was confirmed by indicating white precipitate or turbidity

WAGNER’S REAGENT
The presence of alkaloid was confirmed by indicating yellow to orange colouration.

7.1. B. PRESENCE OF FLAVONOIDS

ASHINODA TEST
Pink or red colour shows the presence of flavonoids

b. Formation of brown colour confirms the presence of flavonoids

7.1. C. TEST FOR STEROLS AND STEROIDS

a. SALKOWSKI’S TEST
The upper layer turns red reveals presence of sterol and steroids compound in the extract.

b. LIBERMAN-BURCHARD TEST:
Appearance of red to violet color indicates the presence of triterpenoids

7.1. D. TEST FOR TRITERPENOIDS:

a. LIBERMAN-BURCHARD TEST:
Appearance of red to violet color indicates the presence of triterpenoids

7.2. TEST FOR CARBOHYDRATES

a. SALKOWSKI’S TEST
Presence of reddish brown color at the interface indicates the presence of cardiolglycosides).

7.1. E. TEST FOR ANTHROQUINONES
Presence of red or violet color in the lower phase indicates the presence of free anthroquiones.

b. Development of red or violet color in the lower phase indicates the presence of anthroquione derivatives in the extract.

7.1. F. TEST FOR PHLOBATANNINS:
Deposition of a red precipitate taken as a evidence for the presence of phlobatannins.

7.2. COFORMATION OF ALKALOIDS BY UV AND TLC ANALYSIS

7.2.1. UV ANALYSIS
The fluorescence bands were also observed when the chromatogram was viewed under UV light.

7.2.2. TLC ANALYSIS OF ALKALOIDS

b. The presence of alkaloids, flavonoids, steroids, triterpenoids, phenols, cardio glycosides and anthraquinones like compounds.
7.2.3. HPTLC ANALYSIS FOR ALKALOID PROFILE DETECTION:
Bright orange color zone presence in sample for various Rf value in the chromatograph and while like after derivatization (peak table 1), Which belongs to alkaloid class compounds present in the given sample.

HPTLC:
From peak table 1 Rf value, height, area, and compound were determined. Track E (Aristolochia bracteata) has an alkaloid compound with Rf values of 0.71. Track F (Aristolochia bracteata methanol extract of root) has two alkaloid compound 0.3 to 0.70.

DETECTION
Chromatograms after derivatization, which confirmed the Presence of Alkaloid in the given sample. Bright orange colored zones were present in sample E and F at various Rf values in the chromatogram at White light after derivatization (peak table 1), which belongs to alkaloid class compounds present in the given sample. From the standard Nicotine track NCT, it was confirmed that Nicotine is present in Sample E and F with other alkaloids. (Fig 1) From peak table 1 given and from the graph the peak, Rf value, area and substance are determined. Track E and F (Aristolochia bracteata stem and root) has Nicotine with the Rf value of 0.67.

HPTLC analysis and quantation was carried out at an UV 254nm

Table 1: Showing the enzymatic and nonenzymatic antioxidant activity of Aristolochia bracteata

<table>
<thead>
<tr>
<th>Enzymatic Assays</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Super oxide dismutase</td>
<td>4U/ gm tissue</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>4U/ gm tissue</td>
</tr>
<tr>
<td>Ascorbate oxidase</td>
<td>24U/ gm tissue</td>
</tr>
<tr>
<td>Catalase</td>
<td>36U/ gm tissue</td>
</tr>
<tr>
<td>Glucose – 6 – phosphate dehydrogenase</td>
<td>113U/gm tissue</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non enzymatic assays</th>
<th>Samples</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing power</td>
<td>Stem</td>
<td>0.277</td>
</tr>
<tr>
<td></td>
<td>leaf</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>0.426</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>Stem</td>
<td>0.48mg</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>0.108</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>0.326</td>
</tr>
<tr>
<td>Super oxide radical scavenging activity</td>
<td>Stem</td>
<td>85.13</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>66.86</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>70.12</td>
</tr>
<tr>
<td>Total phenolic assay</td>
<td>Stem</td>
<td>2.78</td>
</tr>
<tr>
<td></td>
<td>leaf</td>
<td>3.71</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>3.78</td>
</tr>
<tr>
<td>DPPH assay(%)</td>
<td>Stem</td>
<td>60</td>
</tr>
<tr>
<td>Hydroxyl radical scavenging activity(%)</td>
<td>Leaf</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>92</td>
</tr>
</tbody>
</table>

Peak Table I HPTLC Analysis

<table>
<thead>
<tr>
<th>Track</th>
<th>Peak</th>
<th>Rf</th>
<th>Height</th>
<th>Area</th>
<th>Assigned substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>1</td>
<td>0.02</td>
<td>16.4</td>
<td>105.7</td>
<td>Unknown</td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td>0.10</td>
<td>16.4</td>
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<tr>
<td>E</td>
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<td>34.4</td>
<td>532.1</td>
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<tr>
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<td>47.5</td>
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</tr>
<tr>
<td>E</td>
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<td>66.6</td>
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<tr>
<td>E</td>
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<td>0.40</td>
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<td>E</td>
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<td>117.3</td>
<td>6703.4</td>
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</tr>
<tr>
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<td>158.9</td>
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</tr>
<tr>
<td>E</td>
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<td>0.71</td>
<td>220.8</td>
<td>12462.2</td>
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<tr>
<td>E</td>
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<td>0.91</td>
<td>76.6</td>
<td>4822.2</td>
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<td>681.1</td>
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<tr>
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<tr>
<td>F</td>
<td>4</td>
<td>0.32</td>
<td>29.5</td>
<td>614.5</td>
<td>Alkaloid 1</td>
</tr>
<tr>
<td>F</td>
<td>5</td>
<td>0.40</td>
<td>33.1</td>
<td>1212.4</td>
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<tr>
<td>F</td>
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<td>0.54</td>
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<tr>
<td>F</td>
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<tr>
<td>F</td>
<td>8</td>
<td>0.70</td>
<td>53.6</td>
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<td>0.71</td>
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<td>0.90</td>
<td>58.0</td>
<td>3207.0</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

7.3. b.CATALASE ACTIVITY:
The catalase activity was observed to be maximum in the Aristolochia bracteata (36U/g tissue). The activity of this enzyme is to degrade H2O2 to non toxic form. So in our results the activity of the enzyme was higher in the Aristolochia bracteata. Which detoxify more H2O2 molecule. It was reported that in plants the antioxidant enzymes namely catalase have been shown to increase when subjected to stress condition (Hertwig et al 1999, Mouci 1993), Aristolochia bracteata showed a definite, positive effect on wound healing with a significant increases in the level of two powerful antioxidant enzymes superoxide dismutase and catalyses the one electron disputation of superoxide into hydrogen peroxide and oxygen (Fridovich 1997)

7.3. c.SUPEROXIDE DISMUTASE ACTIVITY:
The superoxide dismutase activity was observed to be mini ascorbate oxidase mum in Aristolochia bracteata (40U/g tissue.)Rani et al(2004) reported that the superoxide dismutase activity was maximum activity may be due to the total phenol content. So the SOD activity is higher which means they can scavenge more superoxide ion.

7.3. d.ASCORBATE OXIDASE:
The ascorbate oxidase activity in Aristolochia bracteata (24.70g tissue) which means they have higher capacity to degrade ascorbate. Glucose-6- phosphate dehydrogenase was also higher. Since NADPH is an ultimate reducing equivalent utilized by dehydro ascorbate reductase to maintain the balance between dehydroascorbate and ascorbate through GHG-GSSG system, the increase in ascorbate oxidase activity in orange has been compensated with increase in glucose 6 phosphate dehydrogenase activity in the same which is correlating with our results (Rani et al, 2004).

7.3. e.GLUCOSE – 6 - PHOSPHATE DEHYDOGENASE:
Glucose-6- phosphate dehydrogenase (14 U/g tissues) was also higher. Since NADPH is an ultimate reducing equivalent utilized by dehydro ascorbate reductase to maintain the balance between dehydro ascorbate and ascorbate through GHG-GSSG system, the increase in ascorbate oxidase activity in orange has been compensated with increase in glucose 6 phosphate dehydrogenase activity in orange which is correlating with our results(Rani et al, 2004).

7.3. f. PEROXIDASE ACTIVITY
The peroxidase activity was higher than ascorbate oxidase in Aristolochia bracteata (3.173U/g tissue) Table 1. The activity has been shown increased when subjected to stress conditions.


7.3. h. NON ENZYMATIC ANTIOXIDANTS:
7.3. 1. REDUCING POWER
It has been reported that reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity (Chan Yen, Huang &Duh, 2002; Yen &Duh, 1993). Aristolochia bracteata exhibited a higher reducing power, suggesting that the antioxidant had strong electron-donating capacity

7.3. 2. DPPH RADICAL-SCAVENGING ACTIVITY:
Thirunagaran sampandan et al reported that anti oxidant activity may be affected by different parameter such as and amount of phenolic compounds. There is a Correlation between antioxidant and DPPH activity Aristolochia bracteata antioxidant investigation of the methanol extract along with its two successive fractions using nitric oxide and DPPH induced free radical assay method showed good free radical scavenging activity (sharmaukar and somashkar 2003).

Yildiz et al(2001) have suggested that there may be relationship between phenolic compound and reducing powers. Presence of phenolic compound might be the reasons for reducing power.Aristolochia bracteata contain aristolochic acid and terpenoids. The antioxidant effect is
Fig-2 HPTLC Analysis Chromatogram Before derivatization

NCT – Nicotinic acid (Standard)
E INDICATING: Aristolochia bracteata stem
F INDICATING: Aristolochia bracteata root

Fig-3 HPTLC Analysis Chromatogram Before derivatization

NCT – Nicotinic acid (Standard)
E INDICATING: Aristolochia bracteata stem
F INDICATING: Aristolochia bracteata root

Fig-4 HPTLC Analysis Chromatogram after derivatization

NCT – Nicotinic acid (Standard)
E INDICATING: Aristolochia bracteata stem
F INDICATING: Aristolochia bracteata root

Fig-5 HPTLC Analysis Chromatogram after derivatization

NCT – Nicotinic acid (Standard)
E INDICATING: Aristolochia bracteata stem
F INDICATING: Aristolochia bracteata root

NCT – Nicotinic acid (Standard)
E INDICATING: Aristolochia bracteata stem
F INDICATING: Aristolochia bracteata root

Fig-6 HPTLC Analysis Chromatogram after derivatization

Fig-7 HPTLC Analysis Track E-Baseline display (Scanned at 366nm)

Fig-8 HPTLC Analysis Track E- Peak densitogram display

Fig-9: HPTLC Analysis Track F- Baseline display (Scanned at 366nm)

Fig-10: HPTLC Analysis Track F- Peak densitogram display

1-superoxide dismutase activity
2-peroxidase activity
3-ascorbate activity
4-catalase activity
5-glucose 6 phosphate dehydrogenase activity

Fig: 11: Antioxidant properties of Aristolochia bracteata by Enzymatic Analysis mainly due to phenolic compounds, such as phenolic acid and phenolic diterpenes (Shahidi et al 1992).

The antioxidant activity of phenolic compound is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radical, quenching single and triplet O2 or decomposing peroxides.

7.3 b.3 ASCORBIC ACID ASSAY
Vitamin c content was high in Aristolochia bracteata. Ascorbate has been found in chloroplast, vacuoles and extracellular compartments of plant cell and shown to function as a reluctant for many free radicals (Foyer, 1993).
1-Reducing power
2-Ascorbic acid
3-Superoxide radical scavenging activity
4-Total phenolic activity
5-DPPH activity
6-Hydroxyl radical scavenging activity

FIG: 11: Antioxidant properties of Aristolochia bracteata by non Enzymatic Analysis

7.3. b. SUPEROXIDE RADICAL-SCAVENGING ACTIVITY
Superoxide radical are produced by a number of cellular reactions, including various enzyme systems, such as Lipoygenases, Peroxidase, NADPH oxidase and Xanthine oxidase. Superoxide radical plays an important role in many cells and tissues and is involved in the formation of other cell-damaging free radicals (Blokuna et al. 2003). In the present study, superoxide radical was generated by illuminating a solution containing riboflavin. As with the reducing power and ascorbic acid Aristolochia bracteata exhibited an excellent super oxide radical scavenging activity, which was much higher than those of ascorbic acid. Further superoxide radicals also known to indirectly to initiate lipid per oxidation as result of H₂O₂ formation, creating precursors of hydroxyl radicals (Mayer and Isaksen, 1995). These results clearly suggest that the antioxidant activity of Aristolochia bracteata is also related to its ability to scavenge superoxide radical scavenging activity.

7.3.5. HYDROXYL RADICAL SCAVENGING ACTIVITY
Hydroxyl radical can be formed by the Fenton reaction in the presence of reduced transition metals, such as Fe²⁺ and H₂O₂, which is known to be the most reactive of all the reduced forms of dioxynogen and is thought to initiate cell damage. It is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every living cell. Thus is considered Aristolochia bracteata to be one of the quick initiators of the lipid per oxidation process, abstracting hydrogen atoms from unsaturated fatty acids (Kappas, 1999).

7.3.6. TOTAL PHENOLIC ASSAY
The total phenolic assay was found to contain medium level (3.34 mg/100ml) TableI in Aristolochia bracteata. The antioxidant activity of polymeric compounds is reported to be mainly due to their redox properties. (Galato et al.,2001).

8. CONCLUSION:
Recently, there has been an upsurge and interest in the therapeutic potential of medicinal plants as antioxidant in reducing such free radical induced tissue injury. Aristolochia bracteata is a potential source of antioxidant based on the results described we may concluded that methanol extract of A. bracteata possess significant free radical scavenging activity.

9. ACKNOWLEDGMENT
The authors would like to thank the staff members and friends of P.G. Department of Biotechnology, Nallamuthu Gounder Mahalingam College, Pollachi, Tamilnadu and Dalmia Ref. Technology, Nallamuthu Gounder Mahalingam College, Pollachi, Tamilnadu and Dalmia Ref. Technology for their kind cooperation for completing this work.

10. REFERENCE