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FINAL REPORT

Investigation of antioxidant activity of some medicinal plants used in traditional medicine for the treatment of cardiovascular diseases in Sri Lanka.
NSF (RG/2005/HS/10)

Section 1

Information regarding Project/Project Personnel:

i) Contract Number : NSF (RG/2005/HS/10)

ii) Title of the Project : Investigation of antioxidant activity of some medicinal plants used in traditional medicine for the treatment of cardiovascular diseases in Sri Lanka.

iii) Principal Investigator : Dr. M I F P Jayawardene, Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayawardenepura (Ex)

iv) Co-Investigators : Dr. N MV M Ruvin Kumara, Department of Biochemistry, Faculty of Medicine, Karapitiya, Galle (Ex).

v) Institute(s) where research was being carried out : University of Sri Jayawardenepura. India Institute of Technology Madras, Chennai.

vi) Date of award : 13/06/2005

vii) Date of completion of Project : Halted in 15/10/2009

viii) Total allocation of funds : (Rs) 447,000.00

ix) Total spent (Rs) : (Rs) 374,474.00

x) Number of Research Students employed : 01

xi) Post graduate degree completed with dates : Not completed.

xii) Number of Technical Assistants and/or labourers employed and period of service : None

xiii) Publications/Communications arising from the project during the reporting period

1. Ranatunga D N, Jayawardene M I F P and Ruvin Kumara N K V M In- vitro antioxidant activity of some Sri Lankan Medicinal plants used in Ayurveda as cardio- protective agents, International Conference Biomaterials in Regenerative Medicine, Polish Academy of Sciences, Scientific Centre, Vienna, 2006 Abstract p 96-97.

2. Ranatunga D N, Jayawardene M I F P and Ruvin Kumara N K V M and U G Chandrika Determination of in- vitro antioxidant activity by different methods of some Sri Lankan Medicinal plants used in Ayurveda as cardio-protective agents - 4th Joint Meeting Of Free Radical Research Australasia and Japan at Kyoto University Japan. Abstract p 66.

Section 2

Executive Summary of the Project:

The aim of the project was to determine the antioxidant activity of some medicinal plants used in traditional medicine for the treatment of cardiovascular diseases in Sri Lanka Flavanoids and phenols of plants are used in preparations of alternative medicines used in treatment for cardiovascular diseases and peripheral vascular diseases. Its believed that these the active ingredients of these plant extracts confer cardio-protective by anti

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oxidant properties Plant /plant parts of *Terminalia chebula* (Aralu), *Terminalia belerica* (Bulu), *Terminalia cattapa* (Kottamba), *Rauvolfia serpentia* (Ekaveriya), *Cyprus rotundus* (Kaladuru), *Osimum tenuiflorum* (Maduruthala), *Gmelina arborea*(Athdemata), *Alphinia calcarata* (Heen aratha), *Stereospermum suavelone* (Palol) *Azadiarachia indica* (Kohomba), *Ricinus communis* (Beheth endaru), *Orozylum indicum* (Thotila), *Munronia pinnata* (Binkohomba), *Rubia cordifolia* (Walmadata) were screened for antioxidant activity by DPPH free radical assay, hydrogen peroxide scavenging activity test, hydroxyl scavenging activity test, superoxide anion scavenging activity test and by determination of reducing power. Total phenolic compound analysis and determination of total flavonoid content was done. *Gemelina arborea, Ricinis communis and Terminalia chebula showed the maximum antioxidant activity in the above tests.*The Comet Assay for the detection of DNA fragmentation, cell cytotoxicity of the cells by MTT assay and DPPH quenching assay by Electron spin Resonance (ESR). Based on results of all the tests, *Terminalia chebula* (Aralu) fruit showed the best anti oxidant activity. Further it did not show a high cytotoxicity. *Terminalia chebula* should be studied further and active ingredients should be isolated and their structures elucidated. Such identification and quantification of antioxidants ~~in~~ will let researchers understand the nature of antioxidants in the medicinal plant/parts and could popularize the usage and /or lead to formation of drugs.

Section 3 : Report in detail:

i) Introduction/background

Research on traditional medicine is being revalued all over the world. For this reason at present extensive research is based on different plant species and their therapeutic principles.

Free radicals are molecules / atoms with unpaired electrons which are highly reactive. Free radicals are produced in the cells as by-products of normal oxidation. Most of the radicals are reactive oxygen species (ROS) formed during normal cell aerobic respiration in redox reactions involving xanthine oxidase, aldehyde oxidase, membrane associated NADPH oxidases and cytochrome P450system. (Gutteridge and Halliwell, 2000). Free radicals and ROS react with several biomolecules such as proteins, lipids and DNA and begin to form a chain reaction which could lead to aetiology of many diseases including cardio-vascular diseases. In the case of lipids, ROS cause lipid peroxidation. Lipids form an important part of the cell and many foods. The unsaturated sites of polyunsaturated fatty acids are easily attacked by free radicals. Low density lipoproteins (LDL) are oxidized to form atherosclerotic plaques, which are responsible for the development of cardiovascular disease (Halliwell, 1993; Frei, 1999). These reactions continue until free radicals are removed from the system. The generated free radical reacts with another free radical or when it reacts with a chain breaking or primary antioxidant.

Cardiovascular diseases remain as an important cause of death in developed and developing countries including Sri Lanka. Plants produce a lot of antioxidants to counter act oxidative stresses due to sunlight and oxygen. Research conducted in many parts of the world has shown that diets rich in fruits and vegetables protect against formation of peripheral diseases. Further there is evidence that different antioxidant agents in plants act as synergistic cooperative manner therefore using a mixture could be more beneficial than just using one.

Flavanoids and phenols of plants are used in preparations of alternative medicines used in treatment for cardiovascular diseases and peripheral vascular diseases.
It is believed that these the active ingredients of these plant extracts confer cardio-protective and anti oxidant properties.

ii) Scientific scope of the project (overall and specific objectives)

Antioxidants prevent free radicals from the cell environment which play an important role in modifying the development of such diseases. Living organisms have evolved complex defense systems to minimize the damaging effect of excessive exposure to endogenously and exogenously produced free radicals. Biological antioxidants inhibit oxidation of cellular components by directly scavenging reactive oxygen and nitrogen species by metabolizing lipid peroxides to non-radical products and by the chelation of metal ions to prevent generation of oxidizing species.

In Ayruveda, the Sri Lankan traditional health care system *Terminalia chebula* (Aralu), *Terminalia belerica* (Bulu), *Terminalia cattapa* (Kottamba), *Rauvolfia serpentia* (Ekaveriya), *Cyprus rotundus* (Kaladuru),*Osimum tenuiflorum* (Maduruthala), *Gmelina arbórea* (Athdemata), *Alphinia calcarata* (Heen aratha), *Stereospermum suavelone* (Palol) *Azadiarachia indica* (Kohomba), *Ricinus communis* (Beheth edaru), *Orozylum indicum* (Thotila), *Munronia pinnata* (Binkohomba), *Rubia cordifolia* (Walmadata) are used by practitioners as a preventive or theraputic use. Literature showed that the potential anti oxidant activity of these plants have not been undertaken. ?

General objective

To determine the antioxidant activity of some medicinal plants used in traditional medicine for the treatment of cardiovascular diseases in Sri Lanka.

Specific objectives

1. To determine the free radical removal activity.
2. To estimate the inhibition of microsomal lipid peroxidation
3. To estimate the ability to scavenge the hydroxyl radical.
4. To estimate H_2O_2 scavenging activity
5. *To estimate the in-vivo lipid peroxidation in the heart muscle. (Had to deviate from this as the student hesitated to work with animals due to personal issues cell culture test were introduced..*

iii) Materials and methods (including statistical methods)

Materials

Plant materials

In following plant materials were obtained from Bandaranayake Ayurvedic Research Institute. (Nawinna, Haldamulla, Pattipola Herbal gardens)
Terminalia chebula (Aralu), *Terminalia belerica* (Bulu), *Terminalia cattapa* (Kottamba), *Rauvolfia serpentia* (Ekaveriya), *Cyprus rotundus* (Kaladuru)
Osimum tenuiflorum (Maduruthala), *Gmelina arborea*(Athdemata)
Alphinia calcarata (Heen aratha), *Stereospermum suavelone* (Palol)
Azadiarachia indica (Kohomba), *Ricinus communis* (Beheth endaru)
Orozylum indicum (Thotila), *Munronia pinnata* (Binkohomba)
Rubia cordifolia (Walmadata)

The botanical identities of the plants were confirmed by the botanist at the Bandaranayake Ayurvedic research Institute. Voucher Specimens have been deposited the Bandaranayake Research Institute.
The plant materials were cut manually into 1 cm x 1 cm pieces and dried at 40^0C in an oven until constant weights were attained. Dried parts were stored in air tight polythene bags.

Chemicals
NaH_2PO_4, EDTA, NaOH, $FeCl_36H_2O$, sodium acetate trihydrate, L-ascorbic acid, potassium persulfate, cysteine, 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonate (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and gallic acid, Pyrogallol, Ethyl alcohol, glycine, acetone and Sulfuric acid, Folin-Coicalteu reagent, glacial acetic acid

Methods

1. **Preparation of crude extracts.**

For each plant, the dry plant material (10g) was extracted with 200ml water boiled under refluxed for 4hrs. Each extract was then filtered and reduced the volume by rota-evaporation. The aqueous extract freeze dried and the resulting powder stored at 4 ^{0}C.

2. **DPPH free radical assay**

Water extracts of the different parts above plants were used to test the total antioxidant activity by DPPH. Water extracts of the different parts above plants were used to test the antioxidant activity by DPPH (1,1Diphenylhydrayl).The DPPH Free Radical Assay was compared for each extract by using EC_{50} values. The extracts at the concentrations ranging from 2.5µg/ml to 12.5µg/ml, were mixed with 3.2 ml of 20mM DPPH in Methanol. The absorbance was measured at 517nm was monitored for 20 min.

3. **The Hydrogen peroxide scavenging Activity.**

Preparation of the methanolic extracts. Powdered samples of each plant placed in 10ml of methanol at 4 ^{0}C for 24 hrs. The solution was filtered concentrated by rota-evaporation. Hydrogen peroxide scavenging assay was done for each extract by a modified method of Halliwel (1991). The extract volume ranging from 40µl to 240µl dissolved in PBS (1.0 M, pH=7.4) was mixed with 20µl of hydrogen peroxide 10mM in a cuvette and incubated at 37 ^{0}C for 5 min. After the incubation period, 30µl ABTS (1.25 mM) and 30µl peroxidase (1 unit/ml) was added to the mixture and was further incubated at 37 ^{0}C for 10 min.

The absorbance was measured at 405 nm. Scavenging activity was calculated as
$I = ((A_0 - A_x) / A_0) x\ 100\%$: A_x and A_0 were the absorbance at 405nm of sample with and without hydrogen peroxide respectively.

4. **Total phenolic compound analysis.**

The amount of total phenolics in above plants extracts were determined with the Folin-Ciocalteu reagent using the method of Sapanos and Wrolstad (1990) ,as modified by Lister and Wilson (2001). Each sample (three replicates), 2.5ml /10 dilution of Folin – Ciocalteau's reagent and 2ml Na_2C0_3 (7.5% W/V) were added and incubated at 45^0C for 15 min. The absorbance was measured at 765 nm spectrophotometrically.

Results were expressed as milligrames of gallic acid equivalent per grams of Dry weight (mg GAE / gdw).

5. **Determination of Total flavonoid content**

Different concentration series of plant material extracted in to methanol were separately mixed with methanol (1.5ml), 10% aluminum chloride (0.1ml) , 1M potassium acetate and (0.1ml) diluted with distilled water. Left at room temperature for 30 min. Measured the absorbance at 415nm.

6. **Determination of Hydroxyl scavenging activity.**

Powdered sample of each plant was extracted by soxhelet apparatus in to petroleum ether (40-60) for 24hr.The resultant solution was filtered and concentrated by Rota-evaporation.

Hydroxyl Radical assay was done for each extract by a modified method of Chung et al.(1997).The concentration was changed from 2.5μg/ml-12.5μg/ml. Reaction mixture containing 200μl of 10mM FeSO4.7H2O, EDTA, 2-deoxyribose and sample in 1.2 ml of 0.1M phosphate buffer(pH=7.4).Thereafter 10mM 200μl of H_2O_2 was added to the reaction mixture and incubated for 4hr..Later 1ml of 2.8% TCA and 1ml of 1% TBA were added and placed in the water bath at 90^0C for 20 min. Absorbance was measured at 532 nm.

7. **Determination of superoxide anion scavenging activity**

E solution concentrations ranging from 2.5μl-12.5μl. 2.8ml of phosphate buffer (pH=8.2) was added into freshly prepared 0.1 ml of 0.6mmol/L of pyrogallol. The inhibition rate of pyrogallol auto oxidation was measured at 325nm.

8. **Determination of Reducing power of the plant extracts.**

The different sample concentrations was mixed with 2.5ml of phosphate buffer (pH=6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was placed in a water bath for 20 min. at 50^0C.The resulting mixture was cooled to room temperature. 2.5ml of 10% TCA was added and mixed. After centrifugation at 3000rpm for 10min. 5.0 ml of supernatant was mixed with 5ml of distilled water and 1ml of 1% ferric chloride .Absorbance measured at 700nm.

9. **Comet Assay for the detection of DNA fragmentation ***

Comet assay (Collins A R, 2004) was used for determination of fragmentation of DNA in the individual cell. The alkaline comet assay conducted according to the Singh et. with slight modifications. The formation of the comet in the cells determines the strength of the damage of DNA. Cell suspension and 75μl Low Melting point Agarose (LMA) and it was added over slides percoated with 1.0% Normal Melting point Agarose(NMA). Solidified agarose, slides covered with 75μl of 0.5% LMA. These were immersed in lysis solution for 1hr At 4°C. Electrophoresis for 40 min. Stained with ethedium bromide. Image analysis was done under Florescence microscope.

10. **Determination of the cell cytotoxicity of the cells by MTT assay***

For the assessment of the cytotoxicity activity of the studied plant extracts, the cancer cell line (Hela cell line) was used. This assay measures the conversion of the tetrasolium bromide to form an insoluble formazan blue precipitate by mitochondrial dehyrogenase which is only present in the viable cells. HeLa cell lines were maintained in Dulbecco's Modified Eagle Meduim (DMEM) supplemented with 10% Fetal calf serum,

streptomycin (10 μg/ml), penicillin (100 units/ml) in a carbon dioxide incubator at 5% CO_2 incubator. Washed with PBS and tripsinization. Hela cells in a 96 well micro plate was incubated for 24 hr at 37°C . Sample was added to each well (Concentration ranged 2mg/ml, 1mg/ml, 0.1mg/ml 0.01mg/ml) and incubated 48 hrs. 50μl of 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution added in each well, incubated for 4hr at 37°C in the incubator. After removal of the medium added 200μl DMSO to each well plates were shaken for 30 seconds. Absorbance measured at 570 nm using an ELISA reader

11. DPPH quenching assay by Electron spin Resonance (ESR)*

The principal of the method is the conversion of the reaction product in to a paramagnetic species (Noda et al 1999). This is characterized by ESR. 150μl of 0.9Mm DPPH was mixed with 50μl of sample in the Quarts flat cell. ESR spectrum were read.

*** 10,11,12 were done at India Institute of Technology Madras, Chennai.**

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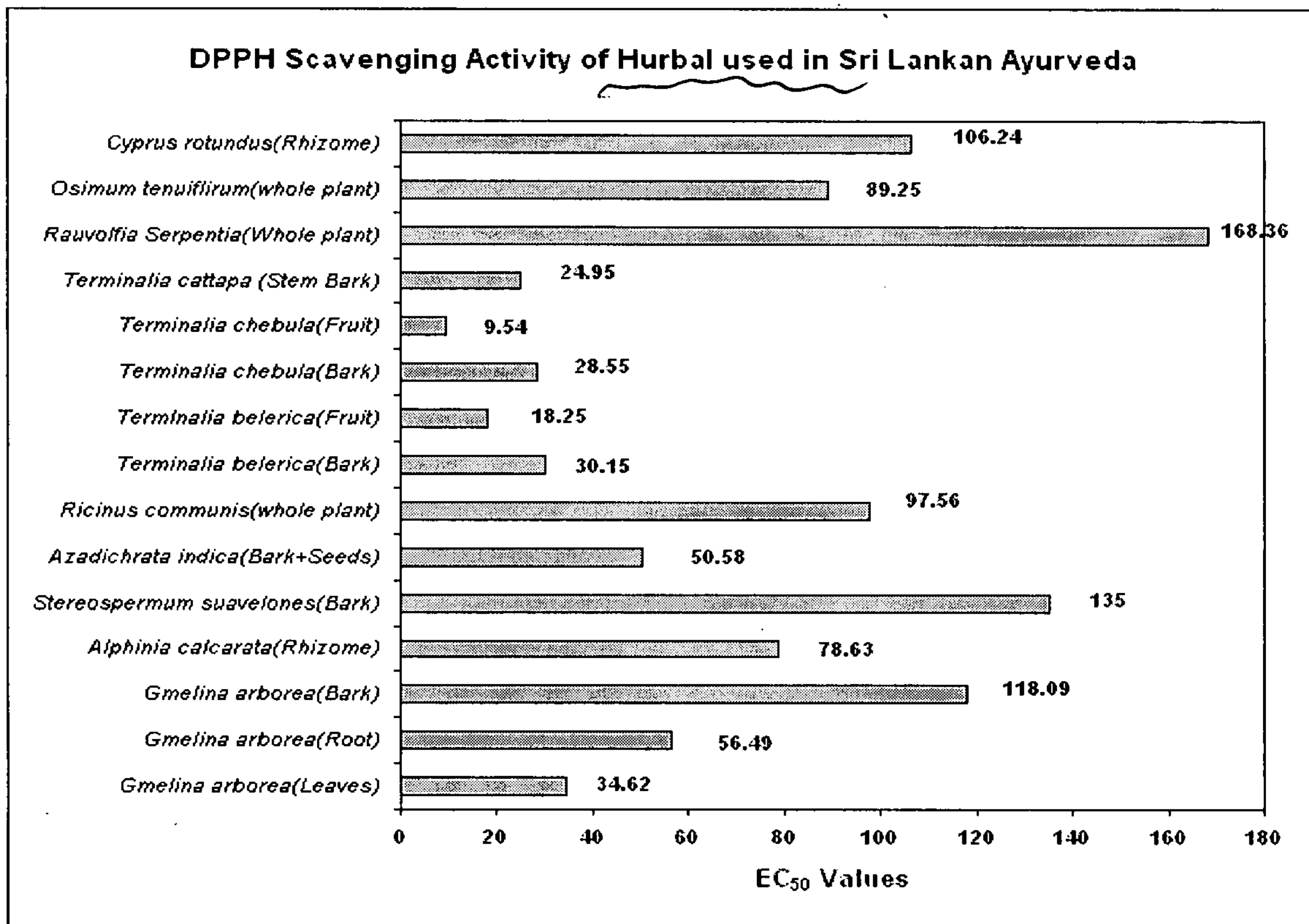
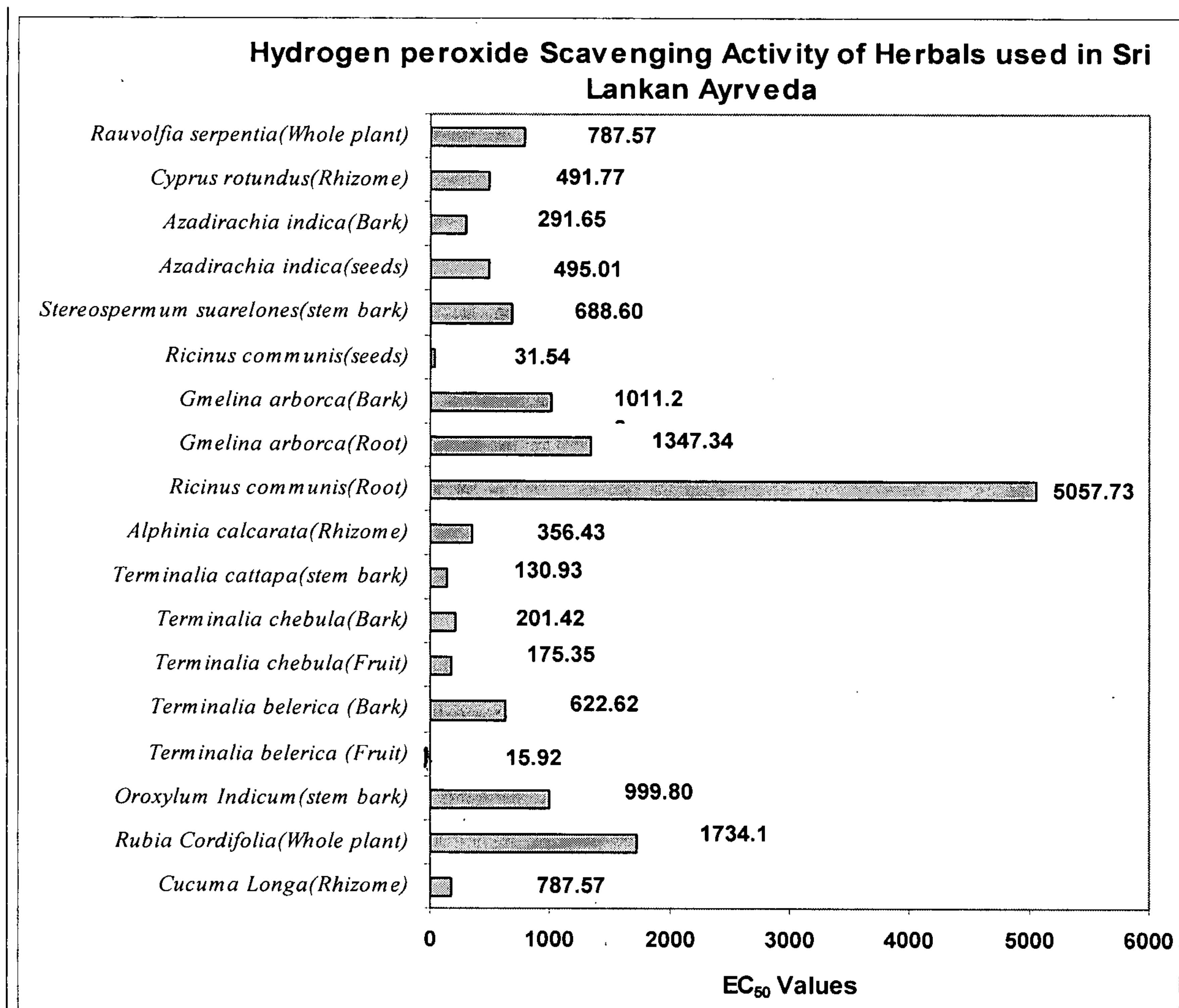
iv) Results/outputs

Figure 01 : DPPH free radical assay



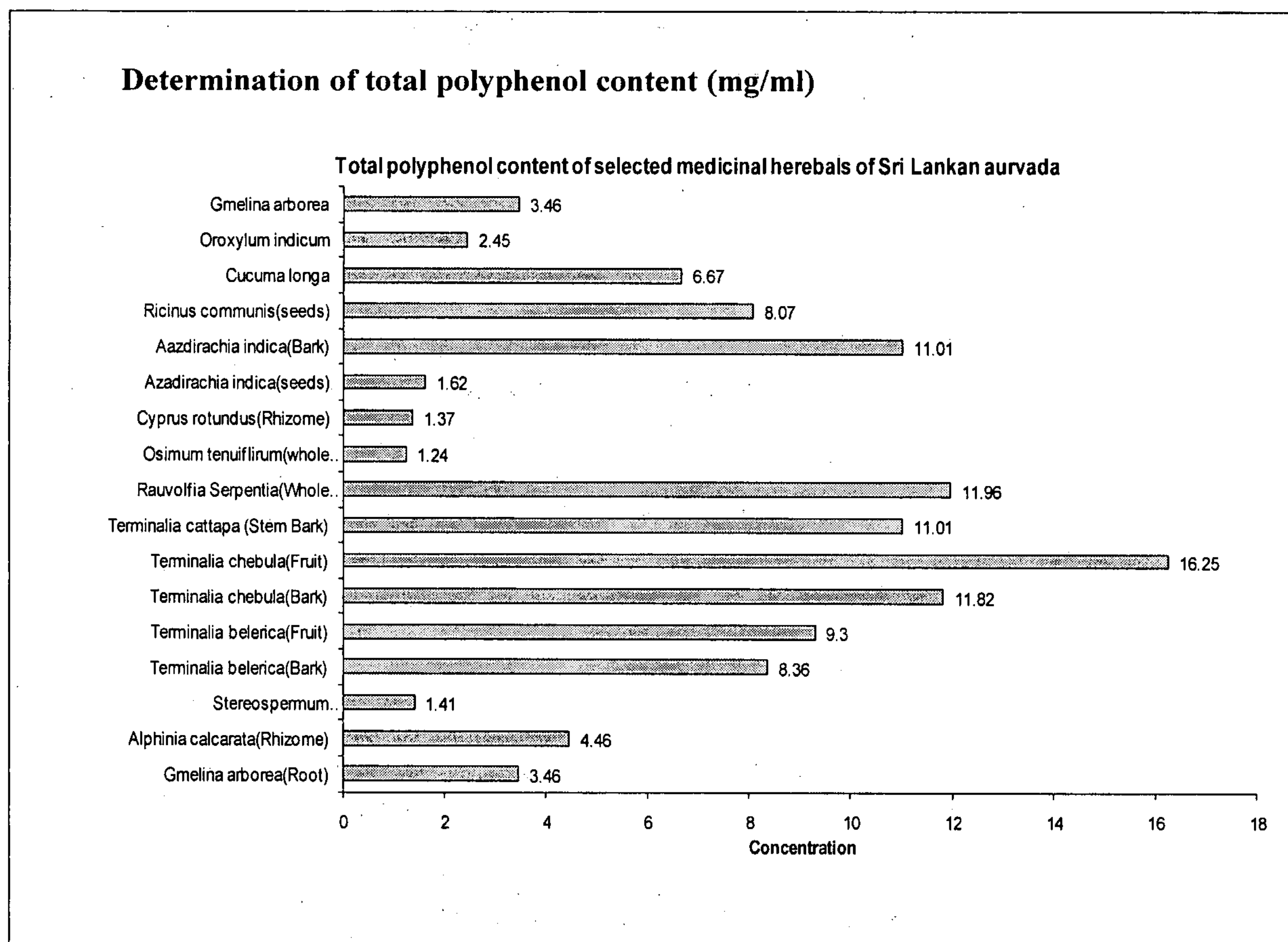
Highest DPPH scavenging activity recorded by *Terminalia chebula* fruit. The activity was greater than the activity of L- ascorbic acid.

Figure 02 : The Hydrogen peroxide scavenging Activity.



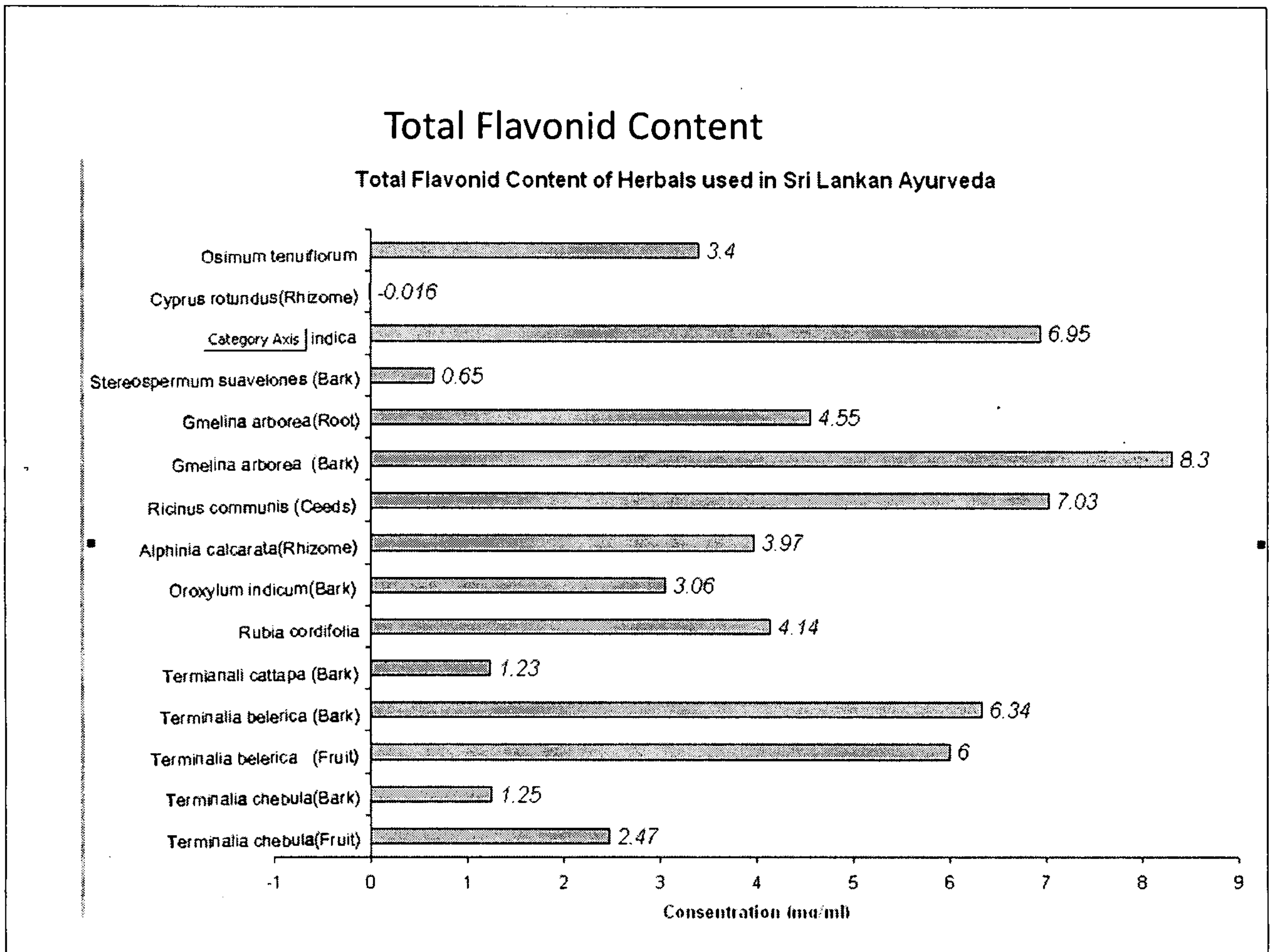
Highest hydrogen peroxide scavenging activity recorded by *Terminalia berelika* fruit with an EC_{50} of 15.92 followed by Ricinus *communis* (seeds) with an EC_{50} of 31.54. Both recorded a activity greater than the activity of Trollox.

Figure 03 : Determination of total phenolic content



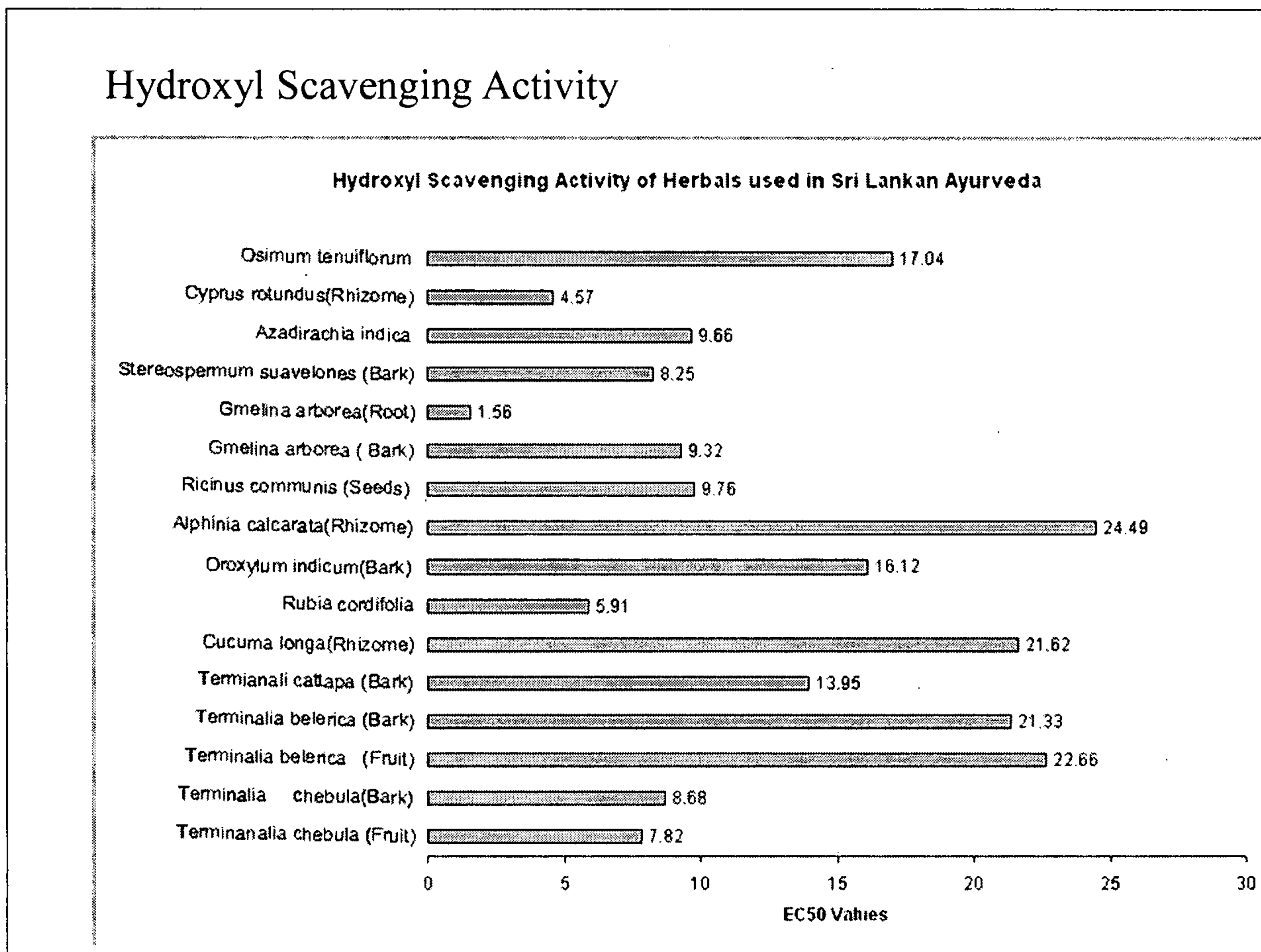
- Highest total polyphenol content recoded by *Terminalia chebula* fruit.
  Results were expressed as milligrames of gallic acid equivalent per grams of dry weight (mg GAE /gdw)

Figure 04 : Determination of Total Flavonoid content



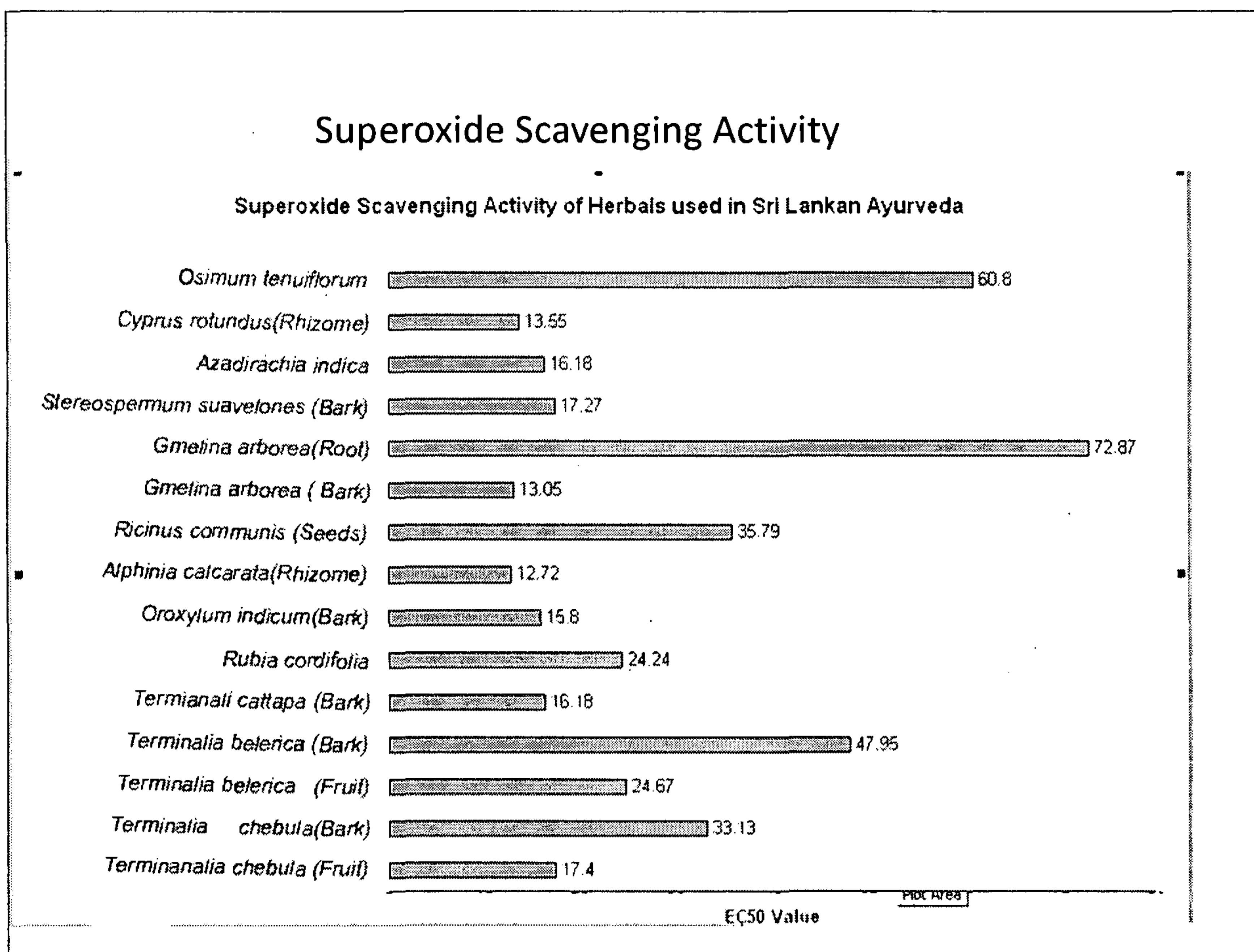
The highest total flavonoid content was recorded in *Gmelina arborera* bark (8.3mg/ml) and followed by was *Ricinis communis* seed (7.03mg/ml).

Figure 05 : Determination of Hydroxyl scavenging activity.



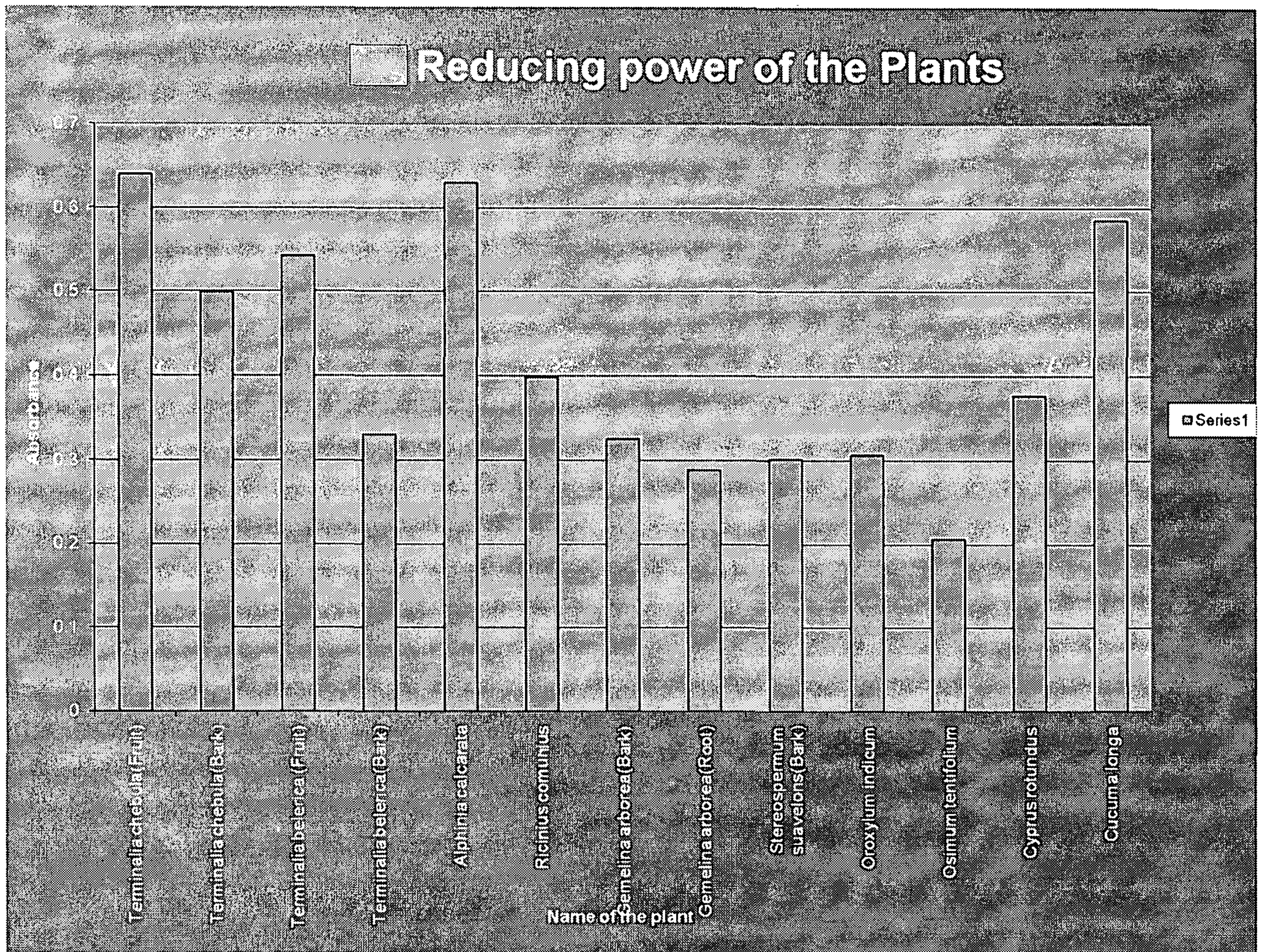
Highest hydroxyl scavenging activity recorded by *Gmelina arborea* root. EC_{50} value 1.56.

Figure 06 : Determination of superoxide anion scavenging activity



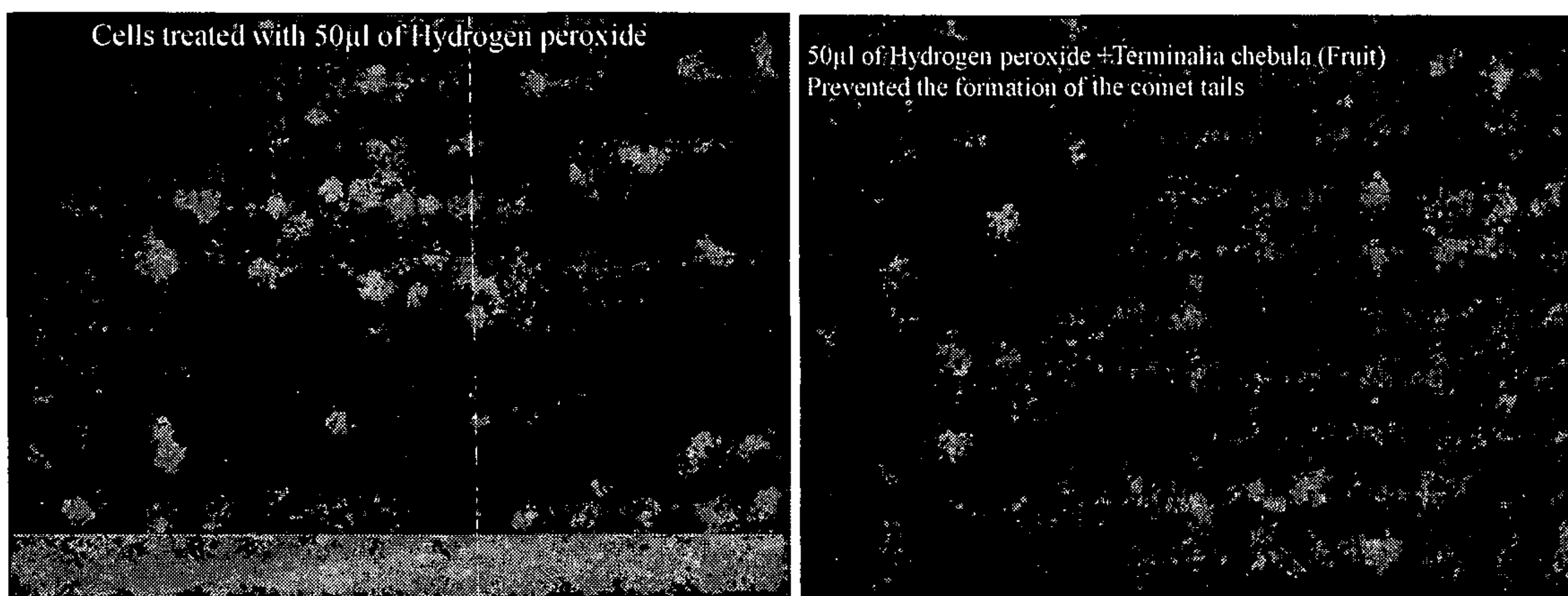
Highest super oxide scavenging activity recorded by *Alphinia calcarata* Rhizome. EC_{50} value 12.72.

Figure 07 : Determination of reducing power of the plant extracts.

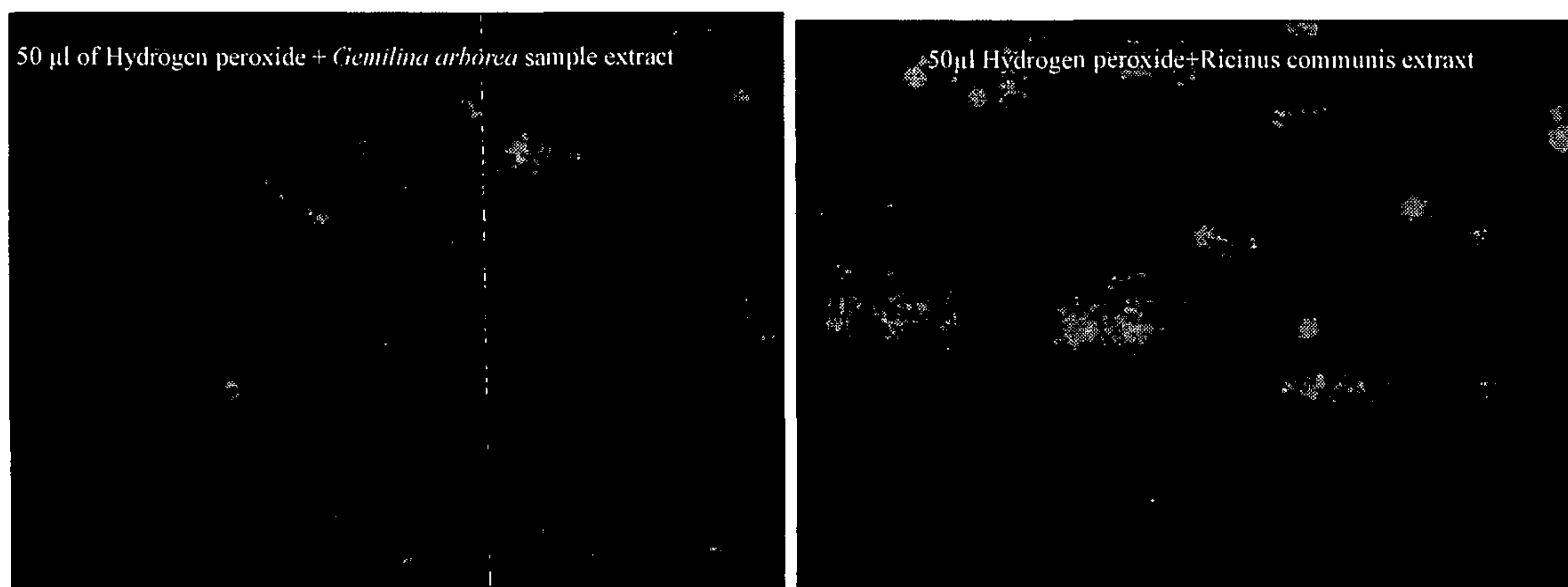


Highest Reducing Power recorded by *Terminalia chebula* fruit, followed by *Alphinia calcarata* rhizome.

Figure 08 : The Comet Assay for the detection of DNA fragmentation



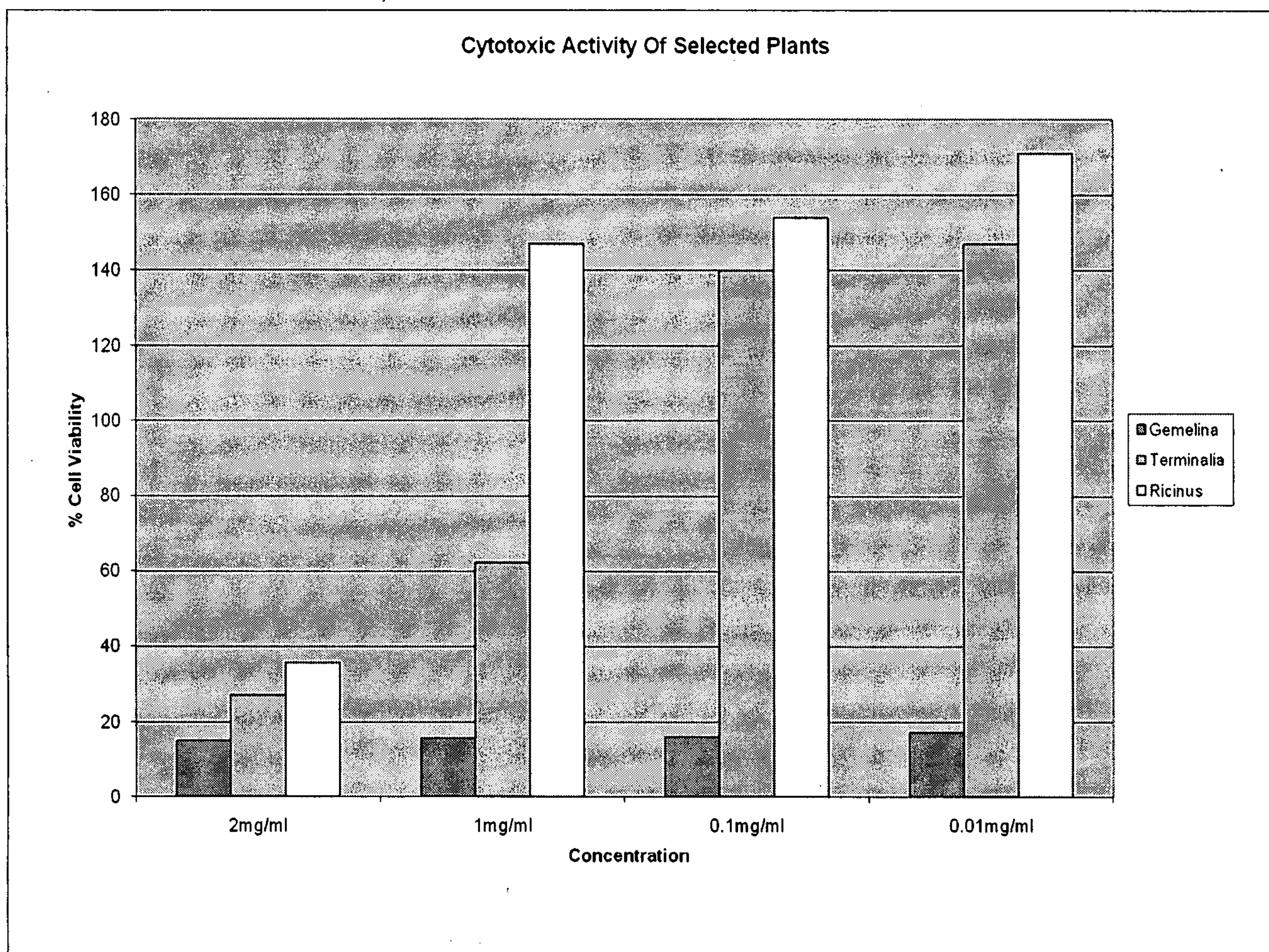
Cells treated with50µl of hydrogen peroxide	50µl of hydrogen peroxide + *Terminalia chebula* fruit extract of prevented the formation of the comet tails.



50µl of hydrogen peroxide + *Gmelina arborea bark* sample extract	50µl of hydrogen peroxide + *Ricinis communis seed*

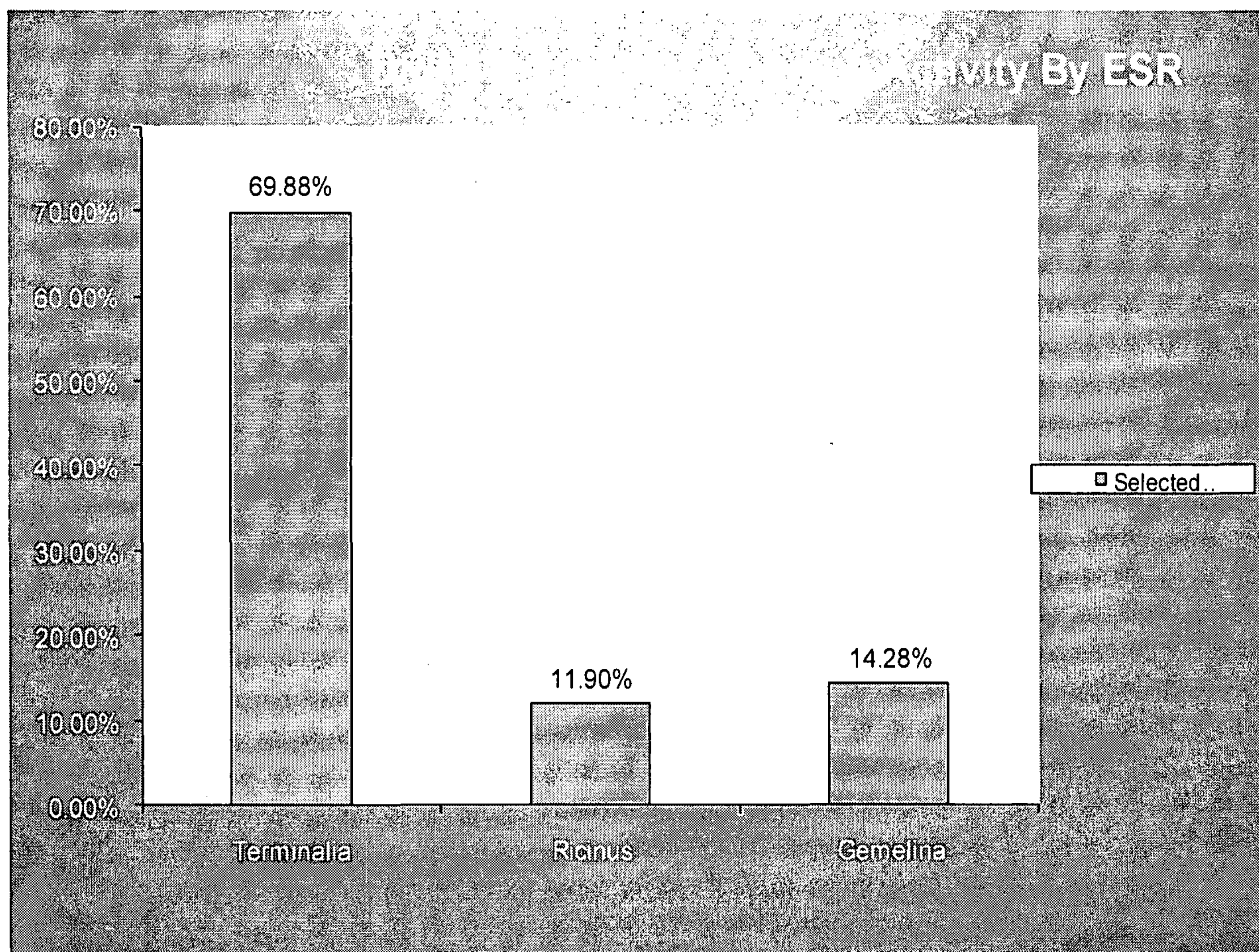
Terminalia Chebula fruit extract prevented fragmentation of DNA significantly.

Figure 09 : Determination of the cell cytotoxicity of the cells by MTT assay



Highest cytotoxic activity recorded by *Gemelina arborea,* while lowest cytotoxic activity recorded by *Ricinis communis.*

Figure 10 : DPPH Radical scavenging activity by ESR.



Terminalia chebula fruit quenched DPPH free radicals significantly.

v) Discussion

Total antioxidant activity of plant extract is a representation of the concerted action of many antioxidant compounds. DPPH assay and ABTS assay was used to determine the total antioxidant activity. These tests are among several methods generally used for the determination of antioxidant capacity of samples such as food extracts. There will be a drop in absorbance of DPPH and ABTS+ radical solution on addition of antioxidants. Total antioxidant activity is measured based on this decrease in absorbance of DPPH and ABTS+• solution. In our experiment highest DPPH scavenging activity recorded by *Terminalia chebula* fruit with an EC_{50} of 9.54 (figure 01). The activity was greater than the activity of L- ascorbic acid.

Hydrogen peroxide scavenging activity recorded by *Terminalia berelika* fruit was highest with an EC_{50} of 15.92 which was followed by *Ricinus communis* seeds with an EC_{50} of 31.54 (figure 02). Both recorded activities greater than the activity of Trollox. In this study it was found that total antioxidant activity values by ABTS assay were different compared with those obtained by DPPH assay. This difference may be due to the presence of different antioxidants in different quantities among the plants and even among the different parts of the same plant.
The extracts of some plants had high levels of DPPH scavenging activity and hydrogen peroxide scavenging activity. This property could inhibit lipid peroxidation, and those could have a potential to prevent cardiovascular diseases.

Total phenolic content Folin-Ciocalteu method used to determine the is based on oxidation of phenolic groups by Folin reagent composed of phosphomolybdic and phosphotungstic acids. Oxidation of Folin reagent produces a green-blue complex that absorbs at 765 nm. The absorbance at 765 nm is proportional to the phenolic compounds present in a sample.
Highest total polyphenol content was recorded by *Terminalia chebula* fruit (figure 03) Results were expressed as milligrames of gallic acid equivalent per grams of Dry weight (mg GAE / gdw). *Terminalia chebula* fruit also recorded the highest DPPH scavenging activity.

Flavonoids are also polyphenolic antioxidants. Flavonoids are divided into six classes and in a plant / plant material these are in different proportions They are flavones, flavanones, isoflavones, flavonols, flavanols, and anthocyanins. Highest total flavonoid content showed by *Terminalia chebula* (bark):8.3mg/ml. Next was *Ricinus communis* seeds:7.03mg/ml (figure 04).

The total antioxidant capacity of a biological sample determined by several methods based on different mechanisms gives the true total antioxidant capacity value. It has been suggested that hydroxyl radical scavenging assay is closely related to *in vivo* antioxidant mechanisms, involves reaction of antioxidants with hydroxyl radicals.In this experiment highest hydroxyl scavenging activity recorded by *Gmelina arborea* root : $EC_{50} = 1.56$ (figure 05).

In the super oxide scavenging activity test the superoxide anions were generated by the oxidation of pyrogallol and the scavenging effects were expressed as the inhibition of pyrogallol autoxidation, so any substance existing in the reaction system that might have effects on the oxidation of pyrogallol affect the test results. It is suggested that flavonoids

including anthocyanins could be involved in scavenging super oxides. Highest superoxide scavenging activity recorded by *Alphinia calcarata* rhizome : EC_{50} = 12.72 (figure 06).

The reducing power of the plant extracts was determined using potassium ferricyanide reduction method. It has been reported that the reducing power was associated with the antioxidant activity and its relationship of phenolic constituents have been well established in several plant sources including vegetables. The yellow colour of the test solution changes to various shades of green and blue depending upon the reducing power of each extract. The presence of antioxidants in the plant extracts causes the reduction of Fe^{3+} / Ferric cyanide complex to ferrous form. Therefore Fe^{2+} complex can be monitored by measuring the formation of Perl's prcussian blue at 700nm. Highest Reducing Power recorded by *Terminalia chebula* fruit, followed by *Alphinia calcarata* rhizome (figure 07).

The comet assay for the detection of DNA fragmentation* is a sensitive and rapid method for DNA strand break detection in individual cells. The principle of break detection, using either the alkaline or neutral version of the assay, makes it a good technique for studying both double and single strand DNA breaks. Furthermore, the possibility of following DNA damage at different time moments also makes it possible to investigate the cell repair mechanisms. *Terminalia Chebula* fruit extract prevented fragmentation of DNA significantly (figure 08).

Determination of the cell cytotoxicity of the cells by MTT assay* is based on a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent (eg. isopropanol) and the released, solubilised formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity measures the viability of cells. Highest cytotoxic activity recorded by *Gemelina arborea,* while least cytotoxic activity recorded by *Ricinis communis* (figure 09).

DPPH quenching assay by Electron spin Resonance (ESR) * is based on the principle is the conversion of the reaction product in to a paramagnetic species which may characterized by ESR.
Terminalia chebula fruit quenched DPPH free radicals significantly (figure 10). Scan diagrams are not attached for this final report.

*** The Comet Assay for the detection of DNA Fragmentation*, Determination of the cell cytotoxicity of the cells by MTT assay* and DPPH quenching assay by Electron spin Resonance (ESR) * were done at India Institute of Technology Madras, Chennai on *Gemelina arborea, Ricinis communis and Terminalia chebula.*.**

vi) Conclusions

The methods used in the present research were well suited to screen the antioxidant activity of the plants used to determine the antioxidant activity of some medicinal plants used in traditional medicine for the treatment of cardiovascular diseases in Sri Lanka. Although some experiments represented what *is in vivo*, the results however cannot be extended directly to *in vivo* systems which are more complex and may differ from *in vitro* systems. The results obtained from this study provide insights into the antioxidant nature of the plants tested.

The present study although not concluded shows that some these plants as a source of natural antioxidants.

In conclusion this research gives an idea about the activity and nature of antioxidants present in the plants tested. *Terminalia chebula* fruit showed the best anti oxidant activity and future studies should be extended to other methods for determination of antioxidant activity. The resulted antioxidant activity would prevent the oxidation of Low density lipoproteins (LDL) to form atherosclerotic plaques, which are responsible for the development of cardiovascular disease. The plant extracts of the plants that showed higher antioxidant activity need to be studied further and active ingredients should be isolated and their structures elucidated. Such identification and quantification of antioxidants in will let researchers understand the nature of antioxidants in the medicinal plant/parts

Structure elucidation of antioxidants would lead to propose reaction mechanism of antioxidant with oxidants or radicals.

vii) References

Gutteridge J.M.C. and Halliwell B. (2000) Free radicals and antioxidants in the year 2000-A historical look to the future. *Ann. N.Y. acad. Sci.*, **899**, 136-147.

Halliwell B. 1993. The role of oxygen radicals in human disease, with particular reference to the vascular system. *Haemostasis*, **23** (S 1), 118-126.

Frei B. 1999. On the role of vitamin C and other antioxidants in atherogenesis and vascular dysfunction. *Proc. Soc. Exp. Biol. Med.*, **222**, 196-204.

Halliwell B (1991). Reactive oxygen species in living systems: Source, biochemistry, and role in human disease. Am. J. Med. 91: S14-S22.

Collins A R (2004). The comet assay for DNA damage and repair: principles, applications, and limitations. Mol biotechnology : 26 (3): 249-261.

Noda Y, Kohno M, Mori A and Packer L (1999). Automated electron spin resonance free radical detector assays for antioxidant activity in natural extracts. Methods enzymol. 299:28-34.

viii) Problems if any, encountered during the implementation of the project.

The investigators were on sabbatical leave and the student could not work up to the end of the project. The student hesitated to do animal studies due to personal issues and the project was deviated towards cell assays.

Section 4

Impact of Research results:

i) Relevance of results achieved to scientific advancement.

The results obtained from this project show the antioxidant activity of the plants tested. The tests were based on different aspects of antioxidant activity. As the total antioxidant activity is a collection of many activities it is a useful set of date to get a broad picture of the antioxidant activity. This is a good start to carry on further investigations on antioxidant activity of some medicinal plants used in traditional medicine for the treatment of cardiovascular diseases in Sri Lanka.

ii) Relevance of results achieved to national/socio-economic development.

When the research findings are concluded such identification and quantification of antioxidants in will let researchers understand the nature of antioxidants in the medicinal plant/parts and could popularize the usage and /or lead to formation of drugs. This would popularize our traditional treatment systems. At present our traditional treatment systems holds a huge draw back as the scientific bases of our treatments are not known.

iii) Dissemination/application of research output.

01 Oral presentation.
01 Poster presentation
01 The results of this study were submitted for publication.

Section 5

Miscellaneous

i) List of major equipment acquired during the project period and their functionality : **Double beam spectrophotometer – Working well**

ii) List of publications/communications arising from the project and/or presentations made at seminars, workshops etc. (Attached copies)

1. Ranatunga D N, Jayawardene M I F P and Ruvin Kumara N K V M In- vitro antioxidant activity of some Sri Lankan Medicinal plants used in Ayurveda as cardio-protective agents, International Conference Biomaterials in Regenerative Medicine, Polish Academy of Sciences, Scientific Centre, Vienna, 2006 Abstract p 96-97.

2. Ranatunga D N, Jayawardene M I F P and Ruvin Kumara N K V M and U G Chandrika Determination of in- vitro antioxidant activity by different methods of some Sri Lankan Medicinal plants used in Ayurveda as cardio- protective agents - 4th Joint Meeting Of Free Radical Research Australasia and Japan at Kyoto University Japan. Abstract p 66.

Section 6 : Summary Statement of Expenditure

Item	**Expenditure Rs.**
Personnel	330,000.00
Equipment	NIL
Consumables	15,019.00
Travel & Subsistence	NIL
Miscellaneous	29,455.00
Other	
Total	**374,474.00**

Section 7

i) Grantees' signatures

ii) Comments of the Head of the Department/signature

iii) Head of the Institution's signature

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