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Evaluation of Antioxidant Effectiveness of a Few Herbal Plants

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We have screened a number of plants from the Indian soil for potential antioxidant properties out of which fifteen extracts were found to be positive. Leaves/bulk from the plants were crushed and extracted with organic solvents by three different ways. *The first group of plants were extracted with* $CHCL_3$:CH_3OH (2:1), evaporated, partitioned between petroleum ether and methanol (9:1), aqueous methanolic part re-partitioned between methanol:H_2O (4:1) and dichloromethane. Methanol was evaporated from the aqueous methanolic part and extracted with n-butanol. The second group of plants were extracted with methanol followed by partitioning between petroleum ether and CH_3OH. The rest of the extraction procedure was the same as above. A third extraction procedure was used for Ocimum sanctum which after extraction with $CHCL_3$:CH_3OH (2:1), partitioned between CCL_4 and CH_3OH:H_2O (9:1). Aqueous methanolic part was repartitioned between CH_3OH:H_2O (4:1) and $CHCl_3$ and $CHCl_3$ soluble part was used for the study. Free radical scavenging activities of the plant extracts were examined by chemiluminescence method. Peroxyl radical was generated from 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), superoxide radical (O_2^-) from xanthine/xanthine oxidase (XO) and hydroxyl radical (OH) from Xanthine/XO/ $FeCl_3$/ EDTA. In addition, O_2^- and OH· scavenging activities were also determined by cytochrome C reduction and deoxyribose oxidation methods, respectively. The results of this study demonstrate that these plant extracts possess potent antioxidant activities.

Keywords: Medicinal plants, Herbs, Antioxidants, Free radicals, India

INTRODUCTION

India has long enjoyed the luxury of an innumerable variety of tropical plants, and inhabitant Indians possess a long tradition of *Kabiraji medicine* utilizing such medicinal herbs and plants. The plant medicine, more commonly known as Ayur-Vedic medicine has been practised in the Indian sub-continent for thousand of years. The word Ayur-Veda comes from two Sanskrit roots: Ayus, meaning life or life span, and Veda, meaning knowledge or science. Ayur-Veda is, therefore, translated as "the science of life," which emphasizes its orientation towards prevention. The Indian Medicine Central Council Act 1970 recognizes Ayur-Vedic medicine; over a hundred colleges for Ayur-Vedic physicians have

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been established; the All India Ayur-Veda Congress has over 300,000 members, and the Indian Government sponsors a programme of research.[1] The World Health Organization also sponsors and encourages research into Ayur-Vedic treatments,[2] and UNICEF and USAID have promoted the use of Ayur-Veda in child survival programmes.

Many of these medicinal plants grow in the tropical rainforests of India. However, of the large variety of such plants grown in the rainforest of India, only a relative handful have been thoroughly studied for all aspects of their potential therapeutic value in medicine. A growing body of evidence suggests that at least part of the therapeutic values may be explained by their antioxidant properties. In this study, we selected seven different plants from India with known therapeutic values and studied their potential antioxidant properties with respect to their abilities to scavenge oxygen free radicals.

MATERIALS AND METHODS

The following plants (Table I) widely cultivated in India with known therapeutic values were selected for our study:

Extraction Methods

Five hundred grams of each, dried and milled plant materials (leaves/ bulk) were crushed and extracted in organic solvents in three groups. The first group of plants were soaked in *5 litres* of $CHCl_3$:CH_3OH (2:1) [CM]. The $CHCl_3$-CH_3OH extract was evaporated, partitioned between petroleum ether and methanol (9:1). The petroleum ether soluble part was discarded. The aqueous methanolic part was partitioned between *100 ml* CH_3OH:H_2O (4:1) and dichloromethane. The dichloromethane soluble part was discarded and aqueous methanolic part was evaporated and extracted with *20 ml* n-butanol (BU). Aqueous part was discarded. The butanol soluble parts [AS/CM/BU, CL/CM/BU, FB/CM/BU, OS/CM/BU, PE/CM/BU, TB/CM/BU, TC/CM/BU] were used for our study.

The second group of plants were soaked in *5 litres* of methanol. The CH_3OH extracts were evaporated, partitioned between petroleum either and CH_3OH (9:1). The petroleum ether soluble parts were discarded. Aqueous methanolic part was partitioned between *100 ml* CH_3OH:H_2O (4:1) and dichloromethane. Dichloromethane soluble parts were discarded while aqueous methanolic parts were evaporated and extracted with *20 ml* n-butanol. Aqueous parts were rejected. Thus, the following n-butanol soluble fractions were obtained : AS/ME/BU, CL/ME/BU, FB/ME/BU, OS/ME/BU, PE/ME/BU, TB/ME/BU, TC/ ME/BU.

A third extraction method was used for Ocimum sanctum (OS) which was soaked in *5 litres* of $CHCl_3$:CH_3OH (2:1). The $CHCl_3$-CH_3OH extract was evaporated, partitioned between *100 ml* CCl_4 and CH_3OH:H_2O (9:1). Aqueous methanolic part was partitioned between *20 ml* CH_3OH:H_2O (4:1) and $CHCl_3$. Aqueous part was discarded. The $CHCl_3$ (CL) soluble part (OS/CM/CL) was used for our study.

Chemiluminescence Measurements

Oxidation of luminol by superoxide, hydroxyl and peroxyl radicals were assayed by the chemiluminescence response of luminol.[4] Delay in the chemiluminescence response was observed by the addition of plant extracts (endogenous antioxidants) with interaction of superoxide, hydroxyl and peroxyl radicals. The amount of free radicals scavenged by antioxidants was determined by comparing with known rate of oxygen free radical generation.

Superoxide radicals (O_2^-) were generated by the action of xanthine (100 μM) on xanthine oxidase (8 mU) in a reaction mixture containing 10 mM phosphate buffer (pH 7.4) and 10 μM EDTA in a total volume of 1 ml. To generate OH· radical, 100 μM $FeCl_3$ and 100 μM EDTA were added to the O_2^- generating system. A water soluble azo-initiator, 2,2'-azobis (2-amidino-

TABLE I

Name	Chemical constituents[3]	Ethanomedical uses[3]
Allium sativum (AS)	allicin, allinase, γ-glutamyl peptides, leucine, allin, methionine, scordine, cyanidin-3-glucoside	anthelmintic,antibacterial,antiepileptic,tubercular, diaphoretic,diuretic,emmenagogue,expectorant, hypoglycemic, possesses potent anticancer and antirheumatic properties, stimulant, tonic
Curcuma longa (CL)	curcumin. 4-hydroxycinnamoyl- and bis-4 (4-hydroxycinnamoyl)-methane	antiarrhythmic,anticancer,antibacterial, antiinflammatory,fungistatic
Ficus bengalensis (FB)	glutathione, quercetin-3-galactoside, rutin, β-sitosterol	topical pain killer, antirheumatic,work against ulcer and sores
Ocimum sanctum (OS)	caryophyllene, eugenol, methyl-eugenol, β-carotene, sterols, fatty acids, ursolic acid	antibacterial,antiperiodic,diaphoretic, stomachic, catarrh, bronchitis, demulcent in genitourinary disorders
Phyllanthus embellica (PE)	ascorbic and gallic acids, corilegin, tannins, gibberelin	antiinflammatory
Terminalia bellerica (TB)	chebulagic, gallic and elegiac acids, ethylgallate, gallylglucose and other common carbohydrates	antibacterial,anticancer, antirheumatics,useful against diarrhea,dropsy,fever,leprosy, piles
Terminalia chebule (TC)	chebulin, ascorbic, chebulinic and tannic acids	antispasmodic, laxative, effective against conjunctivitis and other eye infections

propane) dihydrochloride (AAPH) was used to produce peroxyl radicals at a constant rate. The incubation medium contained 0.1 M phosphate buffer (pH 7.4), AAPH (50 mM) and luminol (400 μM). Luminometer (LKB Wallac 1250) was used for O_2^- and OH· while Luminescent analyzer 633 (Coral Biomedical Inc., San Diego, CA) was employed for peroxyl radicals. Based on the known rate of peroxyl radical generation by AAPH [1.36×10^{-6} (AAPH) mol/l/s at 37°C] and the duration of the lag period, the amount of peroxyl radicals scavenged by endogenous plant antioxidants was calculated.

O_2^- by Cytochrome C Reduction

The generation of O_2^- was measured in the presence of plant extracts by following the reduction of cytochrome C at 550 nm using a spectrophotometers.[5] The final concentration of the assay mixture (in a total volume of 1 ml) was 100 μM cytochrome C, 100 μM hypoxanthine, 10 mM Tris-HCl, and 50 μl of diluted plant extracts. The reaction was initiated with the addition of 8 mU of XO. *Inhibitory effects of each extract against XO was checked to make sure that the plant extracts had no effects on XO activity.* Control experiments were performed by replacing plant extracts by superoxide dismutase (SOD) [100 μM]. Percent inhibition of O_2^- generation was calculated against the SOD reaction which was set at 100%.

OH· by Deoxyribose Oxidation

The OH· scavenging action of the plant extracts were further examined by their abilities to inhibit OH·-catalyzed deoxyribose oxidation using a spectrophotometer.[5] The final concentration of the assay mixture (total volume 1 ml) was 28 mM deoxyribose, 20 mM Tris-HCL, pH 7.4, 100 μM $FeCl_3$, 100 μM EDTA, 1 mM H_2O_2 and 50 μl of diluted plant extracts or dimethyl thiourea (DMTU) [100 μg/ml]. The reaction was incubated at 37°C for 1 hr, after which malonaldehyde formation from deoxyribose oxidation was measured by thiobarbituric acid (TBA) reaction as described elsewhere.[6] In brief, 1 ml of ice-cold 30% HCl and 1 ml of ice-cold 30% HCl and 1 ml of 0.75% of TBA dissolved in 0.5% sodium acetate were added to the reaction mixture. The samples were boiled for 20 min and centrifuged to remove the pellet. The color of the resulting

supernatant was read at 535 nm. The concentration of MDA (in nmol/ml) was calculated by using a molar extinction coefficient at 156 $mM^{-1}cm^{-1}$. Percent inhibition of OH· generation was calculated against the DMTU reaction which was set at 100%.

Evaluation of Antioxidant Effectiveness using Pulmonary Type II cells

Pulmonary type II cells were obtained from adult male Sprague Dawley rats (Charles River Laboratories, Willington, MA). The cells were grown to full confluence in pure culture as described previously.[7] Plant extracts were diluted as described earlier, and then 100 µl was added to the incubation mixture. The cells were then exposed for 24 hours to 95% O_2 /5% CO_2 in an incubator maintained at 37°C. Trolox was used as standard antioxidant whose final concentration in the medium was 10 µM and added to the medium in a separate set of experiments. Matched control experiments were performed by adding 100 µl of the vehicle (in which the plants extracts were diluted) to the cells prior to oxygen exposure. After 24 hr, all experiments were terminated, cells collected by centrifugation, washed, and cell viability was determined by trypan blue exclusion test. To examine the cell injury, a portion of the medium (after the cells were collected by centrifugation) was assayed for lactic acid dehydrogenase (LDH) using a LDH assay kit obtained from Sigma Chemical Company, St. Louis, Mo.

RESULTS

In Vitro Free Radical Scavenging Activities of the Plant Extracts

The results for the chemiluminescence response of the chemically generated superoxide, hydroxyl and peroxyl radicals in the presence of plant extracts and luminol are shown in Table II. Delay in chemiluminescence response was observed after the addition of plant extracts and represented as lag period in the Table II. Inhibition efficiency was calculated as compared to that of trolox whose efficiency at 10 µM towards peroxyl radical was arbitrarily chosen as 1. Our results demonstrate that PE/CM/BU, TB/ME/BU, TB/CM/BU, TC/CM/BU,TC/ ME/BU possess the highest antioxidant property. FB/CM/BU and FB/ME/ BU also showed high degree of oxygen free radical scavenging activities.

These results were further confirmed by the abilities of the plant extracts to scavenge O_2^- and OH· as judged by more specific cytochrome C reduction and deoxyribose oxidation assay, respectively. Figure 1 shows the inhibition of O_2^--dependent reduction of cytochrome C in presence of seven different plant extracts. Our results indicate the highest O_2^--scavenging activities for TB/CM/BU and TB/ME/BU. TC/CM/BU and TC/ME/BU were also found to be almost equally potent O_2^- scavengers. The other antioxidant-rich plant extracts includes FB/ME/BU, PE/CM/BU and PE/ME/BU.

The deoxyribose oxidation assays demonstrated a similar pattern towards OH· scavenging activities of the plant extracts. Again, TB/CM/BU, TB/ME/BU, TC/CM/BU and TC/ME/BU showed the highest degree of OH· scavenging activities. FB/ME/BU was found to be equally effective OH· scavenger. PE/CM/BU and PE/ME/BU also showed high degree of OH· scavenging activity.

Antioxidant Effectiveness of the Plant Extracts on Pulmonary Cells Exposed to 95% O_2

As shown in Table III, exposing the cells to 95% oxygen caused significant cellular injury as evidenced by loss of viability from >97% control as compared to 55.2% after O_2 exposure. Trolox preserved the cells to some extent; the cell viability was increased to 65% and LDH release was 0.012 IU/L compared to 0.023 IU/L for the O_2 exposed cells. A number of plant extracts demonstrated very potent antioxidant properties and protected the cells from cytotoxic reactive oxygen

TABLE II Free radical scavenging effects of tropical plant extracts as determined by chemiluminescence response

PLANT EXTRACT	LAG PERIOD (MINUTES)*			INHIBITION EFFECT (min/μl)		
	O_2^-	OH·	PEROXYL	O_2^-	OH·	PEROXYL
Allium sativum (AS/CM/BU)	4.0 (1600)	7.0 (1000)	7.4 (25)	6.4	7.0	0.19
Allium sativum (AS/ME/BU)	5.8 (4000)	5.6 (1000)	5.2 (2000)	23.2	5.6	10.4
Curcuma longa (CL/CM/BU)	5.0 (8000)	7.1 (2500)	1.6 (5000)	40.0	17.8	8.0
Curcuma longa (CL/ME/BU)	4.3 (3000)	1.2 (2250)	1.0 (22500)	12.9	2.7	22.5
Ficus bengalensis (FB/CM/BU)	4.3 (100000)	7.6 (10000)	7.4 (5000)	430	76.0	37.0
Ficus bengalensis (FB/ME/BU)	3.8 (75000)	4.3 (10000)	7.4 (37500)	285	43.0	277.5
Ocimum sanctum (OS/CM/BU)	4.2 (80000)	7.3 (2000)	1.0 (5000)	336	14.6	5.0
Ocimum sanctum (OS/ME/BU)	4.3 (5000)	5.5 (1000)	1.0 (2500)	21.5	5.5	2.5
Ocimum sanctum (OS/CM/CL)	5.1 (20000)	5.8 (10000)	28.2 (4000)	102	58.0	112.8
Phyllanthus embellica (PE/CM/BU)	4.3 (560000)	5.7 (200000)	0.6 (500000)	2408	1140	300
Phyllanthus embellica (PE/ME/BU)	3.6 (100000)	4.5 (20000)	4.0 (100000)	360	90.0	400
Terminalia bellerica (TB/CM/BU)	3.3 (600000)	5.1 (200000)	11.6 (100000)	1980	1020	1160
Terminalia bellerica (TB/ME/BU)	4.1 (600000)	6.0 (200000)	7.6 (200000)	2460	1200	1520
Terminalia chebule (TC/CM/BU)	3.1 (800000)	4.1 (200000)	2.4 (200000)	2480	820	480
Terminalia chebule (TC/ME/BU)	4.0 (240000)	5.3 (150000)	19.0 (10000)	960	795	190

* Numbers in ( ) following the lag period represent the dilution factor.

metabolites. For example, CL/ME/BU, TC/ME/BU, FB/ME/BU, TC/CM/BU, TB/CM/BU, TB/ME/BU and PE/CM/BU increased the cell viability to 76.1%, 77.7%, 78.5%, 85.4%, 88.5%, 89.5% and 92.0%, respectively. The corresponding LDH release was 0.009, 0.009, 0.009, 0.007, 0.005, 0.004, and 0.003 IU/L, respectively. Most of the remaining plant extracts demonstrated comparable antioxidant effectiveness as that of trolox.

DISCUSSION

In India, like other parts of the world, plant medicines are widely used among the natives of the rainforest region, who have absolutely no knowledge regarding the active components of the plants. Nevertheless, these natives probably have been using these plant medicines to cure diseases for generations after generations. A large number of such plants have been identified for their therapeutic efficacies against cancer, hypertension, inflammation, etc. However, it is practically impossible for general public to acquire knowledge about these plants of potential therapeutic values. Traditionally, the knowledge is transferred within the natives from one generation to another without the knowledge of the rest of the world.

In this study, we used seven different herbal plants with known therapeutic values. The medicinal values of these plants have been extensively studied by the scientists in India. The

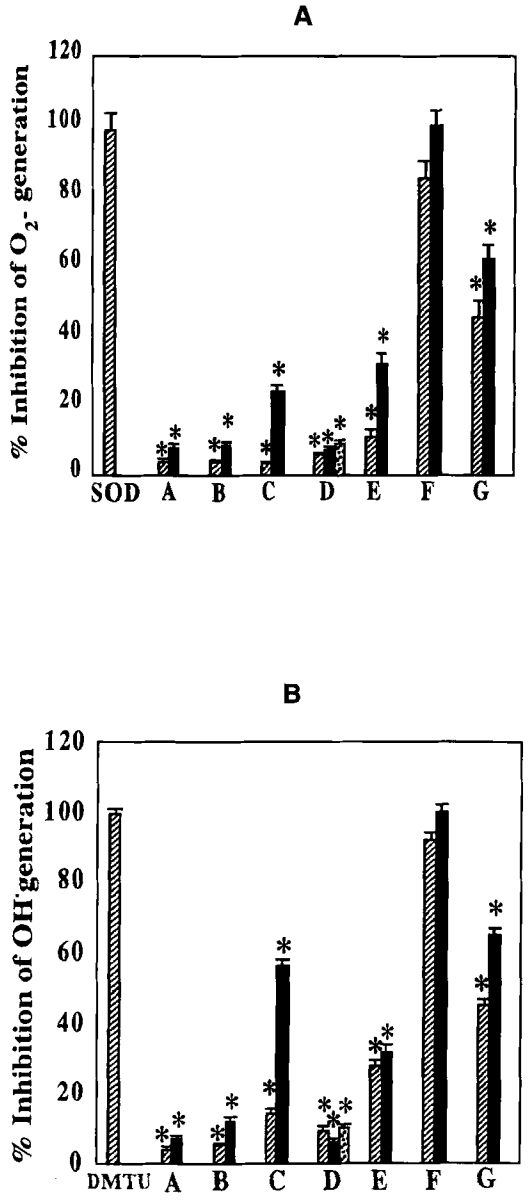

FIGURE 1 Effect of different plant extracts on chemically generated oxygen free radicals. O_2^- was generated by the action of xanthine on XO while OH· was produced by adding $FeCl_3$ and EDTA to the O_2^- generating system. O_2^- generation was measured by cytochrome C reduction assay (**A**) and OH· dependent malonaldehyde formation from deoxyribose was monitored to determine OH· (**B**)

A: AS/CM/BU & AS/ME/BU; **B**: CL/CM/BU & CL/ME/BU; **C**: FB/CM/BU & FB/ME/BU; **D**: OS/CM/BU, OS/ME/BU & OS/CM/CL; **E**: PE/CM/BU & PE/ME/BU; **F**: TB/CM/BU & TB/ME/BU; **G**: TC/CM/BU & TC/ME/BU.

CM BU extracts ▨; ME/BU extracts ■; CM/CL extracts ■.

*$p < 0.05$ compared to either SOD or DMTU reaction

active components of such plants are also known. Based on the existing knowledge of ethanomedical uses and the active components of these seven plants (Table I), we speculated that these plant materials might be sources of natural antioxidants. We, therefore, analyzed the extracts of these plants for their ability to scavenge O_2^-, OH· and peroxyl radicals. The results of our study have indicated that these plants are indeed rich source of natural antioxidants.

The *in vitro* free radical scavenging activities of the plant extracts were confirmed by determining their antioxidant effectiveness as judged for their ability to protect type II lung cells from the oxidant injury. A number of the plants were highly effective in protecting the cells as shown by increased cell viability and reduced LDH release.

The plants included in our study comprise of traditional Ayurvedic medicine for the therapy mentioned in Table I. Sometimes ago a list of plant-derived medicaments of known chemical composition was prepared.[8] These plant medicines derived from 90 different plants have been recognized as valuable non-prescription drugs which found their use in 62 therapeutic categories.[9] In India, these plants are traditionally used as sources of direct therapeutic agents.

The results of our study warrant re-evaluation of their medicinal values. Oxygen derived free radicals are known to play a significant role in the pathophysiology of many diseases that include cardiovascular diseases such as ischemic heart disease, arrhythmias, stroke, brain damage, lung injury such as adult respiratory distress syndrome, liver damage, cancer, influenza, malaria, and many more.[9,10] The chemical constituents of most of these plants examined in our study contain natural antioxidants. Among the seven selected plant species, Ficus bengalensis contains glutathione, Ocimum sanctum contains β-carotene and both Phyllanthus embellica and Terminalia chebule contain ascorbic acid. These antioxidants protect our body from the oxidative

TABLE III Antioxidant effects of tropical plant extracts on type II epithelial cells exposed to 95% oxygen

PLANT EXTRACT	DILUTION	VIABILITY (%)	LDH RELEASE (IU/L)
Control		97.0 ± 3.0	0.001 ± 0.001
95 % O_2		55.2 ± 1.8*	0.023 ± 0.001
Allium sativum (AS/CM/BU)	1600	72.1 ± 2.5*	0.016 ± 0.002*
Allium sativum (AS/ME/BU)	4000	66.5 ± 4.1	0.011 ± 0.003*
Curcuma longa (CL/CM/BU)	8000	68.1 ± 3.7*	0.011 ± 0.003*
Curcuma longa (CL/ME/BU)	3000	76.1 ± 3.2*	0.009 ± 0.001**
Ficus bengalensis (FB/CM/BU)	100000	65.2 ± 2.9*	0.011 ± 0.002**
Ficus bengalensis (FB/ME/BU)	75000	78.5 ± 5.0*	0.009 ± 0.001**
Ocimum sanctum (OS/CM/BU)	80000	66.5 ± 4.7	0.012 ± 0.002*
Ocimum sanctum (OS/ME/BU)	5000	60.0 ± 1.8*	0.014 ± 0.003
Ocimum sanctum (OS/CM/CL)	20000	58.5 ± 2.9	0.012 ± 0.002*
Phyllanthus embellica (PE/CM/BU)	560000	92.0 ± 5.3**	0.003 ± 0.001**
Phyllanthus embellica (PE/ME/BU)	100000	70.0 ± 3.3*	0.009 ± 0.001**
Terminalia bellerica (TB/CM/BU)	600000	88.5 ± 1.8**	0.005 ± 0.001**
Terminalia bellerica (TB/ME/BU)	600000	89.5 ± 2.9**	0.004 ± 0.001**
Terminalia chebule (TC/CM/BU)	800000	85.4 ± 4.4**	0.007 ± 0.002**
Terminalia chebule (TC/ME/BU)	240000	77.7 ± 2.6**	0.009 ± 0.002**

Results are expressed as Means ± SEM of 4 experiments per group. Each assay was run in duplicate. *$p < 0.05$ compared to 95% O_2; **$p < 0.005$ compared to 95% O_2.

stress mediated by a variety of diseases. While it cannot be concluded from this study that the medicinal values of these plants are due to the presence of natural antioxidants, it is not unreasonable to speculate that some of these plants may lead to the development of potential drugs of great therapeutic values against many diseases that involve cytotoxic oxygen free radicals. The peroxyl radical scavenging activities of the plants make them ideal for functioning as membrane antioxidants.

TABLE IV Peroxyl radical scavenging activities of the plant extracts

PLANT EXTRACT	DILUTION FACTOR	PEROXYL RADICAL SCAVENGED (nmol/ml)
Allium sativum (AS/CM/BU)	25	0.8
Allium sativum (AS/ME/BU)	20,000	42.4
Curcuma longa (CL/CM/BU)	5,000	32.6
Curcuma longa (CL/ME/BU)	22,500	91.8
Ficus bengalensis (FB/CM/BU)	5,000	150.9
Ficus bengalensis (FB/ME/BU)	37,500	1132.2
Ocimum sanctum (OS/CM/BU)	5,000	20.4
Ocimum sanctum (OS/ME/BU)	2,500	10.2
Ocimum sanctum (OS/CM/CL)	2,000	230.1
Phyllanthus embellica (PE/CM/BU)	500,000	1224.0
Phyllanthus embellica (PE/ME/BU)	100,000	1632.0
Terminalia bellerica (TB/CM/BU)	100,000	4730.8
Terminalia bellerica (TB/ME/BU)	200,000	6201.6
Terminalia chebule (TC/CM/BU)	200,000	1958.4
Terminalia chebule (TC/ME/BU)	10,000	775.2

Results are expressed as Means ± SEM of 4 experiments per group. Each assay was run in duplicate.

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References

[1] Aswal, B. S., Bhakuni, D. S., Goel, A. K., Kar, K., Mehrotra, B. N. and Mukherjee, K. C. (1984). Screening of Indian medicinal plants. *Indian Journal of Experimental Biology*, **22**, 327–340.

[2] Zaman, H. (1974). The Southeast Asia region. In: *Traditional Medicine* (ed. Bannerman RH) :Published by World Health Organization, Geneva, Switzerland, pp 231–239.

[3] Chatterjee, A, Pakrashi, S. C. (1991). The Treatise on Indian Medicinal Plants. Publications & Information Directorate, New Delhi, India.

[4] Bagchi, D., Das, D. K., Engelman, R. M., Prasad, M. R. and Subramanian, R. (1990). Polymorphonuclear leukocytes as potential source of free radicals in the ischemic-reperfused myocardium. *European Heart Journal*, **11**, 800–813.

[5] Maulik, N., Avrova, N., Denisova, N., Gogineni, M., Cordis, G. and Das, D. K. (1992). Free radical scavenging activities of gangliosides, a sialic acid containing glycosphingolipid. In: *Oxygen Radicals* (K. Yagi, M. Kondo, E. Niki, T. Yoshikawa eds), Excerpta Medica, Amsterdam, pp 765–768.

[6] Cordis, G. A., Maulik, N. and Das, D. K. (1995). Detection of oxidative stress in heart by estimating the dinitrophenylhydrazine derivative of malonaldehyde. *Journal of Molecular and Cellular Cardiology*, **27**, 1645–1653.

[7] Dobbs, L. G., Gonzalez, R. and Williams, M. C. (1986). An improved method for isolating type II cells in high yield and purity. *American Review of Respiratory Diseases*, **134**, 141–145.

[8] Farnsworth, N. R. (1985). Medicinal plants in therapy. Bulletin of the World Health Organization. **63**, 965–981.

[9] Mukhopadhyay, S. N. and Das, D. K. (1994). *Oxygen Responses, Reactivities, and Measurements in Biosystems.* CRC Press, Florida.

[10] Das, D. K. and Maulik (1994). Evaluation of antioxidant effectiveness in ischemia reperfusion tissue injury. *Methods in Enzymology*, **233**, 601–610.