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Research Article

A COMPARATIVE STUDY ON ANTIOXIDANT POTENTIAL OF NINE INDIAN MEDICINAL PLANTS

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ABSTRACT
A comparative antioxidant potential of nine Indian medicinal plants has been evaluated by 2, 2-diphenyl-1-picrylhydrazyl scavenging activity assay in the present study. Our results strongly suggest that out of the nine medicinal plants, seven (*Terminalia chebula*, *Syzygium cumini*, *Saraca indica*, *Butea monospora*, *Aegle marmelos*, *Picrorhiza kurrooa*, and *Albizia lebbeck*) can be promising sources of potential antioxidants. Among the plants evaluated here, *T. chebula* has highest antioxidative potential and was comparable with that of the reference antioxidant, ascorbic acid. Moreover, the present results confirmed that antioxidant activity of these plants is significantly correlated with total phenolic contents (Spearman's rank correlation coefficient, r_ρ= 0.98; P<0.001) but not with flavonoids. We have also confirmed that antioxidative active constituents from *T. chebula* are methyl gallate and gallic acid. In addition, methyl gallate induced significant increase of leukocyte counts in mouse, indicating its immunomodulatory activity.

Key Words: *Antioxidant Activity, Phenolics, Flavonoids, Indian Medicinal Plants, Methyl Gallate, Immunomodulatory Activity*

INTRODUCTION
It is well established that many of the life threatening diseases are due to the oxidative stress that consequences from an inequity between formation and neutralization of pro-oxidants. Human cells, tissue and peripheral systems have innate antioxidant mechanisms to quench the reactive oxygen species (ROS). However, when the generation of ROS exceeds the scavenging capacity of the system, the excess free radicals damage cellular and extracellular macromolecules including proteins, lipids and DNA leading to cancer, cardiovascular diseases, atherosclerosis, diabetes mellitus, myocardial infarction, ageing, inflammatory diseases, and neurodegenerative disorders such as Alzheimer's disease (Smith *et al.,* 1996; Weinbrenner *et al.,* 2003; Kong *et al.,* 2004 and Lee *et al.,* 2005). Hence, antioxidants are of interest to the food industry and administration of antioxidants provides an adjunct to endogenous mechanism for removing ROS. Some synthetic antioxidants are commercially available such as butylated hydroxy anisole and butylated hydroxyl toluene but are quite unsafe and their toxicity is a problem of concern (Sreelatha *et al.,* 2009). Ethnopharmacology and natural product drug discovery remains an important expectation in the current target-rich, lead-poor scenario. Ayurveda, the science of life, is a comprehensive medical system that has been the traditional system of healthcare in India. This comprehensive medical system has been prescribed many preparations from Indian medicinal plants. It has been documented that several Indian medicinal plants are used as nutritional supplements and possess strong antioxidant activity (Sharma *et al.,* 1992). However, several of them have not been investigated from the pharmaceutical point of view to investigate their antioxidant properties, which could support their use in ROS mediated diseases such as cancer, cardiovascular and Alzheimer's diseases etc. We have reported anti-enterobacterial potential of nine ethnobotanically selected plants traditionally used in different parts of India for the treatment of gastrointestinal disorders in a previous study (Acharyya *et al.,* 2009). In addition, some of these plants are generally used for the treatment of fever, asthma, skin diseases, diabetes, and inflammatory diseases in traditional medical care systems (Kala *et al.,* 2004 and

Kala 2009). In the present study, a comparative antioxidant potential of methanolic extracts of these nine Indian medicinal plants (Acharyya *et al.,* 2009) has been evaluated by 2, 2-diphenyl-1-picrylhydrazyl scavenging activity assay. Phytochemical screening for alkaloids, phenolics, tannins, terpenoids, steroids, and flavonoids was also performed. To the best of our knowledge, no comparative study on antioxidant potential of these nine medicinal plants has been done. Hence, results of the present study on comparative antioxidative potential of these plants could be useful to select them as food supplement or for the treatment of fever; asthma, inflammatory diseases, and skin diseases. Active antioxidant compounds were also isolated and identified from the most active *T. chebula* extract. In addition, immunomodulatory activity of methyl gallate isolated from *T. chebula* has been demonstrated here for the first time.

MATERIALS AND METHODS

Plant materials

Nine Indian medicinal plants [*Albizia lebbeck* (L.) Benth. (Mimosaceae) (voucher number, BSI/CDM/019), *Terminalia chebula* Retz. (Combretaceae) (voucher number, BSI/CDM/424), *Syzygium cumini* (L.) Skeels (Myrtaceae) (voucher number, BSI/PKB-SA/03), *Solanum nigrum* L. (Solanaceae) (voucher number, BSI/PKB-SA/04), *Picrorhiza kurrooa* Royle ex Benth. (Scrophulariaceae) (voucher number, BSI/CDM/315), *Butea monosperma* (Lam.) Taub. (Papilionaceae) (voucher number, BSI/CDM/063), *Saraca indica* auct.non L. (Leguminosae) (voucher number, BSI/PKB-SA/09), *Aegle marmelos* (L.) Correa ex Roxb. (Rutaceae) (Voucher number, BSI/PKB-SA/10), and *Withania somnifera* (L.) Dunal (Solanaceae) (voucher number, BSI/CDM/458)] were included here to compare their antioxidant potential. The assessed plants or plant parts were collected during the month of June 2008 by the authors from the local area (West Bengal) and another region (Uttaranchal) in India. The botanical identification of the plant samples was done by Dr. A. B. D. Selvan and Dr. Madhusudan Mandal, Botanical Survey of India (BSI), Central National Herbarium, Howrah, India. The voucher specimens are conserved at Central National Herbarium, BSI, Botanic Garden and Howrah, India.

Plant Extract Preparation

Plant extracts were prepared by macerating air dried plant materials (500 g) with 2500 ml of methanol in a Soxhlet apparatus for 18 h as described previously (Thakurta *et al.,* 2007; Acharyya *et al.,*, 2009). The freeze-dried material obtained after lyophilization was stored at - 20°C pending further use. Test samples were prepared by re-suspending lyophilized powder in methanol.

Isolation of Active Constituents from T. *Chebula*

Extract was prepared by macerating air dried fruit (1000 g) with methanol and lyophilized powder was obtained as described above. Lyophilized powder was partitioned into water, methanol and ethyl acetate. The ethyl acetate portion (129.1 g) was further portioned into water, methanol, chloroform and ethyl acetate. The ethyl acetate portion (24 g) was chromatographed on silica gel column (Merck, 60-120 mesh, 2 ×25 cm) and successively eluted with a stepwise gradient of chloroform-ethyl acetate (50:50 to 0:100). Active fractions were analyzed by TLC ($CHCl_3$: MeOH:HCOOH, 85:15:1), and fractions with identical TLC patterns were combined. Combined fraction a (1.15 g) and B (4.6 g) were obtained which showed antioxidant activity. Fraction A and B were further chromatographed on silica gel column (Merck, 60-120 mesh, 2 ×25 cm) and successively eluted with a stepwise gradient of chloroform-ethyl acetate (100:0 to 0:100). Column fractions were analyzed by TLC ($CHCl_3$/MeOH/HCOOH, 85/15/1), and fractions with identical TLC patterns were combined. Thus fractions A1 (180 mg) and B1 (360 mg) were obtained. After lyophilization, active fraction A1 was subjected to preparative TLC ($CHCl_3$/MeOH/HCOOH, 90:10:1, and $CHCl_3$/EtOAc/HCOOH, 90:10:1). Active band (R_F 0.6) was eluted with EtOAc and crystallized with hot $CHCl_3$-EtOAc (1:1) and thus active compound A2 was obtained (59 mg). Fraction B1 was further chromatographed on silica gel column (Merck, 60-120 mesh, 2 ×25 cm) and successively eluted with a stepwise gradient of chloroform-ethyl acetate (50:50 to 0:100). Active fractions with

identical TLC pattern (R_F 0.37) were pooled and crystallized with hot $CHCl_3$-EtOAc (1:1) and thus active compound B2 (65 mg) was obtained.

NMR Spectra

The NMR spectra were recorded on a Bruker AV300 Supercon NMR spectrometer equipped with 5 mm BBO probe fitted with pulse field gradient using Topspin 1.3 software version. The ^{1}H and ^{13}C NMR spectra at 300.13 MHz and 75.47 MHz respectively were measured at 295 K. Chemical shift are given on the δ scale and referenced to TMS. Coupling constants are in hertz. Proton spectra were taken using the pulse program zg30 with the set up spectral width (SW) = 4496.403 Hz, TD= 65536, Acquisition Time (AQ) =7.2877 s, NS= 32, relaxation delay (RD) = 1.0 s, 90° pulse width =13.70 μs, DS = 2; for the ^{13}C NMR spectrum, AQ=1.8220 s, RD= 2.0 s, SW=17985.611 Hz, NS= 4800, Line Broadening=1.00 Hz. HSQC experiments were run with hsqcetgpsi2 and hmbcgplpndqf programme respectively. For the HSQC spectrum F1=222.305 ppm, F2=15 ppm, NS=48, DS= 16, AQ=0.1139 s, RG=256. UV spectra were measured using a JASCO V-630 spectrophotometer. Melting points were determined by using a Fisher-Johns melting point apparatus.

Phytochemical Screening

Phytochemical screening for alkaloids, phenolics, tannins, terpenoids, steroids, and flavonoids was performed as described previously (Gülçin *et al.,* 2002 and Mosquera *et al.,* 2007).

Determination of Total Phenolic and Total Flavonoid Contents

Determination of total phenolic and total flavonoid contents was performed following the method described previously (Mosquera *et al.,* 2007 and Acharyya *et al.,,* 2009). Phenolics was determined spectrophotometrically at 650 nm (model U-2000, Hitachi), and expressed as mg/g of gallic acid equivalents (GAE) per lyophylisate. The flavonoids content of the supernatant was measured spectrophotometrically at 415 nm, and expressed as mg/g, based on rutin as standard.

Antioxidant Activity

The antioxidant activity was determined using the previously described method of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Gülçin *et al.,* 2002 and Mosquera *et al.,* 2007). A solution (0.5 mL) of 0.2 mM DPPH in methanol was mixed with the test sample (0.5 mL) in methanol (1.25μg/mL – 1000μg/mL). The reaction mixture was vortexed thoroughly and kept in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm (Jasco V-630, Japan). A solution of 0.2 mM DPPH in methanol was used as control. The ability to scavenge DPPH radical was calculated using the following equation: DPPH radical scavenging activity (%) = $[1-(A_s - A_0)/A] \times 100$ where A is the absorbance of DPPH + methanol; as is the absorbance of DPPH + sample (Extract/pure compound/standard) dissolved in methanol; and A_0 is the absorbance of sample dissolved in methanol. Ascorbic acid was used as positive control. IC_{50} value was determined as the sample concentration required to scavenge 50% of the DPPH free radicals (Mosquera *et al.,* 2007).

Effect on Total Leukocyte Count

Swiss albino mice (20-22 gm) were used in this study. The animals kept in wire-mesh cages were acclimated to laboratory conditions (12 h dark: 12 h light cycles; 24±1 ◦C) and had been free access to food and water *ad libitum*. The study obtained prior approval from the Institutional Animal Ethical Committee, University of Calcutta, India (approval reference no. 797/09/CPCSEA). Animal handling was followed the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The mice were fed by gavage either with methanol (vehicle control) or methyl gallate at different concentrations (0.1, 1.0 and 10.0 mg/kg bodyweight) orally. On day 8, the mice were anesthetized by the intraperitoneal administration of ketamine hydrochloride (5 mg/kg) and xylazine hydrochloride (2 mg/kg) and blood sample was collected by retro-orbital puncture in vial containing Alsevier's solution. The sample was mixed with white blood cells (WBC) diluting fluid in fixed ratio and leukocytes were counted in a haemocytometer chamber.

Statistical analysis

Results are expressed as the mean ± SD. The data were analyzed for statistical significance by one-way ANOVA test; P values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Phytochemical screening for alkaloids, phenolics, tannins, terpenoids, steroids, and flavonoids was performed. The phenolics, tannins, terpenoids and flavonoids were found to be present in the flower extracts of *Butea monospora* and *Saraca indica.* Alkaloids were present in the extracts of *Albizia lebbeck* bark, *Solanum nigrum* fruit, *Aegle marmelos* leaf and *Withania somnifera* root. The phenolics, tannins and flavonoids were present in the extracts of *Terminalia chebula* and *Syzygium cumini.* The steroids were found to be present in the extracts of *Picrorhiza kurrooa* rhizome and *A. marmelos* leaf. Tannins and terpenoids were found also in *A. lebbeck* bark and *P. kurrooa* rhizome. *A. marmelos* contained also phenolics, tannins and flavonoids. Total phenolics and flavonods were determined in the crude extracts.

The antioxidant activity of antioxidative potential of glycoprotein from *S. nigrum* L. (SNL glycoprotein) and antioxidant effect of aqueous extract of *T. chebula* was demonstrated previously (Lee *et al.*,, 2005). The free radical scavenging potential of *P. kurrooa* extract was demonstrated previously by lipid peroxidation assay (Govindarajan *et al.*, 2003). *P. kurrooa*, *T. chebula*, *Butea monosperma*, and *S. nigrum* are generally used for the treatment of gastrointestinal disorder, fever, asthma, inflammatory diseases, and skin diseases in Indian traditional medical care systems (Kala *et al.*, 2004; Kala 2009). Antioxidant activity was evaluated by DPPH assay in the present study. We have compared the antioxidant potential of nine medicinal plants here (Table 1, Table 2 and Table 3). Among them, methanolic extract of *T. chebula* showed the strongest antioxidant activity.

Table 1: The percentage of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity values for each plant extract evaluated at different concentrations

Plant species	DPPH radical scavenging activity (%) (mean ± S.D. of triplicates) at concentration (µg/ml)				
	1.25	**2.5**	**5**	**10**	**20**
Ascorbic acid (positive control)	12.53±1.18	21.61±0.45	40.58±3.12	84.55±1.47	89.58± 0.30
S. cumini	5.03±1.14	15.93±0.01	33.34±3.69[a]	61.53±1.45 [a]	85.96±0.63
S. indica	0	8.67±4.26	13.55±4.52 [a]	25.22±2.68 [a]	49.77±1.52
T. chebula	15.74±3.18	35.03±1.07	67.82±0.07	85.88±0.35	86.48±0.24
Methyl gallate	29.70±0.72[b]	62.86±4.10[b]	85.06±0.27[b]	85.39±0.25	ND
Gallic acid	45.43±0.33[b]	83.66±0.55[b]	86.81±0.21[b]	87.22±0.04	ND

ND, not determine. [a]vs T. chebula with p<0.001; [b] vs Ascorbic acid with p<0.001

Although *S. cumini*, *S. indica*, *A. marmelos* and *B. monospora* showed significant antioxidant activity, they exhibited lower activity than *T. chebula*. Two of the plants (*T. chebula* and *S. cumini*) gave scavenging activity >50% at a concentration of 10 µg/mL. Three of the plants (*B. monospora*, *A. marmelos* and *P. kurrooa*) showed scavenging activity >50% at concentrations between 62.5 to 250 µg/mL. *W. somnifera* and *S. nigrum* gave weakest antioxidant activity showing scavenging activity > 50% at a concentration of 1000 µg/mL. The strongest IC_{50} values were given by the extracts of *T. chebula* (3.67 µg/mL) followed by *S. cumini* (8.0 µg/mL) (Table 3).

Table 2: The percentage of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity values for each plant extract evaluated at different concentrations

10.5	DPPH radical scavenging activity (%) (mean ± S.D. of triplicates) at concentration (µg/ml)				
	31.5	**62.5**	**125**	**250**	**500**
A. lebbeck	10.75± 0.81	18.53± 1.75	28.90±6.56[a]	48.90±12.14[c]	73.34±11.60
A. marmelos	30.97±0.50	48.00±4.75	76.92±5.60 [b]	88.98±0.37	90.41±0.16
B. monospora	24.26±2.00	49.29±6.85	81.65±5.07	87.66±0.89	88.52±1.29
P. kurrooa	14.45±1.53	34.25±7.12	54.70±1.56 [a]	82.80±3.91[d]	89.85±0.06
S. nigrum	5.51±0.73	12.41± 9.41	19.63±10.33 [a]	31.13±11.27	42.21±4.88
W. somnifera	6.36±0.33	8.91±2.25	13.31±2.29 [a]	24.57±1.78	42.33±2.12

ND, not determine. [a] vs B. monospora with p<0.001; [b] vs P. kurrooa with p<0.003; [c] vs A. marmelos and B. monospora with p=0.005; [d] vs A. lebbeck with p<0.02. DPPH radical scavenging activity (%) of Ascorbic acid (positive control) at 20 µg/ml was 89.58± 0.30.

Table 3: IC_{50} values for each of the plant materials and purified compounds obtained with DPPH test

Characteristics of the samples	Samples	IC_{50} (µg/ml) (Mean ± S.D. of triplicates)
Positive control	Ascorbic acid	5.91 ± 0.20
Methanol crude extract	*T. chebula*	3.67 ± 0.01[a]
	S. cumini	8.00 ± 0.30[b, c]
	S. indica	22.78 ± 1.01 [b, d]
	A. marmelos	69.22 ± 7.09 [b, e]
	B. monospora	70.28 ± 5.26 [b, f]
	P. kurrooa	128.74 ± 1.68 [b, g]
	A. lebbeck	306.73 ± 67.86 [b, h]
	W. somnifera	664.26 ± 27.03 [b]
	S. nigrum	664.34 ± 94.61 [b]
Purified compounds from	Methyl gallate	2.25 ± 0.08 [i]
T. chebula	Gallic acid	1.395 ± 0.007 [i]

[a] vs ascorbic acid with p<0.001; [b] vs T. chebula wit p<0.001; [c] vs S. indica with p<0.001;
[d] vs A. marmelos with p<0.001; [e] vs P. kurrooa with p<0.001; [f] vs P. kurrooa with p<0.001;
[a] vs ascorbic acid with p<0.001; [b] vs T. chebula with p<0.001; [c] vs S. indica with p<0.001; [d]vs A. marmelos with p<0.001; [e] vs P. kurrooa with p<0.001; [f] vs P. kurrooa with p<0.001; [g] vs A. lebbeck with p<0.02; [h] vs W. somnifera and S. nigrum with p<0.002; [i] vs Ascorbic acid with p<0.001.

In addition, none of the extracts tested in the present study released haemoglobin and hence were not cytotoxic to human erythrocytes at concentrations of up to 32 mg/ml. The crude methanol extracts of these plants contained significant amounts of phenolics (10.6±0.9 - 365.9±16.1 mg/g) and flavonoids (3.0 ± 1.0 - 65.6±2.8 mg/g). It has been previously reported that polyphenolic compounds are widely distributed in plant kingdom and they have shown to have antioxidant properties (Badami *et al.*, 2003; Dar *et al.*, 2005). Phenolic and flavonoid contents of the nine plant extracts were plotted separately against their corresponding antioxidant activity (Figure 1). Antioxidant activity was expressed as rank of each IC_{50} value among the IC_{50} values of nine plants. The rank 9 (IC_{50} value=3.67 µg/ml) indicates the strongest antioxidant activity and rank 1 (IC_{50}, 664.34 µg/mL) indicates the weakest antioxidant activity. Statistical analysis (Das 1980) of the data shows that the Spearman's rank correlation coefficient (r_ρ) between the phenolics content and antioxidant activity is 0.98 and that this value is statistically significant

(P<0.001). However, r_ρ between the flavonoids content and antioxidant activity is 0.65, which is not statistically significant (P>0.05).

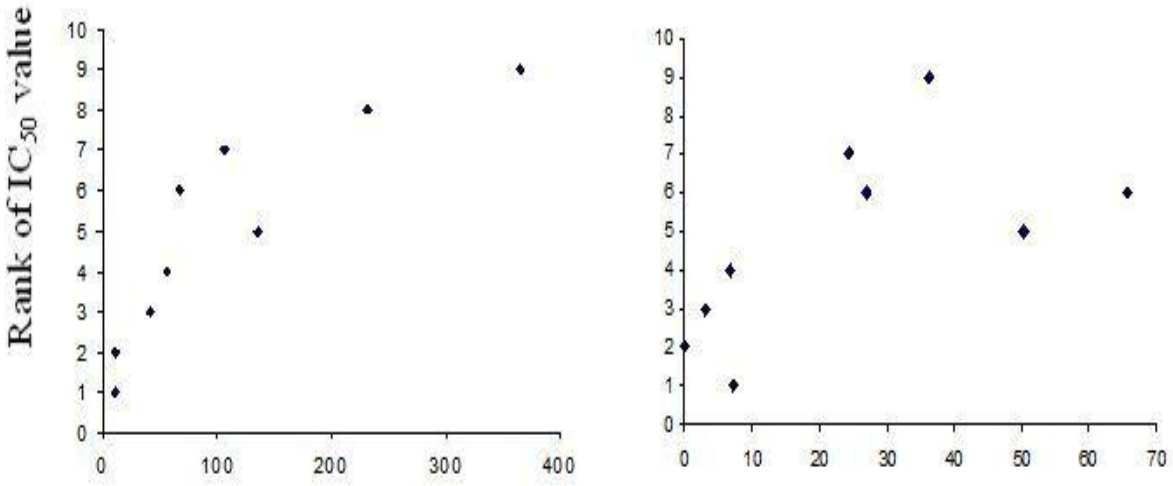

Figure 1: Correlation between total phenolic contents (A) and total flavonoid contents (B) of the 9 plant extracts and their antioxidant activities. Antimicrobial activity was expressed as rank of each IC_{50} value among the IC_{50} values of 9 plants. The rank 9 (IC_{50} value=3.67 µg/ml) indicates the strongest antioxidant activity and rank 1 (IC_{50} value=664.34 µg/ml) indicates the weakest antioxidant activity among the plant extracts tested. Total phenolic content and total flavonoid contents are expressed as gallic acid equivalent (mg of GAE/g) and rutin equivalent (mg of rutin/g), respectively.

Since the methanolic extract of *T. chebula* showed the strongest antioxidant activity, we attempted to purify the active principles from this plant. We isolated two active compounds from the extract of *T. chebula*, which were identified as methyl gallate (compound A2) and gallic acid (compound B2) by 1D and 2D NMR spectral studies and HSQC experiment. Methyl gallate was identified based on the following evidence: white crystals ($CHCl_3$-MeOH 80:20); mp 201-202^{o}C; UV (MeOH) λ_{max} (log ε): 276 (0.568) nm, 220 (1.187); ^{1}H NMR (MeOH-d4): 3.69 (3H, s, -$COOCH_3$), 6.92 (1H, s); ^{13}C NMR: 169.0, 146.5, 139.8, 121.5, 110.1, 52.3. From HSQC experiment it was also evident that methine carbon resonances at δ_c 110.1 and 52.3 showed correlation with the signals at δ_H 6.92 and 3.69, respectively. Gallic acid (compound B2) was identified based on the following evidence: B2 **:** white crystals ($CHCl_3$-MeOH 80:20); mp 249-250 ^{o} C; UV (MeOH) λ_{max} (log ε), 272 (0.131), 218 (0.528). ^{1}H NMR (MeOH-d4): 6.99 (1H, s); ^{13}C NMR: 170.39, 146.38, 139.58, 121.96 and 110.32. From HSQC experiment it was also evident that methine carbon resonances at δ_c 110.32 showed correlation with the signal at δ_H 6.99.

The anticancer, antianaphylaxis, antioxidant and antidiabetic activities of *T. chebula* have been reported previously (Shin *et al.*, 2001; Sabu *et al.*,, 2002; Saleem *et al.*,, 2002; Lee *et al.*, 2005). However, to the best of our knowledge, active antioxidant constituents of *T. chebula* have so far not been extensively investigated. In the present study, methyl gallate and gallic acid were isolated as the active constituents from *T. chebula* and showed significant antioxidant activity (> 50%) at a concentration of 2.5 µg/mL (Table 3). Among the purified compounds and crude extracts, gallic acid (1.39 µg/ml) showed the strongest IC_{50} values followed by methyl gallate (2.25 µg/ml) (Table 3). In addition, leukocyte counts were increased significantly, when the mouse was treated with methyl gallate at a concentration of 10 mg/kg body weight daily for 7 days, suggesting its immunomodulatory activity (Figure 2).

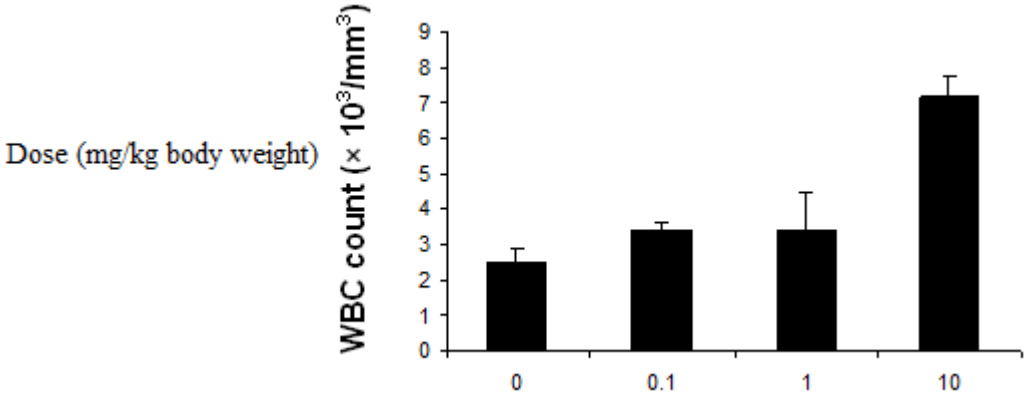

Figure 2: Effect of methyl gallate on total leukocyte count in Swiss albino mice. Mouse was orally administered with methyl gallate. Values were mean ± SD for 5 adult Swiss albino mice. For mice treated with 10 mg/kg body weight, leukocyte counts were increased significantly ($p<0.05$ vs. normal control).

Conclusion

Our results strongly suggest that out of the nine medicinal plants included here, seven (*T. chebula*, *S. cumini*, *S. indica*, *B. monospora*, *A. marmelos*, *P. kurrooa*, and *A. lebbeck*) can be promising sources of potential antioxidants. Among the plants tested here, *T. chebula* has highest antioxidative potential. In addition, the free radical scavenging effect of *T. chebula* and *S. cumini* was comparable with that of the reference antioxidant, such as ascorbic acid. Therefore, in addition to their therapeutic applications, these plant extracts could be used as dietary supplements to protect humans from oxidative damage. Furthermore, the present results confirmed that antioxidant activity of these plants is significantly correlated with total phenolic contents but not with flavonoids. We have also confirmed that antioxidative active constituents from *T. chebula* are methyl gallate and gallic acid that could serve as free radical inhibitors or scavengers. Results of the present study on comparative antioxidative potential of these plants could be useful to select them as food supplement or for the treatment of fever; asthma, inflammatory diseases, and skin diseases. In addition, immunomodulatory activity of methyl gallate isolated from *T. chebula* might be useful for the treatment of some of the immunological disorders associated with decrease in leukocyte counts.

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REFERENCES

Acharyya S, Patra A and Bag PK (2009). Evaluation of the antimicrobial activity of some medicinal plants against enteric bacteria with particular reference to multi-drug resistant Vibrio cholerae. *Tropical Journal of Pharmaceutical Research* **8** 231-237.

Badami S, Moorkoth S, Rai RS, Kannan E and Bhojraj S (2003). Antioxidant activity of Caesalpinia sappan hearwood. *Biological and Pharmaceutical Bulletin* **26** 1534-1537.

Dar A, Faizi S, Naqvi S, Roome T, Zikr-Ur-Rehman S, Ali M, Firdous S and Moin ST (2005). Analgesic and antioxidant activity of mangiferin and its derivatives: The structure activity relationship. *Biological and Pharmaceutical Bulletin* **28** 596-600.

Das D (1980). Statistics in biology and psychology, volume 1 and 2 *Academic Publishers, Calcutta,* India.

Govindarajan R, Vijayakumar M, Rawat AK and Mehrotra S (2003). Free Radical Scavenging Potential of Picrorhiza kurrooa Royle ex Benth. *Indian Journal of Experimental Biology* **41** 875-879.

Gülçin Ý, Oktay M, Küfrevioglu ÖI and Aslan A (2002). Determination of antioxidant activity of lichen Cetraria islandica (L) Ach. *Journal of Ethnopharmacology* **79** 325-329.

Kala CP, Farooquee NA and Dhar U (2004). Prioritization of medicinal plants on the basis of available knowledge, existing practices and use value status in Uttaranchal, India. *Biodiversity and Conservation* **13** 453-469.

Kala CP (2009). Aboriginal uses and management of ethnobotanical species in deciduous forests of Chhattisgarh state in India. *Journal of Ethnobiology and Ethnomedicine 5* 20.

Kong X, Wang R, Xue Y, Liu X, Zhang H, Chen Y, Fang F and Chang Y (2004). Sirtuin 3, a new target of PGC 1alpha, plays an important role in the suppression of ROS and mitochondrial biogenesis. *PLoS One 5* 11707.

Lee H, Won NM, Kim KH, Lee H, Jun W and Lee K (2005). Antioxidant effects of aqueous extract of Terminalia chebula in vivo and in vitro. *Biological and Pharmaceutical Bulletin* **28** 1639-1644.

Mosquera OM, Correa YM, Buitrago DC and Niño J (2007). Antioxidant activity of twenty five plants from Colombian biodiversity. *Memórias do Instituto Oswaldo Cruz Rio de Janeiro* **102** 631-634.

Sabu MC and Kuttan R (2002). Antidiabetic activity of medicinal plants and its relationship with their antioxidant property. *Journal of Ethnopharmacology* **81** 155-160.

Saleem A, Husheem M, Harkonen P and Pihlaja K (2002). Inhibition of cancer cell growth by crude extract and the phenolics of Terminalia chebula Retz fruit. *Journal of Ethnopharmacology* **81** 327-336.

Sharma HM, Hanna A, Kauffman EM and Newman HAI (1992). Inhibition of human low-density lipoprotein oxidation in vitro by Maharishi Ayurveda harbal mixtures. *Pharmacology Biochemistry and Behavior* **43** 1175-1187.

Shin TY, Jeong HJ, Kim DK, Kim SH, Lee JK, Kim DK, Chae BS, Kim JH, Kang HW, Lee CM, Lee KC, Park ST, Lee EJ, Lim JP, Kim HM and Lee, YM (2001). Inhibitory action of water soluble fraction of Terminalia chebula on systemic and local anaphylaxis. *Journal of Ethnopharmacology* **74** 133-140.

Smith MA, Perry G, Richey PL, Sayre LM, Anderson VE, Beal MF and Kowall N (1996). Oxidative damage in Alzheimer's. *Nature* **382** 120-121.

Sreelatha S and Padma PR (2009). Antioxidant Activity and Total Phenolic Content of Moringa oleifera Leaves in Two Stages of Maturity. *Plant Foods Human Nutrition* **64** 303-311.

Thakurta P, Bhowmik P, Mukherjee S, Hajra TK, Patra A and Bag PK (2007). Antibacterial, antisecretory and antihemorrhagic activity of Azadirachta indica used to treat cholera and diarrhea in India. *Journal of Ethnopharmacology* **111** 607-612.

Weinbrenner T, Cladellas M, Covas MI, Fito M, Tomas M, Senti M, Bruguera J and Marrugat J (2003). High oxidative stress in patients with stable coronary heart disease. *Atherosclerosis* **168** 99-106.