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Evaluation of antioxidant potential in selected green leafy vegetables

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ABSTRACT

Green leafy vegetables represent a class of underexploited plants that are stipulated to be rich sources of natural antioxidants. A fundamental study of free radical-scavenging activity in four plant species, namely *Trigonella foenum-graecum, Centella asiatica, Sauropus androgynus and Pisonia alba,* was carried out by measuring the ability of methanol extracts of these plants to scavenge radicals generated by *in vitro* systems and by their ability to inhibit lipid peroxidation. The levels of non-enzymatic antioxidants were also determined by standard spectrophotometric methods. Correlation and regression analysis established a positive correlation between some of these antioxidants and the *in vitro* free radical-scavenging activity of the plant extracts. The conclusions drawn from the study indicate that *in vivo* studies, isolation and analysis of individual bioactive components will reveal the crucial role that these plants may play in several therapeutic formulations.

1. Introduction

Reactive oxygen species (ROS) is an entire class of highly reactive molecules derived from the metabolism of oxygen and, is often generated as byproducts of biological reactions or from exogenous factors. *In vivo*, some of these ROS play positive roles in cell physiology; however they may also cause great damage to cell membranes and DNA, inducing oxidation that causes membrane lipid peroxidation, decreased membrane fluidity, and other DNA mutations, leading to cancer, degenerative, and other diseases (Finkel & Holbrook, 2000). Thus, there is an increased evidence for the participation of these free radicals in the aetiology of diseases like cancer, diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative disorders, ageing, etc.

Mammalian cells possess elaborate defence mechanisms for radical detoxification. Antioxidants are agents, which scavenge the free radicals and prevent the damage caused by them. Some of these compounds are of exogenous nature and are obtained from food. Examples include antioxidants like α-tocopherol, β-carotene, and ascorbic acid, and some micronutrient elements such as zinc and selenium (Halliwell & Gutteridge, 1998).

Drugs for treatment of diseases, like arthritis, cancer, diabetes, etc., formulated on synthetic antioxidants like butylated hydroxytoluene, butylated hydroxyanisole and tertiary butylhydroquinone can be very expensive. Providing modern healthcare to rural people is still a far-reaching goal, due to economic constraints (Grover, Yadav, & Vats, 2002). Hence, it is mandatory that we resort to forms of medicine that mainly depend on the locally available plant materials to cure various health disorders.

Green leafy vegetables constitute a major part of any balanced diet and are good sources of minerals and vitamins. The ethnobotanical reports offer information on the medicinal properties of green leafy vegetables which include details on their antidiabetic (Kesari, Gupta, & Watal, 2005), antihistaminic (Yamamura, Ozawa, Ohtani, Kasai, & Yamasaki, 1998), anticarcinogenic (Khanna, Rizvi, & Chander, 2002), and antibacterial activities (Kubo, Fijita, Kubo, Nehei, & Gura, 2004). These beneficial effects of green leafy vegetables are attributed, at least in part to, antioxidants. The major active antioxidant compounds are flavonoids, flavones, isoflavones, lignans, catechins, and isocatechins. Hence, attention has been devoted to the commonly available green leafy vegetables, which though underexploited in most cases, possess a tremendous potential to help people overcome the deadly diseases of modern society.

In this context, we have attempted to find the bioactive components that might be responsible for the medicinal properties of four such green leafy vegetables, namely *Centella asiatica, Trigonella foenum-graecum, Sauropus androgynus and Pisonia grandis*. These plants have been employed in traditional medicine since time immemorial. Although preliminary research work has been carried out in *Trigonella foenum-graecum* and *Centella asiatica*, their intrinsic medicinal value remains underexploited to this date, and the other two plant species have not been exposed to exhaustive research work. Therefore, we have attempted to evaluate the antioxidant potential of these plants by carrying out different *in vitro* free radical-scavenging assays and correlate the results obtained with the levels of certain non-enzymatic antioxidants present in the plants. The results obtained can be crucial in understanding the

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role played by these green leafy vegetables in the prevention and treatment of several diseases.

2. Materials and methods

2.1. Samples

The leaves of the plants, *Centella asiatica, Trigonella foenum-graecum, Sauropus androgynus and Pisonia grandis* were collected locally from various places in Coimbatore (Tamil Nadu, India). The plant specimens were further authenticated at the Botanical Survey of India, Coimbatore. Voucher specimens of the sample (DBT 01, DBT 02, DBT 03 and DBT 04) have been deposited in the herbarium of the department.

2.2. Chemicals used

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2-azobis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), sodium nitroprusside, sulphanilamide, naphthyl ethylenediamine dihydrochloride, 2-deoxyribose, thiobarbituric acid (TBA), trichloroacetic acid (TCA), sodium dodecyl sulphate (SDS) and ammonium molybdate were all of analytical grade.

2.3. Preparation of crude plant extracts

The leaves were shade-dried for about a week and then crushed to make a coarse powder. The dried powder was weighed and solvent extraction using methanol was performed at a 10% concentration. Exhaustive extraction was carried out for about 36 h in a shaker at 37 °C with a gentle shaking. The extracts were then evaporated at room temperature. The residues obtained were re-evaporated to remove impurities and were used to carry out radical-scavenging assays. The remaining residue was stored in desiccators for further use.

2.4. Free radical-scavenging assays

2.4.1. Total antioxidant capacity assay

Suitable working standards (50–250 μg/ml) of the plant residues were prepared by dissolving the extracts in water. Aliquots (0.1 ml) of the sample were mixed with 1 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped with silver foil and incubated at 95 °C for 90 min (Umamaheswari & Chatterjee, 2008). The tubes were cooled to room temperature and the absorbance was measured at 695 nm against a blank. Ascorbic acid was used as a standard. Total antioxidant capacity was expressed as equivalents of ascorbic acid (Raghavan et al., 2003).

2.4.2. DPPH radical-scavenging assay

DPPH scavenging activity was measured by the slightly modified spectrophotometric method of Brand-Williams, Cuvelier, and Berset (1995). A solution of DPPH in methanol (6×10^{-5} M) was prepared freshly (Mensor et al., 2001). A 3 ml aliquot of this solution was mixed with 100 μl of the samples at varying concentrations (50–250 μg/ml). The solutions in the test tubes were shaken well and incubated in the dark for 15 min at room temperature. The decrease in absorbance was measured at 517 nm. The

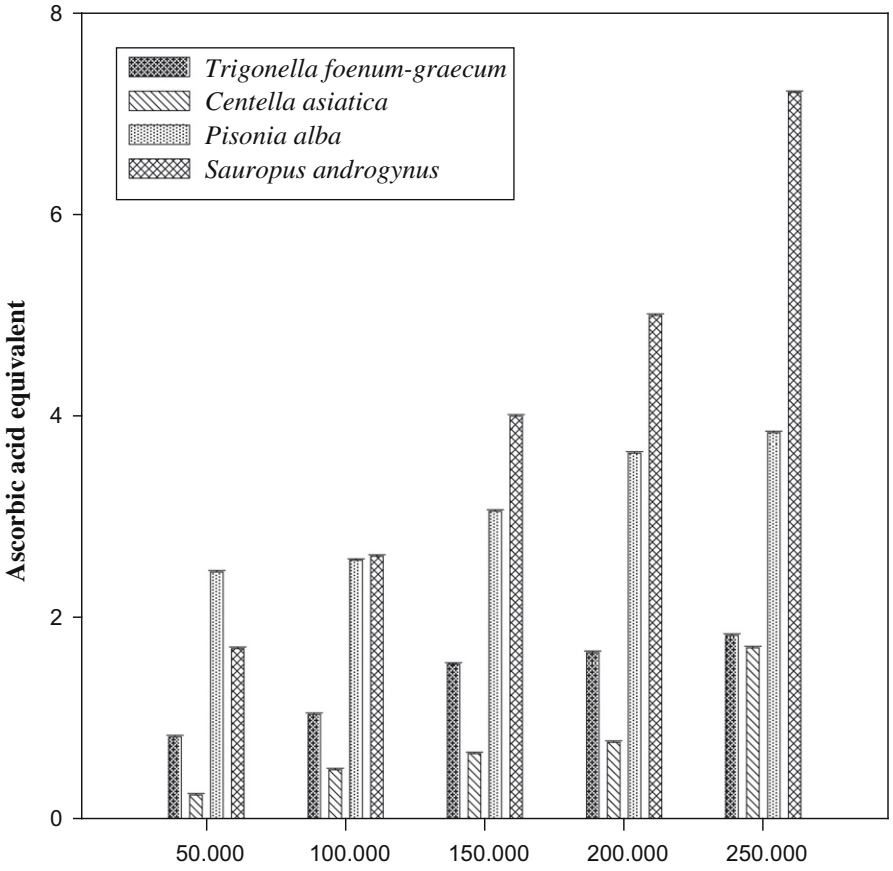

Fig. 1. Total antioxidant capacity of methanol extracts of various plant species expressed as concentrations in terms of ascorbic acid equivalents. The values represent the mean of triplicates ± S.E. for each concentration.

percentage inhibition of the radicals due to the antioxidant property of the extracts was calculated using the formula:

$$\%\text{inhibition} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100\%$$

2.4.3. ABTS radical cation-scavenging assay

The assay was performed by a slightly modified protocol of Re et al. (1999). ABTS solution (7 mM) was reacted with ammonium persulphate (2.45 mM) solution and kept for 12–16 h in the dark, to produce a dark coloured solution containing ABTS radical cations. The initial absorbance was measured at 745 nm. This stock solution was diluted with methanol to give a final absorbance value of about 0.7 (±0.02) (Mensor et al., 2001) and equilibrated at 30 °C. Different concentrations of the sample (50–250 μg/ml) were prepared by dissolving the extracts in water. About 0.3 ml of the sample was mixed with 3 ml of ABTS working standard in a microcuvette. The decrease in absorbance was measured exactly one minute after mixing the solution, then up to six minutes. The final absorbance was noted. The percentage inhibition was calculated according to the formula:

$$\%\text{inhibition} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100\%$$

2.4.4. Lipid peroxidation inhibition assay

The lipid peroxidation assay was carried out by a modified procedure of the spectrophotometric determination of Ohkawa, Ohishi, and Yagi (1979). Goat liver was washed thoroughly in cold phosphate buffered saline (pH 7.4). The liver was then homogenised in a homogeniser to give a 10% homogenate. The homogenate was filtered using cheesecloth to remove the unwanted debris. The filtrate was centrifuged at 10,000 rpm for 10 min under refrigerated conditions. The supernatant was then used to carry out the assay. To 0.5 ml of the 10% homogenate, 0.5 ml of the extracts at various concentrations (50–250 μg/ml) was added. To this, 0.05 ml of 0.07 M ferrous sulphate was added. The solution was incubated at room temperature for 30 min. To the incubated solution, 1.5 ml of 20% acetic acid (pH 3.5), 1.5 ml of 0.8% TBA (in 1.1% SDS) and 0.05 ml of 20% TCA were added. The tubes were vortexed to ensure appropriate mixing. The tubes were then incubated at 100 °C for 1 h and cooled to room temperature. About 5 ml of butanol was added to each tube. The solution was mixed well and then centrifuged at 3000 rpm for 10 min. The upper layer was used to read the absorbance at 532 nm. The percentage inhibition was then calculated.

$$\%\text{inhibition} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100\%$$

2.5. Determination of non-enzymatic antioxidants

2.5.1. Determination of flavonoids

A slightly modified version of the spectrophotometric method was used to determine the flavonoid contents of samples. Fresh leaves (1 g) were weighed and ground with 20 ml of 80% aqueous methanol in a mortar and pestle. The ground sample was filtered with Whatman filter paper No. 42 and a clear filtrate was obtained. A 0.5 ml aliquot of this sample was taken in a test tube and 3 ml of distilled water and 0.3 ml of 5% sodium nitrite were added. The solution was mixed well and allowed to stand at room temperature for 5 min. To this solution, 0.6 ml of 10% aluminium chloride was added. After 6 min, 2 ml of 1 M sodium hydroxide was added to the test tube. The solution was then diluted with distilled water to make the final volume up to 10 ml. The absorbance was read at 510 nm. Flavonoid contents were then calculated using a stan-

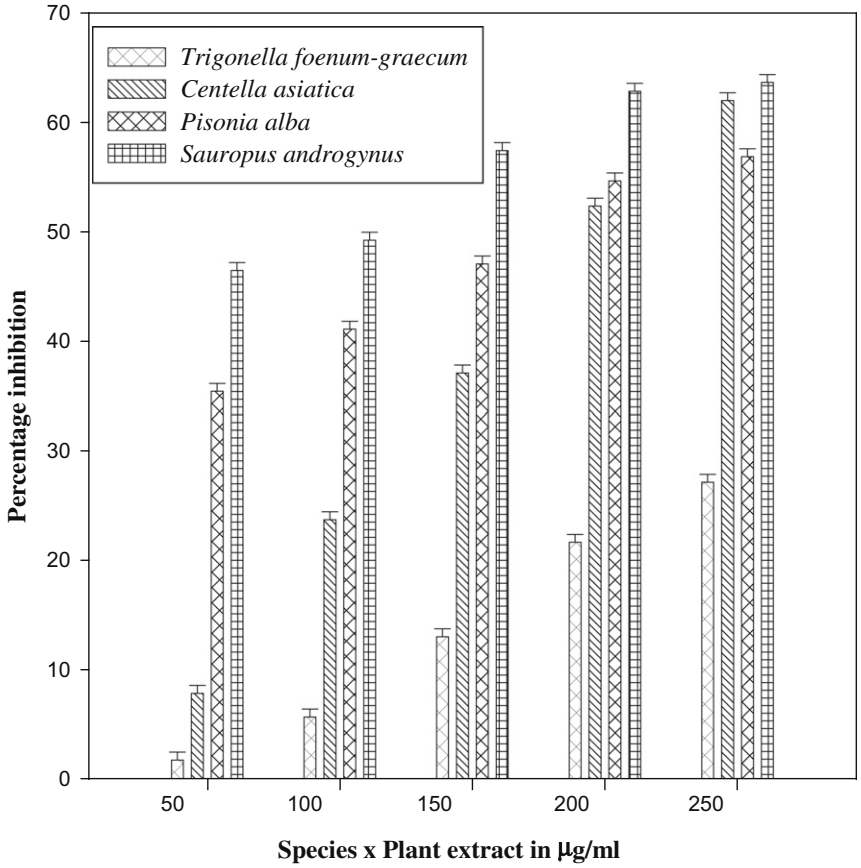

Fig. 2. Percentage inhibition of the DPPH radicals by the methanol extracts of plant species. The values represent the mean of triplicates ± S.E. for each concentration.

dard calibration curve, prepared from rutin (Karadeniz, Burdurlu, Koca, & Soyer, 2005).

2.5.2. Determination of total polyphenol content

Fresh leaves (1 g) were weighed and ground with 10 ml of 80% aqueous ethanol in a mortar and pestle. The ground sample was then centrifuged at 10,000 rpm for 20 min. The supernatant was removed and the pellets were re-extracted in 5 ml ethanol. This sample was again centrifuged at 5000 rpm for 20 min. The supernatants were pooled and then allowed to boil to remove the ethanol. The residue obtained was dissolved in 10 ml distilled water.

Total phenolic contents were determined according to the spectrophotometric methods of Tanner and Brunner (1979), and Kaur and Kapoor (2002). To 0.5 ml of a methanolic solution of the extracts, 7 ml of distilled water and 0.5 ml of Folin–Ciocalteau reagent were added and mixed well. After 3 min, 2 ml of 20% sodium carbonate was added and mixed well again. Absorbance of the resultant solution was read at 720 nm, after 1 h in a water bath at 25 °C (Karadeniz et al., 2005). The total polyphenol content was calculated from the standard calibration curve obtained from gallic acid.

2.6. Statistical analysis

Analysis of Variance (ANOVA) and Duncan's Multiple Range Test were carried out on the values obtained in the experiment. Correlation and regression analysis were also carried out to determine the relationship between the free radical-scavenging activity of the plant extracts and the natural non-enzymatic antioxidants present in the green leafy vegetables. The software SigmaStat 3.5 (Aspire Software International, Ashburn, VA) was used to perform the statistical analysis.

3. Results

The methanol extracts of the four plant species at different concentrations ranging from 50 to 250 μg/ml were tested for their ability to scavenge free radicals generated by *in vitro* systems. The percentage inhibition for each concentration and IC_{50} values of the extracts were calculated. An IC_{50} value is the concentration of the sample required to scavenge 50% of the free radicals present in the system or to inhibit 50% of lipid peroxidation. Statistical analysis of the results obtained by ANOVA indicates that there is a statistically significant interaction between the species and concentration of the extracts at the level of $p \leqslant 0.001$. Statistical analysis was also used to determine the mean value across the concentration range for each assay, to facilitate the comparison of the free radical-scavenging activities of the methanol extracts of the four plant species. From the results, it has been inferred that the free radical-scavenging activity of the plant extracts increases in a concentration-dependent manner.

3.1. Total antioxidant activity of plant extracts

The grouped bar chart (Fig. 1) represents the total antioxidant capacity of the methanol extracts of the plant species at each concentration, expressed in terms of equivalents of ascorbic acid. The results obtained imply that the methanol extracts of *Sauropus androgynus* exhibit the maximum total antioxidant capacity.

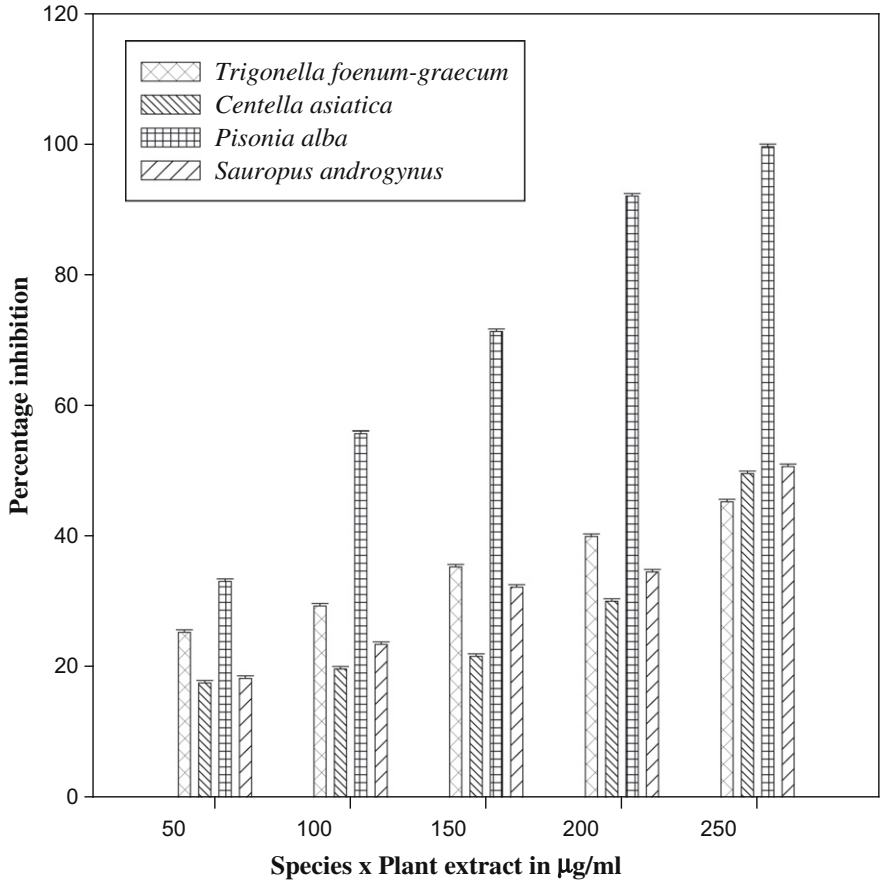

Fig. 3. Percentage inhibition of the ABTS cation radicals by the methanol extracts of plant species. The values represent the mean of triplicates ± S.E. for each concentration.

3.2. DPPH scavenging activity of plant extracts

The bar chart for DPPH radical-scavenging activity of the methanol extracts of the plant species (Fig. 2) elucidating the mean values across the concentration range, indicates that the methanol extracts of *Sauropus androgynus* are more potent in scavenging the DPPH radicals generated *in vitro*, when compared to the extracts of the other plant species.

3.3. ABTS radical-scavenging activity of plant extracts

The grouped bar chart (Fig. 3) indicates the ABTS radical cation-scavenging potential of the methanol extracts of the four different plant species at each concentration, in the range of 50–250 μg/ml. The mean values across the concentration range indicate that the methanol extracts of *Pisonia alba* are highly potent in neutralising ABTS cation radicals.

3.4. Lipid peroxidation inhibition activity of plant extracts

The bar chart (Fig. 4) depicts the inhibition of lipid peroxidation activity of the methanol extracts of the plant species. The mean values across the concentration range indicate that the methanol extracts of *Centella asiatica* exert a better inhibition of lipid peroxidation, when compared to the other plant extracts.

The IC_{50} values of the methanol extracts of the four plant species for each assay are tabulated in Table 1, which indicate the ability of the plant extracts to scavenge free radicals. In addition, the IC_{50} values of ascorbic acid for the assays were also computed. The values are as follows: for DPPH radicals the IC_{50} value is 33.2 μg/ml, for ABTS cation radicals the IC_{50} value has been found to be 8 μg/ml and the IC_{50} value for inhibition of lipid peroxidation is 19.2 μg/ml. A comparison of these values with those laid out in the table however, indicates the plant species are not as potent as ascorbic acid. It has been well established that ascorbic acid is one of the most potent free radical scavengers.

Table 1
Comparison of the IC_{50} values of the methanol extracts of four plant species.

Plant species	IC_{50} values for the free radical-scavenging assays (μg/ml)		
	DPPH radicals	ABTS cation radicals	Inhibition of lipid peroxidation
Trigonella foenum-graecum	435	300	240
Centella asiatica	200	115	90
Pisonia alba	175	80	505
Sauropus androgynus	85	350	350

3.5. Flavonoid and total phenol content of plant species

Fig. 5 represents the levels of flavonoids and total phenols present in the fresh leaves of the plant species. From the figure, it is inferred that *Sauropus androgynus* has been found to possess the maximum levels of flavonoids and total phenols.

3.6. Correlation and regression analysis

A correlation and regression analysis was performed to determine the relationship between the non-enzymatic antioxidants

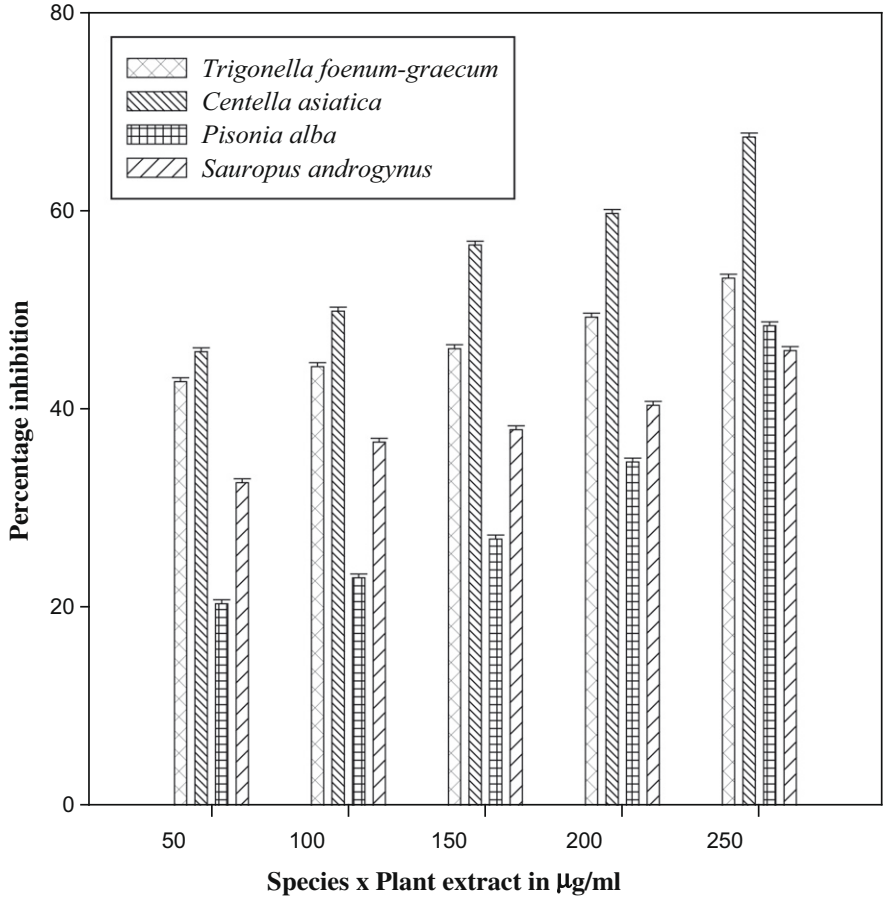

Fig. 4. Percentage inhibition of the lipid peroxidation by the methanol extracts of plant species. The values represent the mean of triplicates ± S.E. for each concentration.

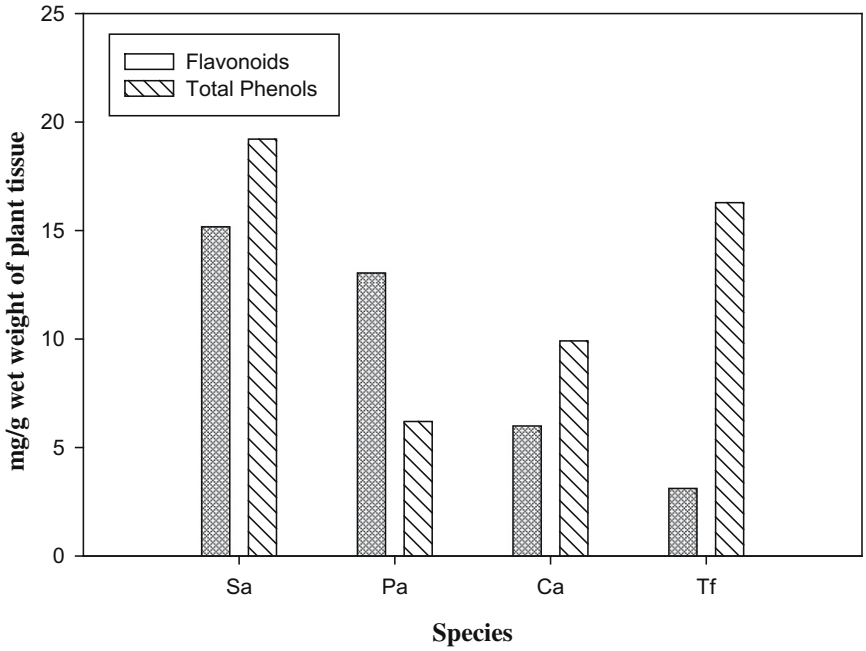

Fig. 5. Levels of flavonoids and total phenols present in various plant species. The values represent the mean of triplicates ± S.D. Sa – *Sauropus androgynus*; Pa – *Pisonia alba*; Ca – *Centella asiatica*; Tf – *Trigonella foenum-graecum*. For flavonoids, results are expressed as mg of rutin per kg of fresh material. For total phenols, results are expressed as mg of gallic acid per kg of fresh material.

and the total antioxidant capacity of the plant extracts. The flavonoids have been found to have a statistically significant influence on the antioxidant activity of the methanol extract of the plant species (Methanol extracts: $r^2 = 0.799$ and $p < 0.01$). On the other hand, there seems to be no statistically significant relation between the total phenols and the antioxidant activity of the plant extracts.

The results obtained suggest that the *in vitro* free radical-scavenging activity of these plants is strongly dependent on the non-enzymatic antioxidants present in the plants, though the degree of contribution of each phytochemical group to the free radical-scavenging activity varies considerably in each extract of the four plant species.

4. Discussion

Oxidative stress has been implicated in the pathology of many diseases, inflammatory conditions, cancer and ageing. Antioxidants may offer resistance to oxidative stress by scavenging free radicals, inhibiting lipid peroxidation, etc., and thus prevent the onset of deadly diseases. Apart from the antioxidants synthesised naturally, the body requires a supplement of dietary antioxidants, which can be obtained only by the consumption of an antioxidant-rich diet. Green leafy vegetables, some of which, underexploited, are proven sources of essential nutrients like vitamin A and vitamin C, carotenoids, flavonoids, phenols, etc. Their tremendous potential as dietary therapeutic agents has remained in the dark and needs extensive documentation.

4.1. In vitro free radical-scavenging assays

The phosphomolybdate method has been routinely used to evaluate the total antioxidant capacity of extracts (Preito, Pineda, & Aguliar, 1999). In the presence of the extracts, Mo (VI) is reduced to Mo (V) and forms a green-coloured phosphomolybdenum V complex, which shows maximum absorbance at 700 nm. From the results obtained, it can be seen that methanol extracts of all the plants possessed significant antioxidant activity which increased in a concentration-dependent manner.

DPPH assay is one of the most widely used methods for screening the antioxidant activity of plant extracts. The assay is based on the measurements of the antioxidants' ability to scavenge the stable radical DPPH. DPPH is a stable nitrogen-centred free radical, which produces violet colour in methanol solution. DPPH radicals react with suitable reducing agents, during which the electrons become paired off and the solution loses colour stoichiometrically depending on the number of electrons taken up (Blois, 1958). In the experiment, the solution progressively reduced to a yellow coloured product, diphenylpicryl hydrazine, with the addition of the extracts in a concentration-dependent manner. The absorbance was measured at 517 nm. The results obtained clearly indicate the potential of the plant extracts in scavenging free radicals as high percentage inhibition in the range of 27–64% was observed at 250 μg/ml for the methanol extracts of the four plant species.

ABTS assay is based on the inhibition of the absorbance of the radical action $ABTS^+$, which has a characteristic long wavelength absorption spectrum (Sanchez-Moreno, 2002). The results obtained clearly imply that the methanol extracts of plant species inhibit the radical or scavenge the radical in a concentration-dependent manner. The methanol extracts of the four plants exhibited an inhibition ranging from 45% to 99% at 250 μg/ml. The extracts of *Pisonia alba* showed an inhibition of 99.63% at this concentration.

Lipid peroxidation is the oxidative degradation of polyunsaturated fatty acids (PUFA) and involves free radicals. This is a basic membrane damage process and results in deleterious effects. Initiation of lipid peroxidation was carried out by the addition of ferrous sulphate. This occurs by the formation of hydroxyl radicals by Fenton's reaction (Braughler, Duncan, & Chase, 1986). These produce malondialdehyde (MDA), which reacts with thiobarbituric acid (TBA) to form a pink chromogen. This inhibition could be caused by the absence of ferryl–perferryl complex or by scavenging the hydroxyl radical or the superoxide radicals or by changing the

Fe^{3+}/Fe^{2+} ratio or by reducing the rate of conversion of ferrous to ferric or by chelating the iron itself (Baskar, Rajeswari, & Sathish Kumar, 2007). The results obtained show a fairly constant high percentage inhibition. At 250 μg/ml concentration, the methanol extracts showed an inhibition of 45–68%. *Centella asiatica* proves to be the most potent and correlates with the previously established results that expose its role as a potent inhibitor of lipid peroxidation (Mahanom, Abdul-Hamid, Suhaila, Nazamid, & Maznah, 2007).

4.2. Correlation and regression analysis

Flavonoids are large compounds occurring ubiquitously in food plants. They occur as glycosides and contain several phenolic hydroxyl groups on their ring structure. Many flavonoids are found to be strong antioxidants capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups (Cao, Sofic, & Prior, 1997). In our study, the flavonoids showed a significant positive correlation with the antioxidant activity of the plant extracts. Phenols are secondary metabolites in plants and are known to possess a wide range of therapeutic uses, such as antioxidant, antimutagenic, anticarcinogenic, free radical-scavenging activities and also decrease cardiovascular complications (Yen, Duh, & Tsai, 1993). The scavenging ability of the phenols is mainly due to the presence of hydroxyl groups. From the results obtained, it is inferred that total phenols were present in reasonable amounts in the leaves of the plant species. However no significant correlation was observed between the total phenol content and the antioxidant activity of the extracts. Previous reports also suggest negative correlation existing between the phenol content and the antioxidant activity of *Trigonella foenum-graecum, Lawsonia inermis and Piper cubeba* (Aqil, Ahmad, & Mehmood, 2006). The above result might be the consequence of the phytochemical diversity in the antioxidant phytocompounds.

4.3. Antioxidant components of plants

Our results of various free radical-scavenging assays have established the antioxidant potential of the four plants and the major groups that might be responsible for the observed *in vitro* free radical-scavenging activity of these plants. In this context, it emphasises the fact that each plant has its own unique arsenal of flavonoids, polyphenols and other compounds that contribute to this activity. The following segment throws light on the individual molecules in plants that constitute the broader category of flavonoids and phenols, their previously ascertained biological roles and tries to establish their influence in the observed antioxidant potential.

Ample research has been carried out previously on the seeds of *Trigonella foenum-graecum*. Until now five different flavonoids, namely vitexin, tricin, naringenin, quercetin, and tricin-7-O-ß-D-glucopyranoside, have been reported in fenugreek seeds (Shang, Cais Han, & Li, 1998) and in some cases have been found to exert beneficial effects. Polyphenolic flavonoids from the seeds have been shown to protect various cell types from oxidative stress-mediated cell injury. For example, the phenolic antioxidant probucol protects human umbilical vein endothelial cells and rat neuronal cells (PC12) exposed to the highly toxic lipid peroxide, linoleic acid hydroperoxide (Sasaki, Toda, & Kaneko, 2002). Quercetin, one of the constituents of the extract, has been reported to prevent the cytotoxicity of oxidised low-density lipoproteins in human lymphoid cell lines (Negre-Salvayre & Salvayre, 1992). The leaves have remained a little less exploited and are known to contain at least 7 saponins, known as graecunins. These compounds are glycosides of diosgenin (Elujoba & Hardman, 1987). It is believed that the presence of the same or similar compounds in the leaves may be responsible for its role in the treatment of both types of diabetes, promotion of lactation, prevention of kidney stone formation and lowering of cholesterol levels.

Centella asiatica has been found to possess several chemical constituents, like vallarine, centellin, asiaticin, centellicin, pentacyclic triterpenes (asiatic acid, asiaticoside madecassic acid and madecassoside), used in the treatment of leprosy (Inamdar, Yeole, Ghogare, & de Souza, 1996), other triterpenes with healing properties (terminolic acid, asiaticoside-B), sceffoleoside A, tannins with antiprotozoal effects and saponins (centellasaponins B, C and D) (Oyedeji & Afolayan, 2005). These might have crucial roles in the observed antioxidant potential of the leaves, which have been shown to be able to significantly improve the antioxidant status of ageing organisms. It is taken as a panacea drug and can have a role in increasing intelligence. Further research is essential to understand the influence of these compounds on the prospective therapeutic utilisation of the plant.

Sauropus androgynus is an underexploited shrub, that is an excellent source of vitamins A, B and C. It is also known as a blood-building agent and a cell rejuvenator. Available literature suggests that lignan diglycoside, (−)-isolariciresinol 3-alpha-*O*-beta-apiofuranosyl-(1—>2)-*O*-beta-glucopyranoside, and a megastigmane glucoside, sauroposide were isolated from the aerial part of *Sauropus androgynus* together with (+)-isolariciresinol 3-alpha-*O*-beta-glucopyranoside, (−)-isolariciresinol 3-alpha-*O*-beta-glucopyranoside, (+)-syringaresinol di-*O*-beta-glucopyranoside, guanosine and corchoionoside (Kanchanapoom, Chumsri, Kasai, Otsuka, & Yamasaki, 2003). *Pisonia grandis* leaves are used in the treatment of arthritis in traditional medicine. However, no scientific literature to our knowledge exists pertaining to the compounds responsible for the antioxidant capacity of these plants. These plants have however been found to be as potent as the two former plants and hence the study has allowed us to unearth two more of the thousands of plant species that could influence the health sector in the future.

5. Conclusion

The results of the study imply the enormous nutritive value of the four plant species and their significance in the prevention of free radical-induced diseases. The observations may be used to substantiate the scientific reasoning that free radical-scavenging is indeed the mode of operation of these plants in the treatment or prevention of the onset of deadly disorders like arthritis, breast cancer, atherosclerosis, etc. The conclusions if established by *in vivo* studies on biological systems can open up new avenues in the search for natural antioxidants that can be employed successfully in further clinical trials.

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