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Phytochemical constituents and antioxidant activities of some plants commonly used in Indian traditional diet

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ABSTRACT

Dietary antioxidants are known to play a key role in combating the cellular oxidative stress imposed by the free radicals as well as in inhibiting the undesirable changes in nutritional quality of food. The present investigation was designed to evaluate the *in vitro* antioxidant properties and the major phytoconstituents of some plants regularly used in Indian traditional diet. Methanolic extracts of *Costus igneus*, *Foeniculum vulgare*, *Leucas aspera*, *Muntingia calabura*, *Physalis minima* and *Sauropus androgynus* were prepared by maceration. Antioxidant activities were assessed using phosphomolybdenum method and ferric reducing power assays. Total antioxidant activities of methanolic extracts ranged between 4.89 µg/ml to 46.0 µg/ml ascorbic acid equivalents for the tested extracts. *F. vulgare* methanolic extract possessed maximum antioxidant activity. This investigation revealed the potential antioxidant activity of the tested extracts and therefore, their use in the regular diet will be helpful in combating free radical associated health effects. Also, antioxidant rich formulations can be prepared using these plant materials.

INTRODUCTION

The generation of Reactive Oxygen Species (ROS) has been considered as a significant criterion in complications induced by oxidative stress. Under normal circumstances, cellular physiology is regulated in such a way that the oxidative stress is combated by enhanced activity of intracellular antioxidant enzymes. However, under circumstances of cellular dysfunction, increased levels of ROS eventually lead to pathogenesis of certain disorders such as cardiovascular disease, cancer, diabetes, autism and aging. Prolonged oxidative stress can trigger apoptotic and necrotic pathways in cells.

ROS imposed stress activates downstream signaling molecules such as PI3K, PKC and MAPK, thereby phosphorylating the transcription factor Nrf2 which then binds the antioxidant responsive element within the genes encoding antioxidant enzymes (Circu and Aw, 2010). Oxidative damage imposed by ROS can be reduced by various enzymatic/non-enzymatic antioxidant entities.

In addition, an adequate antioxidant status in the body can be maintained through the intake of dietary antioxidants (Lu *et al.*, 2010). Many studies have indicated that increased cellular oxidative damage is associated with premature aging and early onset of certain disorders. ROS levels can be decreased with the use of dietary antioxidants (Rahman, 2007). An enormous interest is focused on the intake of natural antioxidants due to the toxicological concerns associated with the use of synthetic antioxidants.

Several efforts have identified certain plants as a source of antioxidants that can be used under circumstances of enhanced antioxidant demand. Major compounds belonging to phenolics, flavonoids, carotenoids, phytosterols and thiols present in the plants confer antioxidant activity to the plant products. Natural antioxidants in foods have attracted greater attention as they are presumed to be safe and potentially nutritional (Khalaf *et al.*, 2008). Regular intake of antioxidants in the di*et al*so has a role in modulating the lifestyle disorders and aging (Nunez *et al*.,2013). This investigation was designed to evaluate the antioxidant potential of certain commonly used plants in Indian traditional diet. *Foeniculum vulgare* (fennel) is a highly aromatic and flavorful herb with culinary and medicinal uses. Fennel seeds are used as flavoring materials in baked foods, meat, fish dishes, ice cream, alcoholic beverages and herb mixtures (Rather *et al.*, 2012).

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The whole plant is consumed as a vegetable in many European and Asian countries (Kaur and Arora, 2009). Similarly, leaves of *Leucas aspera* are regularly used in North India as a fragrance aid for food materials and is also used in the treatment of inflammation, dyspepsia and jaundice (Ali *et al.*, 2013). *Muntingia calabura*, commonly known as Singapore cherry, is consumed as fresh fruits by certain ethnic groups (Gomathi *et al.*, 2013). Fruits of *Physalis minima*, commonly known as native gooseberry, are used in the preparation of sharbat and also are added to desserts, fruit preserves or dried raisins. It is a good source of vitamin C and is considered to be a purgative, diuretic and cure for spleen inflammations (Chothani and Vaghasiya, 2012). *Sauropus androgynus*, commonly known as vitamin leaves or star gooseberry is a perennial plant grown in India and leaves are used as salad or steamed, added to egg dishes, casseroles and prepared as chutney (Kanchanapoom *et al.*, 2003). *Costus igneus* (Family: Costaceae) leaves are consumed for the management of diabetes as a dietary supplement. This herb is also encountered in market in dried form or ground powder (Satareddi and Nandibewoor, 2012). Hence, considering the beneficial effects of these plants, the antioxidant potential of *C. igneus*, *F. vulgare*, *L. aspera*, *M. calabura*, *P. minima* and *S. androgynus* were studied.

MATERIALS AND METHODS

Chemicals

Sodium dihydrogen phosphate crystals, sodium hydroxide pellets, disodium hydrogen phosphate anhydrous, potassium ferricyanide, potassium hydroxide pellets, ascorbic acid, pyridine, methanol, sulphuric acid, hydrochloric acid, ammomium molybdate tetrahydrate and potassium iodide were procured from Merck (India). Sodium nitroprusside dihydrate and ferric chloride anhydrous were obtained from HiMedia (India). All the chemicals used were of analytical grade.

Plant materials

Leaves of *C. igneus, F. vulgare, L. aspera, M. calabura, P. minima and S. androgynus* were collected from local environs of Mangalore, Karnataka. Plant identification was done according to database on medicinal plants used in Ayurveda (Sharma *et al.*, 2000). Plants were washed, shade-dried and powdered using a kitchen blender. The plant materials were stored at 4 $^{\circ}$C till use for further analyses.

Extraction

Methanolic extracts of the plant materials were prepared by macerating 10 g of the powder with 40 ml methanol at room temperature using an orbital shaker. The suspension was filtered using Whatmann No. 1 filter paper. The filtrate was concentrated to obtain the extract.

Preliminary phytochemical screening

The extracts were subjected to preliminary phytochemical scrrening using the standard procedures (Raman, 2006) in order to determine the presence of alkaloids, carbohydrates**,** glycosides, saponins, steroids, proteins, phenolics and flavonoids.

Total antioxidant activity

Antioxidant activities of the extracts were evaluated using phosphomolybdenum method (Prieto *et al.*, 1999). The assay is based on the reduction of Mo (VI)–Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at an acidic pH. Different concentrations of extracts were combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Methanol was used as blank. The tubes containing the reaction solution were capped and incubated in a boiling water bath at 95 °C for 90 min. After cooling to room temperature, the absorbance of the solution was measured at 695 nm using a spectrophotometer (UV 1800, Shimadzu, Japan). Total antioxidant activities of the tested extracts were expressed as µg/ml ascorbic acid equivalents (AAE).

Reducing Power assay

The reducing power of the extracts was determined according to Oyaizu *et al* (1978). Different concentrations of the extracts in 1 ml of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The reaction contents were incubated at 50 °C for 20 min. To this mixture, 2.5 ml of trichloroacetic acid (10 %) was added and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as standard. Phosphate buffer (pH 6.6) was used as blank. Ferric reducing powers of the extracts were expressed as µg/ml AAE.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Preliminary phytochemical screening of the methanolic extracts indicated the presence of alkoloids in the extracts of *P. minima*, and *S. androgynus* whereas, phytosterols were present in the extracts of *C. igneus*, *F. vulgare*, *L. aspera* and *S. androgynus*. *L. aspera* extract also showed the presence of saponins. Phenolics and flavonoids were detected in the extract of *F. vulgare*.

Total antioxidant activity

Antioxidant activity of the methanolic extracts of plants increased in a concentration dependent manner (Figure 1). At lower tested concentrations (10 and 15 µg/ml), the highest antioxidant activity was observed in *P. minima* (15.26 and 21.0 µg/ml AAE) and *L. aspera* (15.25 and 19.0 µg/ml AAE) extracts. However, *F. vulgare* possessed the maximum antioxidant activity which ranged from 12.12 to 46.0 µg/ml AAE for the tested concentrations of 10 to 25 µg/ml. Other extracts also showed good antioxidant activity ranging from 18.6 to 40.0 µg/ml ascorbic acid equivalents at a concentration of 25 µg/ml.

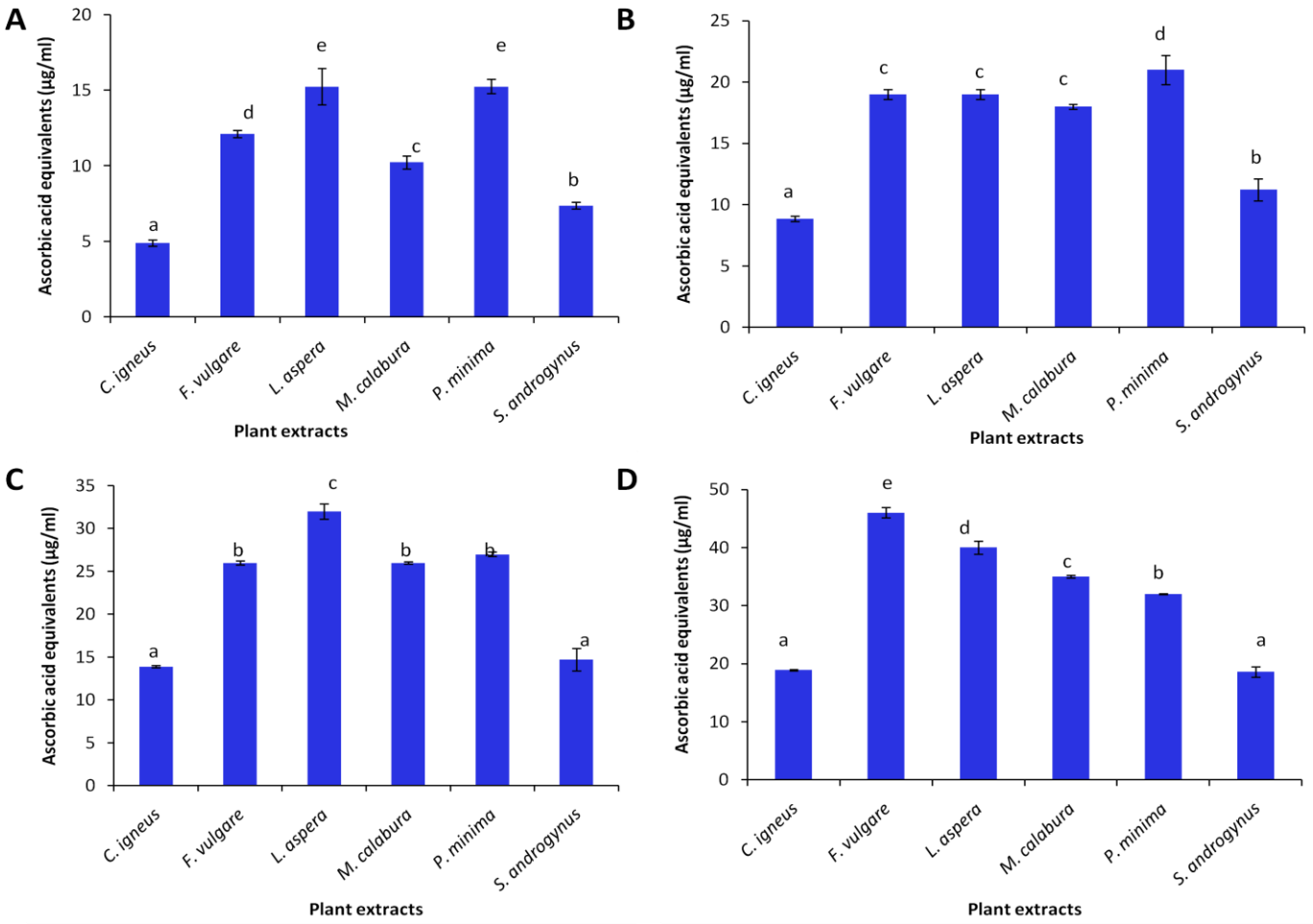

Fig. 1: Total antioxidant activity of tested extracts at (A) 10 µg/ml (B) 15 µg/ml (C) 20 µg/ml and (D) 25 µg/ml concentrations. Values are mean ± SD (n = 3). Data points indicated by different alphabets are significantly different from each other ($p < 0.01$).

Ferric Reducing Power

Ferric reducing power of the tested extracts was found to follow a concentration dependent trend (Figure 2). Among the extracts studied, highest activity was present in *F. vulgare*, followed by *L. aspera*, *M. calabura*, *P. minima*, *C. igneus* and *S. androgynus*. At the highest tested concentration of 25 µg/ml, *F. vulgare* extract showed a reducing activity of 60.18 µg/ml AAE which was 2.3 % higher than that of the standard antioxidant ascorbic acid.

Among the methanolic extracts of the plants studied, *F. vulgare* extract showed maximum antioxidant activity which could be contributed to the presence of phytoconstituents such as phytosterols and flavonoids, which are well known to possess antioxidant activity (Rather *et al.*, 2012). The free radical scavenging activity of the flavonoids are reported both *in vitro* and *in vivo*. Even though most ingested flavonoids are degraded into phenolic acids *in vivo*, their antioxidant activity was found to persist still. Absorbed flavonoids and their metabolites displayed antioxidant activity evidenced by increase of plasma antioxidant status *in vivo* (Pietta, 2000). Acetone and petroleum ether extracts of *F. vulgare* are known to possess potent activity against malodialdehyde production and this property was attributed to the presence of cis-miyabenol C, d-limonene and fenchone (Purkayastha *et al.*, 2012). Phenolic compounds from fennel including rosemarinic acid, quercetin and kaempferol are known for their antioxidant properties (Parejo *et al.*, 2004). Ethanolic extract of *L. aspera* was found to be a potent DPPH free radical scavenger, which was attributed to the presence of nectandrin B, apigenin, myristagenol-B and licarin in it (Sadhu *et al.*, 2003). Chloroform extracts of *M. calabura* were reported to be rich in polyphenolics, steroids and flavonoids and have been shown to exert strong ferric ion reducing activity (Sindhe *et al.*, 2013). The presence of triterpenoids and polyphenolics in *P. minima* extract was correlated with its antioxidant activity (Karpagasundari and Kulothungan, 2014). The activity reported in this study by the plants are higher than that reported for *Mimusops elengi* (Saha *et al.*, 2008), *Acanthus ilicifolius*, *Suaeda meritima* and *Avicennia alba* which are known for their antioxidant activities (Banerjee *et al.*, 2008). *C. igneus* and *S. androgynus* extracts also displayed good activity due to the presence of alkaloids and phytosterols. The antioxidant activities displayed by the studied plants can be attributed to more than one class of phytocompounds.

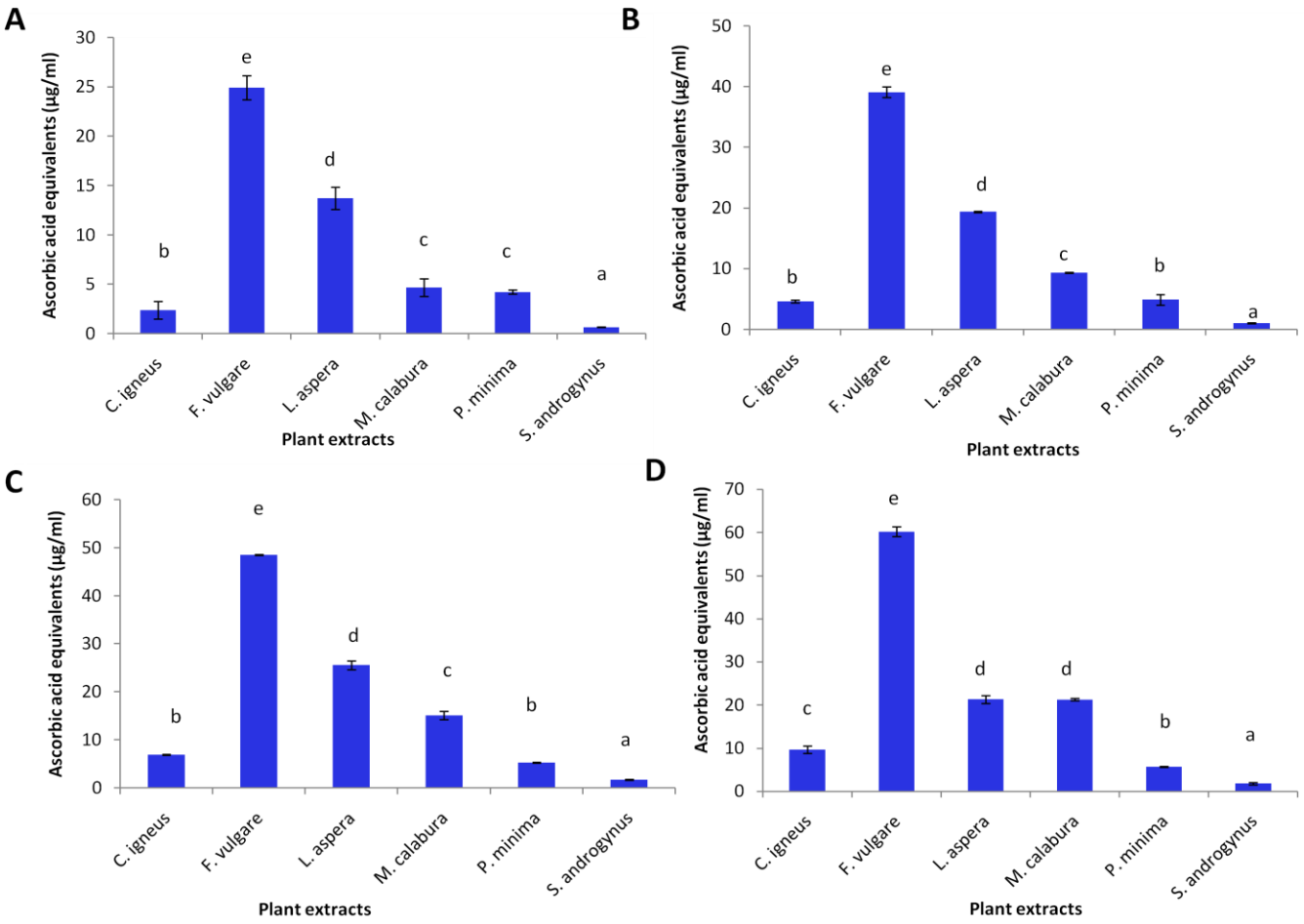

Fig. 2: Ferric reducing power of tested extracts at (A) 10 µg/ml (B) 15 µg/ml (C) 20 µg/ml and (D) 25 µg/ml concentrations. Values are mean ± SD (n = 3). Data points indicated by different alphabets are significantly different from each other ($p < 0.01$).

CONCLUSION

This study provides the antioxidant activity of six plants used in indigenous traditional diet. The antioxidant activity of *F. vulgare* extract was higher than that of the reference antioxidant, ascorbic acid, thereby indicating its possible usage as a potential source of dietary antioxidants. Consumption of functional foods is thought to play a better role than neutraceutical consumption. Prolonged intake of neutraceuticals may impose a health concern because of their negative attributes such as presence of adulterants and inorganic pollutants. Most of the neutraceutical products need to be supported with clinical data, which is not the case for functional whole foods. In our study, the tested plant extracts were found to be antioxidant, thereby implementing their prospective use as nutritional whole food supplements for gaining health benefits against oxidative stress related disorders.

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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