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Research Article

Total antioxidant potential of selected indigenous plants and culinary spices

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ABSTRACT

The total antioxidant activity of selected indigenous plants and culinary spices was determined *in vitro* by phosphomolybdenum method. The antioxidant activity of each extract was expressed as ascorbic acid equivalents. Among the spices tested, *Illicium verum* (hook fruit) revealed strongest antioxidant potential followed by *Cinnamomum zeylanicum*, Bl. (Bark), *Pimpinella anisum* (seed), *Myristica fragrans* Houtt (seed), *Terminalia chebula*, Retz. (seed), *Mentha piperata* (leaf), *Cuminum cyminum*, L. (Seed) and *Nigella sativa*, Linn (seed). All the tested spices have exhibited greater antioxidant potential than that of ascorbic acid except for some concentrations of *Mentha piperata* (leaf), *Cuminum cyminum*, L. (Seed) and *Nigella sativa*, Linn (seed). Among the indigenous plants, antioxidant potential observed were in the following descending order; *Amaranthus gangeticus* (leaf), *Gloriosa superba*, L. (Tubers), *Mangifera indica*, L., *Achyranthes aspera*, L. (whole plant), *Calotropis gigantea*, R. Br. (Leaf) and *Sesbania grandiflora*, L. Pers (Leaf). The present study revealed that the tested plants could be used as natural sources of antioxidants or as nutritional supplements to counter the deleterious effects of the oxidative stress.

Keywords: Ascorbic acid; Culinary spices; indigenous plants; Oxidative stress; Total antioxidant activity

INTRODUCTION

During the process of oxygen utilization in normal physiological and metabolic processes, approximately 5% of oxygen gets reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. All these radicals were known as reactive oxygen species (ROS) that exert oxidative stress (Lata and Ahuj, 2003). In living organisms, the oxidative stress is confronted by antioxidative enzymes, reduced glutathione, vitamins, pigments, phenols and polyphenols (Bahadori and Yazdani-Biuki., 2003). When generation of ROS overtakes the antioxidant defenses of the body, the free radicals start attacking the proteins, lipids and carbohydrates of the cell (Yu et al., 1994; Campbell and Abdulla., 1995; Cotran et al., 1999) and this is implicated in the pathogenesis of inflammation, cardio-vascular diseases, diabetes, cancer, neurological disorders, and in the process of aging (Tsa et al., 2004; Losso et al., 2007). Therapy using free-radical scavengers or antioxidants has the potential to prevent, delay

or ameliorate many of these disorders (Delanty and Dichter, 2000).

Synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), are well known for their ability to stop the chain reactions of lipid peroxidation (Escobar et al., 1995). There is increased evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress by protective biochemical functions of natural antioxidants contained in spices, herbs and medicinal plants (Noda et al., 1997). In recent times, research on the extraction of natural antioxidants that would be less toxic and more effective than the commonly applied synthetic antioxidants to fight the so-called "oxidative stress", is gaining momentum (Bahadori and Yazdani-Biuki., 2003). Hence, the present study was undertaken to explore the antioxidant potential of some commonly used indigenous plants and culinary spices.

MATERIALS AND METHODS

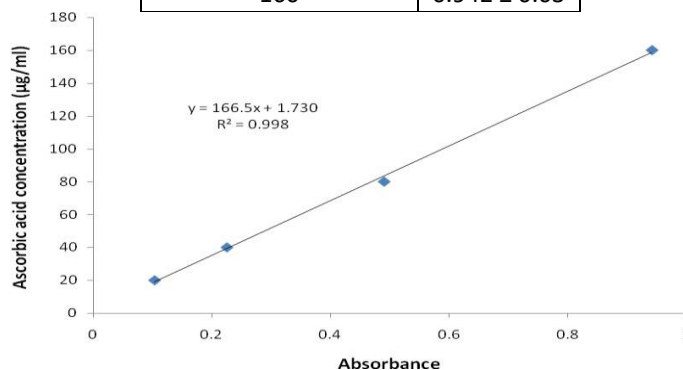
Plant Material Collection and Authentication

Culinary spices *viz.* *Cinnamomum Zeylanicum* Bl. (bark), *Cuminum cyminum* L.(seed), *Illicium verum* (hook fruit), *Mentha piperata* (leaf), *Myristica Fragarn*, Houtt.(seed), *Nigella sativa* Linn. (seed), *Pimpinella anisum* L. (seed), *Terminalia chebula* Retz. (seed) and indigenous plants *viz.* *Achyranthes aspera* L. (whole

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Table 1: Absorbance values of ascorbic acid at various concentrations (Mean \pm SD, n=3)

Ascorbic acid Concentration ($\mu\text{g/ml}$)	Absorbance
20	0.103 \pm 0.06
40	0.225 \pm 0.04
80	0.49 \pm 0.48
160	0.942 \pm 0.03

**Figure 1: Standard calibration curve for ascorbic acid (standard antioxidant)****Table 2: Total antioxidant activity of acetone extract of culinary spices expressed as ascorbic acid equivalents**

Extract	Concentration			
	20 $\mu\text{g/ml}$	40 $\mu\text{g/ml}$	80 $\mu\text{g/ml}$	160 $\mu\text{g/ml}$
<i>C.zeylanicum</i>	66.0346 \pm 1.00 ^g	85.0906 \pm 1.22 ^f	132.2542 \pm 1.88 ^g	208.1606 \pm 2.66 ^f
<i>C. cyminum</i>	16.3302 \pm 1.56 ^a	27.9226 \pm 1.62 ^a	51.5838 \pm 1.64 ^b	87.7902 \pm 1.78 ^c
<i>I.verum</i>	95.5714 \pm 1.55 ^h	166.7138 \pm 2.20 ^g	279.4618 \pm 3.23 ^h	440.485 \pm 3.88 ^g
<i>M. piperata</i>	40.309 \pm 1.55 ^d	49.6782 \pm 1.90 ^b	62.3822 \pm 2.02 ^c	79.3738 \pm 2.64 ^b
<i>M. fragrans</i>	45.073 \pm 1.03 ^e	62.2234 \pm 1.24 ^c	100.1766 \pm 1.36 ^e	166.3962 \pm 1.87 ^e
<i>N. sativa</i>	27.9226 \pm 0.10 ^b	28.0814 \pm 0.31 ^a	38.2446 \pm 0.44 ^a	54.601 \pm 0.83 ^a
<i>P. anisum</i>	37.927 \pm 1.61 ^c	67.7814 \pm 1.88 ^d	122.7262 \pm 2.87 ^f	212.7658 \pm 3.02 ^f
<i>T. chebula</i>	58.2534 \pm 1.13 ^f	73.9746 \pm 1.29 ^e	87.3138 \pm 1.57 ^d	118.7562 \pm 1.80 ^d

Values are Mean \pm SD, (n=3)

Means with different alphabets as superscripts in a column differ significantly (P < 0.05)

plant), *Amarantus gangeticus* Linn (leaf), *Calotropis gigantea* R. Br. (Leaf), *Gloriosa superba* L. (tubers), *Mangifera indica* L. (seed kernel), and *Sesbania grandiflora* L. Pers (Leaf) were brought from local sources. The plant materials were identified and authenticated by the Department of Botany, Vemulapalli Kodanda Ramaiah College, Buddhavaram, Gannavaram Mandal, Krishna District, Andhra Pradesh, India.

Extraction procedure

Shade dried plant parts were reduced to fine powder and 10g of powder was taken into a 250 ml conical flask and 100 ml of acetone was added. After thorough mixing the flask was kept on a rotary shaker at 190 – 220 r/min for 24 hours and it was filtered with whatman filter paper No1. The filtrate was evaporated until dry in a water bath at 80°C. The stock solution of crude extracts (10mg/ml) was prepared by dissolving a known amount of dry extract in DMSO. Ascorbic acid stock solution (1 mg/ml) was prepared in distilled water. The working solutions (20, 40, 80 and 160 $\mu\text{g/ml}$) of the extracts and ascorbic acid were prepared from the stock solution using suitable dilution.

Chemicals

Acetone (sd fine-chemicals Limited, Mumbai, India); Ammonium molybdate (Thermo fisher scientific India Pvt. Ltd., Mumbai, India); Dimethyl sulfoxide (DMSO), Disodium hydrogen phosphate and Sulfuric acid (Merck Specialties Private Limited, Mumbai, India).

METHODS

Phosphomolybdenum antioxidant assay

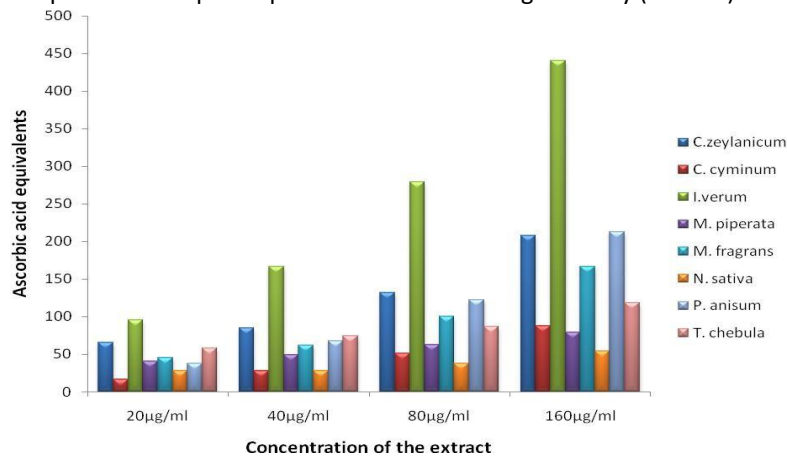
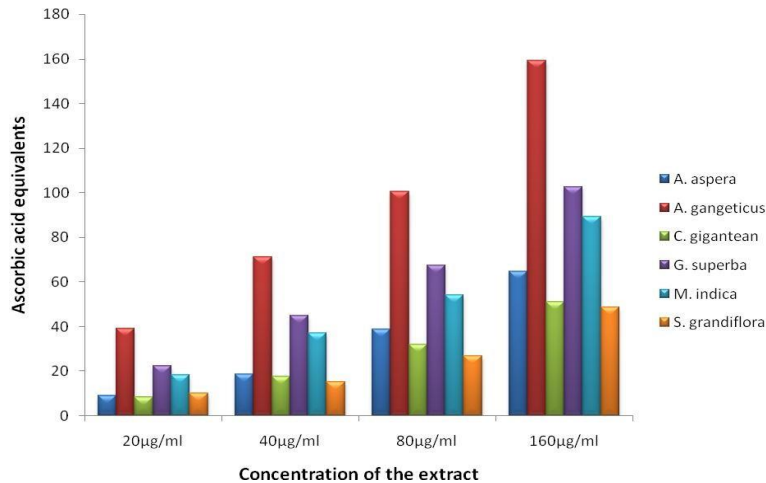
The total antioxidant activity of the extracts was evaluated by the phosphomolybdenum assay method (Prieto, et al., 1999). It was based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate-Mo (V) complex in acidic condition. Three hundred micro liters of extract was combined with 3ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The reaction mixture was incubated at 95°C for 90min. Then, the absorbance of the solution was measured at 695nm using a UV-visible spectrophotometer against a blank (0.3ml DMSO + 3ml reagent) after cooling to room temperature. The total antioxi-

Table 3: Total antioxidant activities of acetone extract of indigenous plants expressed as ascorbic acid equivalents

Extract	Concentration			
	20 µg/ml	40 µg/ml	80 µg/ml	160 µg/ml
<i>A. aspera</i>	9.2302 ± 1.16 ^a	18.8217 ± 2.23 ^a	38.6748 ± 2.44 ^c	64.8603 ± 2.55 ^b
<i>A. gangeticus</i>	39.0402 ± 1.55 ^d	71.3228 ± 1.78 ^d	100.7302 ± 2.12 ^f	159.3972 ± 2.75 ^e
<i>C. gigantean</i>	8.4128 ± 0.25 ^a	17.572 ± 1.0 ^a	32.1722 ± 1.25 ^b	51.2204 ± 1.55 ^a
<i>G. superba</i>	22.3302 ± 0.25 ^c	44.9226 ± 0.88 ^c	67.5838 ± 1.0 ^e	102.7902 ± 1.67 ^d
<i>M. indica</i>	18.206 ± 0.25 ^b	37.1802 ± 0.56 ^b	54.2039 ± 1.0 ^d	89.3405 ± 1.67 ^c
<i>S. grandiflora</i>	10.2958 ± 0.55 ^a	15.2186 ± 0.87 ^a	26.9698 ± 1.20 ^a	48.5666 ± 1.56 ^a

Values are Mean ± SD, (n=3)

Means with different alphabets as superscripts in a column differ significantly (P< 0.05)

**Figure 2: Total antioxidant activity of acetone extract of culinary spices expressed as ascorbic acid equivalents****Figure 3: Total Antioxidant activities of acetone extract of Indigenous plants expressed as ascorbic acid equivalents**

dant activity was expressed as ascorbic acid equivalents by substituting the absorbance of test extracts in the regression equation obtained by plotting the absorbance versus concentrations of standard antioxidant (ascorbic acid).

Statistical analysis: The experiment was repeated at least three times. Results were reported as Mean ± SD and analyzed for statistical significance using ANOVA with Tukeys multiple comparison test using 'Graph pad Instat' software version 3.10. Difference of p<0.05 was considered statistically significant.

RESULTS

The absorbance values at various dilutions of the standard antioxidant ascorbic acid were presented in Table 1 and standard calibration curve was presented Fig 1. The total antioxidant activity of culinary spices and indigenous plants at different dilutions was presented as ascorbic acid equivalents in Table 2 and 3, respectively. Among all the culinary spices tested, *Illicium verum* (hook fruit) revealed strongest antioxidant potential at all the dilutions tested, followed by *Cinnamomum zeylanicum*, *Bl. (Bark)*, *Pimpinella anisum*

(seed), *Myristica fragrans* Houtt (seed), *Terminalia chebula*, Retz. (seed), *Mentha piperata* (leaf), *Cuminum cyminum*, L. (Seed) and *Nigella sativa*, Linn (seed) (Fig 2). Among the indigenous plants, the decreasing order of antioxidant potential was *Amaranthus gangeticus* (leaf) followed by *Gloriosa superba*, L. (Tubers), *Mangifera indica*, L., *Achyranthes aspera*, L. (whole plant), *Calotropis gigantea*, R. Br. (Leaf) and *Sesbania grandiflora*, L. Pers (Leaf) (Fig 3).

DISCUSSION

The human body possesses many defense mechanisms against oxidative stress, including enzymatic and non-enzymatic compounds. Under certain circumstances, the natural antioxidant mechanisms become insufficient and the excess of free radicals may damage the structure and function of the cell membrane in a chain reaction leading to many degenerative diseases (Rahman and Choudhary, 2001). Antioxidants prevent the undesirable oxidation by absorbing and neutralizing the free radicals, quenching singlet and triplet oxygen, or decomposing peroxides but the use of synthetic antioxidants like butylated hydroxytoluene and butylated hydroxyanisole (BHA) are restricted because of their carcinogenicity and other side effects (Gowri and Vasantha 2010). The quest for exploring the antioxidants from natural sources is of great interest in food, cosmetic and pharmaceutical industries to replace the synthetic preservatives with natural ones.

Phosphomolybdenum method was used for determining the antioxidant potential of plant extracts in this study. The assay determines the total antioxidant capacity and is based on the reduction of Mo(VI) to Mo(V) in presence of the antioxidant compounds and subsequent formation of a green phosphate/Mo(V) complex at acidic pH, which is measured at 695 nm by visible spectroscopy. The assay has been successful in the quantification of total antioxidant activity of vitamin E (Prieto *et al.*, 1999; Kumaran and Karunakaran, 2006) and it was found efficient in determining the antioxidant potential of plant extracts (Lu Y and Foo LY 2001).

The culinary spices selected for this study revealed strong antioxidant potential. Among the spices tested, *I. verum* showed strongest activity; presence of flavonoids and phenolics might explain its free radical scavenging and antioxidant effects (Yang *et al.*, 2012). The phenolic contents may directly contribute to the antioxidant action because of their radical scavenging ability due to presence of hydroxyl groups. They donate hydrogen to the free radicals and thus inhibit the chain reaction. They also act as singlet oxygen quenchers and decompose peroxides (Ozen *et al.*, 2011). The antioxidant activity of *C. zeylanicum* was attributed to the presence of essential oils and polyphenolic compounds *viz.*, cinnamaldehyde or cinnamic acid, eugenol, cinnamic acid mucilage, diterpenes and proanthocyanidins (Jayaprakasha *et al.*, 2002). Nickavar and Abolha-

sani (2009) screened antioxidant properties of seven *Umbelliferae* fruits and reported that among them *P. anisum* revealed strongest antioxidant potential and they found a positive correlation between flavonoid content and antioxidant activity. They have also reported antioxidant activity for *C. cyminum*. Flavonoids are responsible for the antioxidant potential of *M. fragrans* and its use in arteriosclerosis (Olaleye *et al.*, 2006). The fruit of *T. chebula* possessed high antioxidant activity and phenolics were found to be responsible for this activity (Chang *et al.*, 2010). The cardioprotective actions of *T. chebula* might be partially attributed to its antioxidant potential (Suchalatha *et al.*, 2005). The antioxidant activity of *N. sativa* obtained was attributed to thymoquinone, which is a potent superoxide anion scavenger that inhibited iron-dependent microsomal lipid peroxidation (Eltahir *et al.*, 1993, Yoruk *et al.*, 2010).

Among the indigenous medicinal plants, *Amaranthus gangeticus* (leaf), *Gloriosa superba*, L. (Tubers) and *Mangifera indica*, L (seed kernel) revealed relatively strong antioxidant activity. *A. gangeticus* (leaf) exhibited greater activity than ascorbic acid (Table 3); higher amounts of vitamin C, carotenoids and phenolic compounds such as flavonoids might be the reason for such activities (Puravankara, 2000; Abdalla *et al.*, 2007). Consequently, the antioxidant activities of plant/herb extracts were often explained by their total phenolics (Ruan *et al.*, 2008). So the discrepancies in total phenolic and flavonoid content decide the antioxidant activity of the plant/spice. By virtue of their antioxidant potential, plants/spices with higher phenolics and flavonoids play a major role in the prevention of various pathological conditions such as cancer, cardiovascular and neurodegenerative diseases believed to be associated with oxidative stress (Losso *et al.*, 2007).

CONCLUSION

The culinary spices tested in this study and indigenous plants like *Amaranthus gangeticus* (leaf), *Gloriosa superba*, L. (Tubers) and *Mangifera indica*, L (seed kernel) revealed strong antioxidant potential, so these plants/spices can be explored to obtain natural sources of antioxidants to alleviate the pathological conditions resulting from oxidative stress and also to replace the synthetic antioxidants, consequently eliminating the side effects caused by them. From the present study, it can be concluded that exploring the antioxidant nutraceuticals from culinary spices and indigenous plants for therapeutic purposes will be a viable option.

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CONFLICT OF INTEREST

None

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