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EVALUATION OF PHYTOCHEMICAL PROPERTIES AND NITRIC OXIDE SCAVENGING ACTIVITY OF LEAFY VEGETABLES OF SOME TREES

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ABSTRACT

The biochemical content and nitric oxide scavenging potential of leafy vegetables of some trees like *Moringa oleifera* L. (Moringaceae), *Sesbania grandiflora* (L.) Pers (Fabaceae) and *Pisonia grandis* R.Br (Nyctaginaceae) were studied. The nitric oxide scavenging activity of ethanolic extracts from shade dried leaves of *Moringa oleifera* showed IC_{50} values 226.24 ± 0.52µg/ml against the standard of BHT 280µg/ml. The rest of the plant extracts showed higher IC_{50} values than BHT indicates their scavenging activities were lesser than BHT in the following order *Sesbania grandiflora* > *Pisonia grandis.* The heirarchy of total poly phenols in different plant extracts were found to be *Moringa olifera* > *Pisonia grandis* > *Sesbania grandiflora* ranging between 34.54 ± 0.99 tannic acid equivalent mg/g in *Sesbania grandiflora* and a maximum amount of 129.85 ± 0.79 tannic acid equivalent mg/g in *Moringa olifera.* Similarly the flavonoids (19.91± 0.81mgQE/g), and flavonols (13.73 ± 0.52 mg QEequivalents /g) were also maximum in *Moringa olifera.* But flavonoids and flavonols were minimum in *Sesbania grandiflora* (13.48 ± 0.74mg QE/g) and *Pisonia grandis* (4.48 ± 0.57) respectively. The concentration of Total Proanthocyanidin in leafy vegetables was in the range of 3.78 ± 0.48 equivalents mg/g in *Sesbania grandiflora* and a maximum of 6.26 ± 0.54 equivalents mg/g in *Pisonia* grandis.

Keywords: Tree leafy vegetables, *Moringa olifera, Pisonia grandis, Sesbania grandiflora, BHT,* Nitric oxide radical scavenging, toatal phenols, flavonoids, flavonols, and Proanthocyanidin.

INTRODUCTION

Reactive oxygen species (ROS) including superoxide (O_2), hydrogen peroxide (H_2O_2), hydroxyl (OH), nitric oxide (NO) exert oxidative stress in the cells of human body rendering each cell to face about 10,000 oxidative hits per second (Halliwell, 1989; Lata and Ahuja, 2003). When generation of ROS overtakes the antioxidant defense of the cells, the free radicals start attacking the cell proteins, lipids and carbohydrates and this leads to development of degenerative diseases. Hence the rationale for the use of antioxidants is well established in prevention and treatment of diseases where oxidative stress plays a major role. Antioxidants may protect the body against ROS toxicity either by preventing the formation of ROS, by bringing interruption in ROS attack, by scavenging the reactive metabolites or by converting them to less reactive molecules (Sen, 1995; Hegde, and Joshi, 2009). Therefore the uses of antioxidants, both natural and synthetic are gaining wide importance in prevention of diseases.

Flavonoids are present in most plant tissues and often in vacuoles (Croteau *et al*.,2000). Flavonoids are divided into classes according to their substitutes and oxidation level on the middle ring. The main subclasses and their respective food sources are anthocyanidins (red, purple and blue berries), flavanols (tea, red grapes and red wine), flavones (green leafy species) flavanones (citrus), and isoflavones (soybeans). Flavonoids in plants can function as color definitions and attractants to pollinators and seed dispersers, as antioxidants to protect plants against UV-radiation, as insect feeding attractants in host-species recognition, as signal molecules to facilitate nitrogen fixation, in inducible defense against bacterial and fungal attack; and as bitter or astringent taste attributes to repel birds and other animals (Croteau *et al*.,2000; Wildman, 2001; Winkel-Shirley, 2001, 2002). For humans, several health beneficial properties of dietary flavonoids are recognized for their antioxidant and anti proliferative effects which may protect the body from various diseases, such as cancers, cardiovascular disease and inflammatory (Middleton *et al.*,2000; Nijveldt *et al.*,2001). Flavonoids and flavonols are also stabilizing lipid oxidation with antioxidant activity (Yen and Duh, 1993).

Polyphenolic substances possess many biological effects which are mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidation and chelating transition metals (Bahman *et al.,* 2007). For examples, flavonols, cinnamic acids, coumarins and caffeic acids are well known polyphenolic compounds with strong antioxidant properties. Hence they play an important role in protecting food, cells and organs from oxidative damage (Osawa, 1999). These compounds (phenolic substances) all share the same chemical patterns, with one or more phenolic groups for hydrogen proton donors and neutralize free radicals (Parejo *et al.,* 2002; Milliauskas *et al.,* 2004; Atoui *et al.,* 2005; Galvez *et al.,* 2005). Keeping the above back ground information in mind the present study was taken up to evaluate the Phytochemical contents and nutritive value of edible greens of tree species and their antioxidant/Nitric oxide radical scavenging potential.

MATERIALS AND MEHODS

Plants selected for this study are *Pisonia grandis* R.Br (synonym: *Pisonia alba, Pisonia morindifolia*) belongs to the family Nyctaginaceae; *Moringa oleifera* L., commonly Known as Drum stick, belongs to the family Moringaceae and *Sesbina grandiflora* (L.) Pers (Leguminosae) commonly called as Agati (Fig. 1).

Fig.1 *Pisonia grandis* *Moringa oleifera* *Sesbania grandiflora*

Preparation of extract

The dried powder (20 g) was extracted with ethanol (200 mL) in an orbital shaker for 48 hours. The extract was filtered using a Buchner funnel and Whatman No.1 filter paper and evaporated to dryness. The resulting extract was reconstituted with sterile distilled water to give desired concentrations used in this study.

Phytochemical screening of the plant extract

Total Phenolics Content

The total phenolics content of the extract were determined by Folin-Ciocalteu method described by Wolfe *et al.* (2003), with little modification. The amount of total phenolic content was expressed as mg/g tannic acid equivalent using the expression obtained from the calibration curve: Y = 0.1216x, R2 = 0.936512, where x is the absorbance and Y is the tannic acid equivalent in mg/g.

Total Flavonoids

The total flavonoids were determined using the method of Ordonez *et al.*, (2006). Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation obtained from the curve: Y = 0.255x, R2 = 0.9812, where x is the absorbance and Y is the quercetin equivalent.

Total Flavonols

The total flavonols content were determined using the method of Kumaran and Karunakaran (2007). Total flavonoids were calculated as quercetin (mg/g) using the following equation based on the calibration curve Y = 0.0255x, R2 = 0.9812, where x is the absorbance and Y is the quercetin equivalent.

Proanthocyanidins

The total proanthocyanidin were determined using the procedure reported by Sun *et al*. (2005). Total proanthocyanidin contents were expressed as catechin (mg/g) using the following equation of the curve: Y= 0.5825x, R2 = 0.9277, where x is the absorbance and Y is the catechin equivalent.

Nitric Oxide Scavenging Activity

The method of Garrat (1964) was used to determine the nitric oxide radical scavenging activity of extracts of sample.The amount of nitric oxide radical was calculated using the equation: % Inhibition of nitric oxide radical scavenging activity = [Ao − A1]/Ao × 100

Estimation of pigments: Chlorophylls and carotenoids

The concentrations for Chl a (C_a) 664nm, Chl b (C_b) 648nm, and the sum of leaf carotenoids ($C_{(x+c)}$) can be calculated (Lichtenthaler, 1987) with the following equations

Ethanol with 5% (v/v) water:

$$c_a\left(\mu g/ml\right) = 13.36\ A_{664.1} - 5.19\ A_{648.6}$$
$$c_b\left(\mu g/ml\right) = 27.43\ A_{648.6} - 8.12\ A_{664.1}$$
$$c_{(x+c)}\left(\mu g/ml\right) = \left(1000\ A_{470} - 2.13\ c_a - 97.64\ c_b\right)/209$$

Anthocyanins

Anthocyanins were estimated by adapting method of Mónica Giusti and Wrolstad, 2001.

Statistical analysis

Experiments were carried out in triplicate and the data were subjected to statistical analysis like mean and standard error using SPSS 10.0.

RESULTS AND DISCUSSION

Over the years, exploration of plant products has been on the increase leading to the identification and improvement of plant products beneficial to mankind. *Moringa oleifera* L. (Moringaceae), *Sesbania grandiflora* (Fabaceae) and *Pisonia grandis* (Nyctaginaceae) are recognized as a multifunctional versatile plants with enormous economic, nutritional and health potentials and hence was considered for the antioxidants study.

Total phenols (TP) and Flavonoid (TF)

Phenolic compounds may contribute directly to antioxidative action and free radical terminators (Duh *et al.*, 1999). Polyphenolic compounds may have an inhibitory effect on mutagenesis and carcinogenesis in humans when as much as 1.0 g is ingested daily from a diet rich in fruits and vegetables (Tanaka *et al.*, 1998). They also involved in retardation of oxidative degradation of lipids (Pourmorad *et al.*, 2006).

The trend of total poly phenol and flavonoid contents in the studied plant extracts were *Moringa olifera* > *Pisonia* > *Sesbania grandiflora* (Table 1). *Moringa olifera* leaf showed 129.85 ± 0.79 tannic acid equivalent mg/g, a maximum among the tested plants. Sharma *et al.*, (2009) reported that the total phenol content in *Moringa olifera* fruit 125 GAE mg/g and the oil has 40.17 ± 0.01mg GAE g^{-1} (Ogbunugafor *et al.*,2011).

The total phenol content in *Sesbania grandiflora* was 34.54 ± 0.99 tannic acid equivalent (TAE) mg/g which is almost similar to the observations made by Padmaja *et al.*, (2011) which was 31.34 ± 0.577 GAE/gm. The content may vary from species to species, as *Sesbania rostrata* - 18mg/g (Dc - Ouattara *et al*. 2011) and *Sesbania pachycarpa* - 47.50 ± 2.0mg/g (Ouattara *et al.*, 2011).

In the Present study Total flavonoid content of *Moringa olifera* was 19.91± 0.81QEmg/g, almost equal in seed oil, 18.24 ± 0.01mg RE g^{1} (Ogbunugafor *et al*.,2011) but slightly lesser than fruit (Sharma *et al.*, 2009). Flavonoids in *Pisonia grandis* and *Sesbania grandiflora* were 14.76 ± 0.75 and 13.48 ± 0.74mg QE/g respectively.

Table1. Total phenol and Flavonoid content of leafy vegetables

Plants	TP mg/g	TF mg/g	TFL mg/g	PAC mg/g
Moringa olifera	129.85 ± 0.79	19.91± 0.81	13.73 ± 0.52	5.56 ± 0.56
Pisonia grandis	36.54 ± 0.89	14.76 ± 0.75	4.48 ± 0.57	6.26 ± 0.54
Sesbania grandiflora	34.54 ± 0.99	13.48 ± 0.74	11.45 ± 0.5	3.78 ± 0.48

Total Flavonol content (TFL)

The concentration of total flavonol in leafy vegetables was in the range of 4.48 ± 0.57 mg QE equivalents/g in *Pisonia grandis* and a maximum of 13.73 ± 0.52 mg QE equivalents /g in *Moringa olifera* (Table 1).

In terms of Polyphenols, Flavonoids and Flavonol contents *Moringa oleifera* shows maximum content among the tested plants and expected to have maximum antioxidant activity possibly due to synergistic effects of different compounds.

Total Proanthocyanidin (PAC)

In the tested plants, unlike the other compounds, the concentration of total PAC in leafy vegetables was maximum in *Pisonia grandis* and least in *Sesbania grandiflora* (Table 1) The unique polyhydroxy phenolic nature of proanthocyanidins and the resulting electronic configuration allows relatively easy release of protons and, as a result, they have substantial antioxidant activity, which is greater than vitamins C and E, the gold standards (Bagchi *et al.*, 1997; Ho *et al.*, 1999; Santos-Buelga & Scalbert, 2000; Bors *et al.*, 2001; Hatano *et al.*, 2002; Beninger & Hosfield, 2003).

Cataract formation and occurrence of retinopathy are two common complications of type 2 diabetes. Cocoa derived proanthocyanidins fed to diabetes-induced (streptozotocin treated) rats nearly completely inhibited cataract formation (Osakabe *et al*., 2002).

Nitric oxide scavenging assay

Nitric oxide is a very unstable species under the aerobic condition. It reacts with O_2 to produce the stable product nitrates and nitrite through intermediates likeNO_2, N_2O_4 and N_3O_4. In the presence of test compound, which is a scavenger, the amount of nitrous acid will decrease. The extent of decrease will reflect the extent of scavenging. Here the extent of inhibition of ethanolic extract of *Moringa oleifera* showed the IC_{50} values 226.24 ± 0.52µg/ml as against the standard of BHT 280µg/ml. while the rest of the plant extracts showed higher IC_{50} values than BHT indicating that their nitrous oxide scavenging activity lesser than BHT in the following decreasing order *Sesbania grandiflora* > *Pisonia grandis* (Table 2).

Table 2. Nitric oxide scavenging activity of leafy vegetables

Standard / Plants	**IC_{50}** (µg/ml)
BHT	280 ± 0.28
Moringa olifera	226.24 ± 0.52
Sesbania grandiflora	550.27 ± 0.48
Pisonia grandis	965.74 ± 0.92

Pigments in Leafy vegetables

Anthocyanins

Among the leafy vegetables *Pisonia grandis* had a maximum concentration of anthocyanin (0.759mg/g) followed by *Moringa olifera* and *Sesbania grandiflora* (fig.2). The phenolic structure of anthocyanins is responsible for their antioxidant activity; i.e., ability to scavenge reactive oxygen species (ROS) such as superoxide (O2 −), singlet oxygen ('O2), peroxide (ROO−), hydrogen peroxide (H2O2), and hydroxyl radical (OH) (Wang and Jiao, 2000) and by chelating metals, direct binding to proteins (Kong *et al*., 2003). They play an important role in the prevention against mutagenesis and carcinogenesis mediating some physiological functions related to cancer suppression (Omenn, 1995). Anthocyanins show inhibitory effects on the growth of some cancer cells (Kamei *et al*., 1995; Koide *et al*., 1997; Nagase *et al*., 1998; Meiers *et al*., 2001) and also inhibit cell transformation (Hou *et al*., 2004).

The antioxidant effects of anthocyanins in vitro have been demonstrated using several cell culture systems including colon (Parry *et al*., 2006; Renis *et al*., 2007), endothelial (Bagchi *et al*., 2004), liver (Meyers *et al*., 2003) and leukemic cells (Feng *et a*l.,2007), and keratinocytes (Afaq *et al*., 2007).

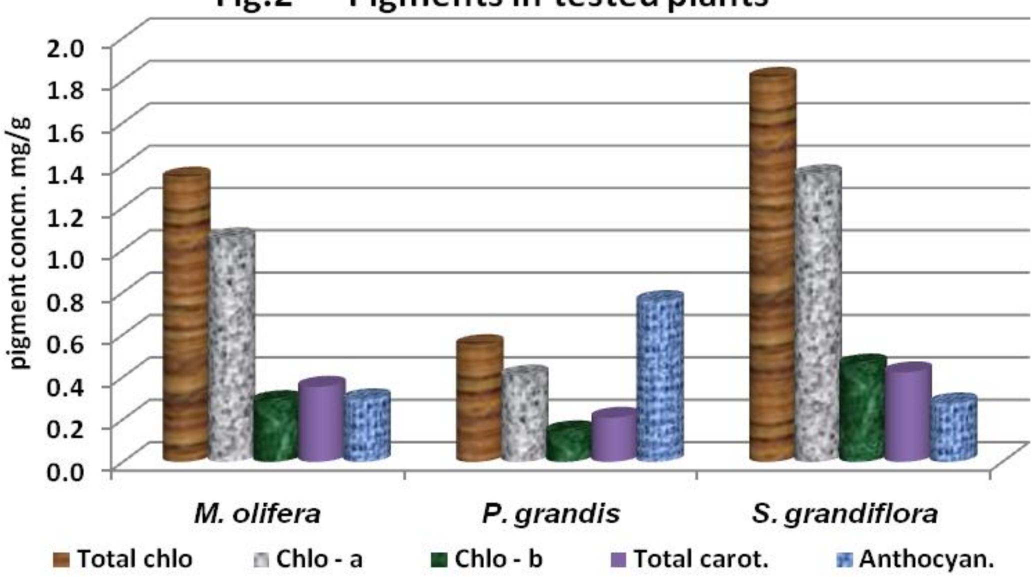
Carotenoids

Among the leafy vegetables *Sesbania grandiflora* Showed a maximum (0.424mg/g) total carotenoid pigments and least in *Pisonia grandis* (fig.2).

Chlorophyll pigments

Among the leafy vegetables *Sesbania grandiflora* Showed (fig.2) maximum (1.812mg/g) total chlorophyll pigments and *Pisonia grandis* observed to have least amount (0.550mg/g) of total chlorophyll pigments.

Fig.2 Pigments in tested plants



Thus, the presence of phenolic compounds and flavonoids in the tested greens are an added value to its nutritional and health potential and having a preventive action against cancer and heart disease. The maximum content of anthocyanin and proanthocyanidin in *Pisonia grandis* possibly have a role in reducing joint pain and arthritis as it has strong anti-inflammatory, antioxidant, and radical scavenging activity. Among all tree leafy vegetables *Moringa olifera* is the cheap and best.

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