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In Vitro Evaluation of Nitric Oxide Scavenging Activity of *Guettarda speciosa* Linn.

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Abstract: ***In-vitro antioxidant activity of various extracts of Guettarda speciosa Linn. leaves were evaluated by using Nitric oxide scavenging activity. The petroleum ether, chloroform, acetone, ethanol and water extracts of the leaves were screened for their antioxidant activity. Total phenolic contents of the plant material were determined. The result showed that water extract of the selected plant leaf had the highest antioxidant activity [IC_{50} value 77.22±0.60 µg/ml] than other extracts and similarly water extract had maximum amount of total phenols [115.81± 0.67 mg TAE/g extract] compare to other extracts. So the present research indicates that Guettarda speciosa leaves have high antioxidant component and scavenging activity in water extract.***

Keywords: Antioxidant, Nitric oxide, *Guettarda speciosa,* Scavenging activity.

1. Introduction

Medicinal plants are boon of nature. Natural products from plants are rich source of primary and secondary metabolites. The contribution of these metabolites is acting as defense mechanism in living flora. There is an increasing interest in utilization of herbal medicine among the global level, due to therapeutic potential of plants and healing power of the plants depends upon metabolites present in it. In modern civilization, there is a need of natural antioxidants for man, intake of these antioxidants helps to reduce the risk of many diseases caused by free radicals. In recent years, as a result of polluted environment (including polluted water, air; pesticide used food etc.) man easily acquired many diseases through free radicals. Free radicals have been shown to be harmful as they react with important cellular components such as proteins, DNA and cell membrane (Mantena *et al.,* 2008). On the other hand, body requires free radicals for immune responses. However, an overload of these molecules had been linked to certain harmful diseases. Atoms of oxygen or nitrogen having central unpaired electron are called as reactive oxygen or nitrogen species (Finkel and Holbrook, 2000; Pietta, 2000).

NO (Nitric oxide) is an important bioregulatory molecule, which has a number of physiological effects including control of blood pressure, neural signal transduction, platelet function, antimicrobial activity. Low concentration of NO, are sufficient in most cases to affect these beneficial functions. However, during infections and inflammations, formation of NO is elevated and may bring about some undesired deleterious effects (Marcocci *et al.,* 1994a, b).

The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidant components such as phenols, flavonoids and tannins are the secondary metabolites in the plants that exhibit antiallergenic, antimicrobial, antiartherogenic, antithrombotic, anti-inflammatory and cardio protective effects (Middleton *et al.,* 2000; Alpinar *et al.,* 2009). Antioxidants are reducing agents and limit oxidative damage to biological structures by passivating free radicals. These compounds are when added to lipids and lipid containing foods increases their shelf-life by retarding the process of lipid per oxidation. Also, these have been widely used as food additives to avoid food degradation and they play an important role in preventing man lifestyle – related diseases and aging, being closely related to the formation of ROS and lipid peroxidation (Gulcin *et al.,* 2004).

Green plants have been utilized by mankind since time immemorial for curing various diseases. Even though many researches are available in many plants, there is no scientific data available regarding Nitric oxide scavenging activity of *Guettarda speciosa* Linn. leaves (Rubiaceae). Hence the present research carried out in this filed. *Guettarda speciosa* is a sacred tree and also promoted to grown as ornamental tree. The tree have therapeutic potential and traditionally used in Indian medicine to treat cough, cold, sore throats, poultice, wounds, dysentery, remedy for boils and head ache, etc. Previously, the preliminary phytochemical screening of the ethanolic extract of inner bark of *Guettarda speciosa* revealed that presence of alkaloids, flavonoids, carbohydrates, tannins, phenols, gums and mucilage and absence of saponins and steroids (Sunil Kumar Reddy *et al.,* 2010).

2. Materials and Methods

2.1. Plant Collection

The plant *Guettarda speciosa* leaves used for investigation was collected from Erode, TamilNadu, India. The plant was authenticated by Dr. G.V.S. Murthy, Scientist and Head, Botanical Survey of India, Southern Regional Circle, Coimbatore, India.

2.2. Preparation of Extract

The collected leaves were dried in shade and powdered. The powdered leaves were subjected to successive soxhlet extraction using a series of solvents of increasing polarity starting from petroleum ether, chloroform, acetone, ethanol and water respectively. Extracts were obtained according to the method described by Kokate *et al.* (2003).

2.3 Estimation of Total Phenolics

The total phenolic content (TPC) was determined according to the method described by Sindduraju and Becker (2003). Ten microlitre aliquots of the extracts (10mg/2ml) were taken in test tubes and made up to the volume of 1ml with distilled water. Then 0.5ml of Folin – Ciocalteu phenol reagent (1:1 with water) and 2.5ml of Sodium arbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min. and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as tannic acid equivalents.

2.4 Nitric oxide radical scavenging activity (Sreejayan and Rao, 1997)

3 ml of 10mM Sodium nitroprusside in 0.2 M phosphate buffered saline (pH 7.4) was mixed with different concentrations (40-1000 µg) of solvent extracts and incubated at room temperature for 150 min. After incubation time, 0.5 ml of Griess reagent (1% sulfanilamide, 0.1 % naphthylethylene diamine dihydrochloride in 2% H_3PO_4) was added. The absorbance of the chromophore formed was read at 546nm. BHA and the same mixture of the reaction without sample extracts were employed as positive and negative control. Percentage radical scavenging activity of the sample was calculated as follows:

% NO radical scavenging activity = (control OD- sample OD/ control OD) x 100

The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC_{50}) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

3. Result and Discussion

The amount of total phenolics, measured by in tannic acid equivalents, varied widely in *G. speciosa* leaf material and ranged from 13.98±0.33 to 115.81±0.67 mg TAE/ g extract (Table 1).The highest level of phenolics was found in water extract (115.81±0.67 mg TAE/g extract), while the lowest was present in petroleum ether (13.98±0.33 mg TAE/g extract). Chloroform, acetone, ethanol extracts exhibited phenolics levels were 28.18±0.69, 39.38±0.51, 27.29±0.67 mg TAE/g extract respectively. Phenolics are known powerful antioxidants. 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 and also cardiovascular complications (Yen *et al.,* 1993). Phenolic compounds and flavonoids have been reported to be associated with anti-oxidative action in living organisms as it acts as scavengers of singlet oxygen and free radicals (Rice-Evans *et al.,* 1997; Jorgensen *et al.,* 1991).

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups (Hatano *et al.,* 1989). According to the recent reports, a highly positive relationship between total phenols and antioxidant activity was found in many plant species (Vinson *et al.,* 1998; Velioglu *et al.,* 1998; Gulcin *et al.,* 2002b; Oktay *et al.,* 2003).

Many reports are available for total phenolic content of plants. According to Memnune Sengul *et al*. (2009) the total phenolic content of *Inula aucherana, Fumaria officinalis, Crocus sativus, Viscum album, Tribulus terrestris Polygonatum multiflorum, Alkanna tinctoria* and *Taraxacum officinale* were in range of 4.04 mg GAE/g (in *Polygonatum multiflorum* L.) to 42.29 mg GAE (in *Crocus sativus*) per g dry weight basis.

In the last decade a number of publications have been published in which antioxidant characteristic of phenol compounds are tested, through different methods (Halvorsen *et al.,* 2002). The responses of the methods are about the number of the hydroxylic groups in phenolic compound. Because of this, it is difficult to compare final results, even though there are the same plant species. In *G. speciosa* leaf the TPC was much higher in water extract (115.81±0.67 mg TAE/g extract) and less in petroleum ether extract (13.98±0.33 mg TAE/g extract). From the results obtained, it is inferred that reasonable amount of total phenol contents were present in water extract of *G. speciosa* leaves.

To know the free radical scavenging activity of test plant, Nitric oxide scavenging activity was carried out in *G. speciosa* leaves. Oxide (NO) scavenging assay is based on the scavenging ability of the extracts (petroleum ether, chloroform, ethanol, acetone and water) as well as BHA, which is used as standard. The scavenging of NO by the various extracts of *G. speciosa* showed a dose dependent elevation in NO scavenging activity. Table 2 illustrates a significant decrease in the NO radical due to the scavenging ability of extracts and BHA. The water extract showed maximum scavenging activity of 77.22±0.60 µg/ml % at 200 µg/ml which indicating an effective capacity for scavenging reactive nitrogen species. At the same time the other fractions (petroleum ether, chloroform, acetone and ethanol) expressed comparatively less scavenging % activity up to 1000 µg/ml and comparatively higher IC_{50} values (342.88±3.37; 319.17±2.96; 284.37±2.32 and 343.89±3.34 µg/ml respectively) show decreasing antioxidant capability which was indicative of weak activity against this radical. The IC_{50} value for water extract was found fairly significant (77.22±0.60 µg/ml) while compared to the IC_{50} value of the reference standard BHA (43.37±1.26µg/ml).

However, the crude extracts of certain plants like *Gingko biloba* (Marcocci *et al.,* 1994a,b), *Sanguisorbae radix*, *Caryophylli flos*, *Coptidis rhizoma*, *Granati cortex*, *Gallae*

rhois, *Rhei rhizoma* and *Cinnamomi cortex* have been reported to inhibit NO generation *in vitro* (Yokozawa *et al.*, 2000). According to Nitai Chand Chaulya *et al.* (2010) the methanolic extract of *Cyperus tegetum* significantly inhibited nitric oxide in a dose dependent manner with the IC_{50} being 65 µg/ml. This nitric oxide scavenging activity could be attributed the presence of phenolic and polyphenolic compounds in the extract

Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity (Hagerman *et al.*, 1998). Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitric oxide (Govindarajan *et al.*, 2003).The nitric oxide scavenging activity of flavonoids and phenolic compounds (Kim *et al.*, 1998; Kim *et al.*, 1999; Crozier *et al.*, 2000; Madson *et al.*, 2000; Jagethia *et al.*, 2004) might be responsible for the observed nitric oxide scavenging activity.

In the present study, the water extract of *G. speciosa* leaves proved more potent than the other solvent fractions (petroleum ether, chloroform, acetone and ethanol).

From the research, it concluded that water extract of *G. speciosa* leaves possesses high phenolic content; hence indirectly it indicated that good antioxidant activity capacity of the leaves. The presence of antioxidant compound leads to good free radical scavenging activity. Hence, NO scavenging activity showed better scavenging activity in water extract. It might be useful for development of new drug.

Table 1: Estimation of Total phenloic content in *G. speciosa* leaves

S. no.	*Various extracts*	*Total phenolics mg TAE/g extract*
1.	Petroleum ether	13.98±0.33
2.	Chloroform	28.18±0.69
3.	Acetone	39.38±0.51
4.	Ethanol	27.29±0.67
5.	Water	115.81±0.67

Values are means of three independent analysis of the extract ± standard deviation (n=3).
TAE – Tannic acid equivalent

Table 2: *In vitro* antioxidant activity of various extracts of *G. speciosa* leaves against Nitric oxide radical

S. no.	Pet. Ether extract		Chloroform extract		Acetone extract		Ethanol extract		Water extract	
	Conc. (µg)	Activity (%)	Conc. (µg)	Activity (%)	Conc. (µg)	Activity (%)	Conc. (µg)	Activity (%)	Conc. (µg)	Activity (%)
1.	200	14.46±0.43	200	13.46±0.32	200	16.90±0.98	200	12.83±0.86	40	10.87±0.42
2.	400	27.32±0.28	400	29.59±0.49	400	33.61±0.85	400	24.17±0.52	80	21.55±0.28
3.	600	35.49±0.28	600	41.56±0.19	600	45.59±0.43	600	37.00±0.72	120	31.03±0.42
4.	800	48.45±0.28	800	51.82±0.49	800	56.64±0.43	800	49.71±0.72	160	42.36±0.16
5.	1000	55.59±0.16	1000	57.91±0.37	1000	66.48±0.58	1000	54.87±0.72	200	50.83±0.73
IC_{50} (µg/ml)	342.88±3.37		319.17±2.96		284.37±2.32		343.89±3.34		77.22±0.60	

IC_{50} value for BHA = 43.37±1.26µg/ml

Values are means of three independent analyses of the extract ± standard deviation (n=3)

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