Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 6, Issue 2, 2013 ISSN - 0974-2441

Research Article

EVALUATION OF ANTIOXIDANT ACTIVITY OF TWO IMPORTANT MEMORY ENHANCING MEDICINAL PLANTS CELTIS TIMORENSIS AND VANDA SPATHULATA

RAJANEEKAR DASARI*, D. SATHYAVATI , SAMPATH KUMAR BELIDE, P.JAYACHANDRA REDDY, K.ABBULU

Mallareddy Institute of Pharmaceutical Sciences, Dhulapally, Maisammaguda, Quthbullapur Rangareddy Dist, Hyderabad, (AP). Jawaharlal Nehru Technological University Hyderabad , Email : rajaneekar.cologist@gmail.com

Received: 29 January 2013, Revised and Accepted: 3 March 2013

ABSTRACT

Background : Free radicals or highly reactive oxygen species are capable of inducing oxidative damage to human body. Antioxidants are the compounds which terminate the attack of reactive species and reduce the risk of diseases. Both Celtis timorensis and Vanda Spathulata are used in treatment of brain disorders in human and have almost similar effects.Objective : The study was conducted to determine the Antioxidant properties of two well known memory enhancer medicinal plants, Celtis timorensis and Vanda Spathulata.Results : The antioxidant activity of these two medicinal plants were evaluated by measuring reducing ability, free radical scavenging activity by DPPH and hydrogen peroxide methods. The antioxidant compounds like ascorbic acid , total phenols and tannins were also evaluated in these plants. Celtis timorensis and Vanda Spathulata exhibited significant differences ($P<0.05$) in their antioxidant values. The methanolic extract of whole leaf powder of Celtis timorensis exhibited significantly higher antioxidant activity than the Vanda Spathulata. The antioxidant components viz. ascorbic acid, total phenols and tannins were also found in a higher concentration in Celtis timorensis as compared to Vanda Spathulata.Conclusion : It can be concluded from the study that regular use of Celtis timorensis as a supplement could be more helpful compared to Vanda Spathulata in treatment of neurological disorders caused by free radical damage.

Keywords: Celtis timorensis ,Vanda Spathulata, DPPH, free radical scavenging activity, antioxidant activity, hydrogen peroxide, reducing ability.

INTRODUCTION

Free radicals or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. These are capable of oxidizing bio-molecules viz nucleic acids, proteins, lipids and DNA and can initiate different degenerative diseases like neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, arthritis etc.[1, 2] Antioxidant are the compounds which terminate the attack of free radicals and thus reduce the risk of these disorders.[3] Almost all organisms are protected up to some extent by free radical damage with the help of enzymes such as super-oxide dismutase, catalase and antioxidant compounds viz. ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids and glutathione.Prior and Cao,[4] reported that antioxidant supplements or dietary antioxidants protect against the damaging effects of free radicals. Presently, much attention has been focused on the use of naturals antioxidants to protect the human body especially brain tissues from the oxidant damage caused by free radicals. In last two decades, several medicinal plants have shown such effectiveness through the traditional methods of psychoneuro- pharmocology.[5] Keeping this in view, the present study has been conducted to evaluate the comparative antioxidant activity of Celtis timorensis and Vanda Spathulata which are traditionally well known for their CNS activity.

Celtis timorensis (cannabaceae/Ulmaceae family) is commonly known (kotibera) as stinwood or stinking wood and is a species of flowering plant. The specific epithet comes from the name of the island of timor, locality of the collection. It has been recommended by several ancient Ayurvedic treatises for the improvement of memory power and treatment of mental disorders. Its extract have also shown anti-depressant activity, anti-convulsive and neuro disorders action. It also enhanced learning and memory (Nootropic) in humans.[6,7] It also helps to repair damaged neurons in specific brain areas. Celtis extract has shown neuroprotective effect against oxidative stress in the hippocampus of rat brain.[8] Vanda Spathulata, a small epiphytic herb (Family Orchidaceae) is commonly known as Svarna-pushpa bandaa or baandaa.[9] Dried flowers are powdered and given for consumption, asthma and maniac troubles, juice of the plant is given to temper the bile and abate frenzy. Its dried flowers are used in the treatment of Asthma, Depression, Nootropic, as a pacifier and as a liver tonic.[10] This plant is also found to improve short-term memory and learning. Vanda Spathulata has also shown protective effect in neurotoxicity. The present study has been conducted to evaluate the comparative antioxidant properties of Celtis timorensis and Vanda Spathulata including the composition of their antioxidant components like ascorbic acid, total phenol and tannins.

MATERIALS AND METHODS

The mature leaves of Celtis timorensis and Vanda Spathulata were collected, dehydrated (in a chamber below 40^0 C), Powdered with a mechanical grinder and stored in an air-tight container. The dried powder material of the plants was extracted with methanol. The solvent was completely removed under reduced pressure and a semisolid mass was obtained. It was dried with lyophilizer and dissolved in methanol for the present study.

Chemicals

Sodium Carbonate, $KMnO_4$, $FeCl_3$, H_2O_2 and 2,6-dichlorophenolindophenol were purchased from E.Merck.BHT. trichloroacetic acid, potassium ferricyanide, catechol, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid, tannic acid and oxalic acid were purchased from Sigma Chemical Co.Ltd, India

Free Radical Scavenging Activity (FRSA) using DPPH

The DPPH method was used for estimating FRSA of the methanolic extracts as suggested by Hatano et al.[11] 2ml of methanolic solution of DPPH (0.1mmol) was mixed with various doses of 20-80 µl of methanolic extract (4 mg/ml) in test tube and final volume of 3 ml was made with methanol. The absorbance of the mixture was measured after 40 min at 517 nm against methanol as blank. Ascorbic acid was used as standard. FRSA (%) of the test samples was evaluated by comparing with control (2 ml DPPH and 1 ml methanol). Each sample was then measured in triplicate and averaged. FRSA was calculated using the formula :

Determination of Ascorbic Acid Content

Total ascorbic acid content in plant extract was determined by 2,6-dichlorophenolindophenol method.[16] 2 g dried powdered sample was extracted with 4% oxalic acid and the volume was made up to 100 ml. It was centrifuged at 10,000 rpm for 10 min. 5 ml supernatant liquid was transferred to a conical flask and 10 ml of 4% oxalic acid was added. It was titrated against standard dye solution (2,6-dichloro phenolindophenol) to a pink end point. The procedure was repeated with a blank solution (without adding sample). 5 ml ascorbic acid of 100 ppm was used as standard. Ascorbic acid content was calculated using the formula :

FRSA = [(Ac-At/Ac-As)]x100
Where , Ac = Absorbance of Control ,
As =Absorbance of Standard and

At =Absorbance of Test.

Reductive Ability

The reducing ability of medicinal plants was determind according to the Oyaizu[12] method. Methanolic extract (0.08-0.4 mg) of plants was dissolved in 1ml of distilled water and then 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide [$K_3Fe(CN)_6$] (1%) were added. The mixture was incubated at 50° C for 20min, after that 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min, 2.5 ml of upper layer of the solution was mixed with 2.5ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%). Absorbance was measured at 700 nm. BHT was used as reference compound, all the Analysis was performed in triplicate.

Free Radical Scavenging Activity (FRSA) using Hydrogen Peroxide

The hydrogen peroxide FRSA of the methanolic extract was done as suggested by Czochra and Widensk.[13] 2 ml of hydrogen peroxide (43mmol) and 1.0ml of methanolic sample [20-100 µl] of methanolic extract (4mg/ml) of plant in methanol followed by 2.4 ml of 0.1 M phosphate buffer (pH 7.4) were added. The resulting solution was kept for 10 min and the absorbance was reported at 230 nm. All readings were repeated three times. Blank was prepared without adding hydrogen peroxide and control was prepared without sample. Ascorbic acid was used as a standard compound. Free radical Scavenging activity of hydrogen peroxide (%) was calculated as

FRSA (%)=[(V_0-V_1/V_0)]x100
where ,V_0 = Absorbance of Control and

V_1= Absorbance of sample

Determination of Total Phenols

The concentration of total Phenols in the plant extract was determined by using Folin-Ciocalteu method.[14] Catechol was used as standard, 0.2 ml ethanolic (80%) extract (4mg/ml) of plants and 0.2ml Folin-Ciocalteu reagent were mixed thoroughly. After 4 min, 1 ml of 15% sodium carbonate was added and the mixture was allowed to stand for 2 hr at room temperature. The absorbance was measured at 760 nm. The concentration of total phenols was measured equivalent to catechol (as a standard drug) by using standard calibration curve of catechol.

Determination of Total Tannin

Total tannin in plant extract was determined by Folin-Denis method.[15] 0.5 g of powdered drug was boiled for 30 min with 75ml of double distilled water. It was cooled, centrifuged at 2000 rpm for 20 min and supernatant was collected in 100 ml volumetric flask and the volume was made up with double distilled water. 1ml of this solution was transferred to a 100 ml volumetric flask containing 75 ml water and 5 ml of Folin-Denis reagent + 10 ml of sodium carbonate solution were added and diluted up to 100 ml with water. After shaking, the absorbance was read at 700 nm after 30 min. Blank solution was prepared with water instead of the sample. Standard graph was prepared by using 0-100 µg tannic acid. Total tannin content of the sample was measured equivalent to tannic acid by standard graph.

Ascorbic acid (mg/100g) = [0.5mg X titer vol.aganist test X 100ml/titer vol. against ref. X 5ml X weight of sample] X 100

Statistical Analysis

Statistical analysis of difference between two medicinal plants was done by one-way ANOVA followed by student's t test. P<0.05 was considered as significant.

RESULTS AND DISCUSSION

The antioxidant properties of Celtis timorensis and Vanda Spathulata have been evaluated by measuring their DPPH FRSA, reducing ability, Hydrogen peroxide FRSA, Total phenols, Total tannins and Ascorbic acid content using crude methanolic extract of aerial parts of these plants.

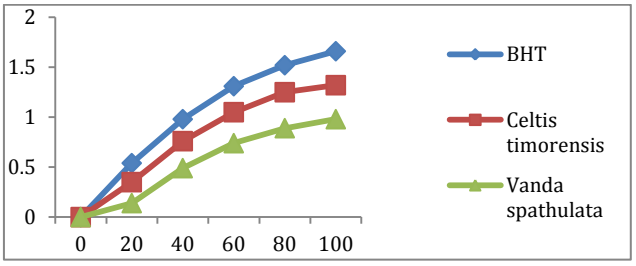
DPPH Free Radical Scavenging Activity Both the plants, Celtis timorensis as well as Vanda Spathulata, exhibited an antioxidant activity in a dose-dependent manner. The methanolic extract of Celtis timorensis leaf at different doses exhibited significantly (P<0.05) higher antioxidant activity as compared to Vanda Spathulata [Table 1].

Volume of Sample	DPPH free radical Scavenging activity (%)		Hydrogen peroxide Free radical scavenging activity(%)	
	Celtis timorensis	Vanda Spathulata	Celtis timorensis	Vanda Spathulata
20 µL	51.97	41.32	56.15	46.75
40 µL	82.54	63.67	85.21	76.22
60 µL	110.25	84.79	109.86	92.99
80 µL	137.55	96.89	122.21	105.89

Reductive Ability

Tanaka et al,[17] have reported that the antioxidant activity is concomitant with the reducing power. The reducing power of Celtis timorensis, Vanda Spathulata and the standard drug (BHT) is shown in [Figure 1]. The extract of Celtis timorensis had shown significantly higher (P<0.001) reducing power than the extract of vanda spathulata in dose-dependent manner. Absorbance of solution was increased with concentration of plant extract, indicating the concentration of hydrogen donating compounds present in the extracts was increased or reducing power of extracts was increased or reducing power of extracts was increased.

Figure 1: Reductive Ability Of Celtis timorensis, Vanda spahtulata and BHT



On X- Axis = Dose (µL) & On Y-Axis = Absorbance.

Hydrogen peroxide Free Radical Scavenging Activity

Hydrogen peroxide FRSA is another useful method for determination of antioxidant activity. Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic due to the increased hydroxyl radicals in the cells.[18] Activity of both plants was evaluated and it was observed that significantly higher (P<0.05) antioxidant activity exists in the extract of Celtis timorensis as

compared to Vanda Spathulata at different concentrations. Standard ascorbic acid at the concentration of 20 µg/ml showed 100% hydrogen peroxide FRSA [Table 1].

Total Phenols

Phenols are another important plant constituent due to their free radical scavenging ability because of hydroxyl groups.Tanaka et al.,[17] had suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when consumed up to 1g per day through diet rich in fruits and vegetables. Total Phenols were estimated to be 0.73% in Celtis timorensis while in Vanda spathulata it was recorded to be 0.45% (catechol equivalent).

Tannin Contents

Tannins also possess very high antioxidant activity due to their tremendous free radical scavenging ability and thus they protect the body from harmful effect of free radicals. Very high tannin contents (2.23%) were recorded in Celtis timorensis leaf while they were moderately low in Vanda Spathulata leaf (1.61%).

Ascorbic Acid Contents

Ascorbic acid is a naturally occurring antioxidant compound found in medicinal plants, vegetables, fruits and whole grains. We observed that dried powder of Celtis timorensis has higher ascorbic acid contents (14.71 mg/100g) as compared to that of Vanda spathulat (5.41 mg/100g).

It is evident from the present study that both Celtis timorensis and Vanda Spathulata have significant antioxidant content and activity, Celtis timorensis better in their regard than Vanda spathulata. The regular use of Celtis timorensis leaf as a natural health supplement can be beneficial in the treatment of neurological disorders associated with free radical damage. Keeping in view its high antioxidant property, this plant can also be used alone or in combination in the form of different herbal formulations to protect the body from deleterious effects of free radicals.

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