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The Antioxidant Effects of *Eupatorium triplinerve*, *Hygrophila triflora* and *Pterocarpus marsupium*-A Comparative Study

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Abstract: *Eupatorium triplinerve* Vahl., *Hygrophila triflora* (Roxb.) Fosberg *and Pterocarpus marsupium* Roxb., are very essential plant species having a broad spectrum of medicinal activities. The present study is designed to produce the comparative data of antioxidant and free radical scavenging effects of the three sources. The free radical scavenging activity was measured by using *in-vitro* DPPH model. The pet ether, chloroform and methanol extract of the leaves had been prepared and study was carried out using different concentration of the extract. The methanol extract showed potential activity than the other two solvent extracts. Among the three plants *Pterocarpus marsupium* showed most significant antioxidant activity, even a more potentiality than the standard drug Vitamin C.

Key word: *Eupatorium triplinerve* · *Hygrophila triflora* · *Pterocarpus marsupium* · Free-radical-scavenging · DPPH · *In-vitro* antioxidant.

INTRODUCTION

Antioxidants are our first line of defence against free radical damage and are critical for maintaining optimum health and wellbeing. Free radicals are fundamentals to any biochemical process and represent an essential part of aerobic life and metabolism. Majority of the diseases / disorders, like atherosclerosis, heart failure, neurodegenerative disorders, aging, cancer, diabetes mellitus and several other disorders are mainly linked to oxidative stress due to free radicals. [1] In a normal cell there is an appropriate pro-oxidant: antioxidant balance. However, this balance can be shifted towards the pro-oxidant when production of reactive oxygen species [e.g: superoxide anion (O_2), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO) and reactive hydroxyl radicals (OH)] is increased or when levels of antioxidants are diminished. This state is called 'oxidative stress' and can result in serious cell damage if the stress is massive or prolonged. [2].

Antioxidants are added to a variety of foods to prevent or deter free-radical-induced lipid peroxidation, which is responsible for the development of off-flavors and the undesirable chemical compounds in food [3]. These ROS cause destructive and irreversible damage to the components of a cell, such as lipids, proteins and DNA. Although normal cells possess antioxidant-defense systems, ROS produced in the cells induces diseases such as cancer and aging [4].

Antioxidant supplements or food-containing antioxidants may be used to help the human body reduce oxidative damage. The most commonly used antioxidants are BHA- Butylated hydroxyl anisole, BHT-Butylated hydroxyl toluene, propyl gallate and *tert*-butyl-hydroquinone [5]. However, they have been suspected of being responsible for liver damage and carcinogenesis in laboratory animals. Therefore, the development and use of more effective antioxidants are desired.

Traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their therapeutic principles. Plants produce antioxidants to control the oxidative stress caused by sunbeams and oxygen; they can represent a source of new compounds with antioxidant activity [6].

Eupatorium triplinerve Vahl. (Asteraceae), commonly known as 'Ayapana' in West Bengal, India, an ascending, slender perennial herb; leaves purple, subsessile, lanceolate, 3-nerved, acuminate, subentire, glabrous. Inflorescence a lax, few-headed corymb, heads pedicellate, about 20-flowered; flowers slaty blue [7]. Antimicrobial, anthelmintic activity of the leaves has been studied [8-10].

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Hygrophila triflora (Roxb.) Fosberg (Acanthaceae) is a dicotyledonous swampweed type of plant. However, remarkable research studies on this species till not carried out.

Pterocarpus marsupium Roxb., is renowned for its antidiabetic property, commonly known as 'Bijayasara' or 'Asana' in Bengal. Traditionally, the plant material has been used as a cooling external application for inflammations and headache, as antipyretic, anti-helminthic, aphrodisiac, alexeteic and in biliousness, mental aberrations and ulcers [11-13]. Parts of the Indian Kino (heart wood, leaves and flowers) have long been used for their medicinal properties in Ayurveda. The heart wood is used as an astringent and in the treatment of inflammation. The wood and bark are known for their anti-diabetic activity [14,15].

MATERIAL AND METHODS

Plant Materials: The mature leaves of *Eupatorium triplinerve, Hygrophila triflora and Pterocarpus marsupium* were collected during November 2011 from South 24 Parganas, West Bengal, India. The plant materials were taxonomically identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen (CNH/82/2011/Tech.II/605, CNH/81/2011/Tech.II/604 and CNH/80/2011/Tech.II/574 respectively) of *Eupatorium triplinerve, Hygrophila triflora and Pterocarpus marsupium* were maintained in our research laboratory for future reference. The plant materials were shade dried at room temperature with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40 and stored in an air-tight container.

Preparation of Plant Extracts: The dried powdered materials of each plant were defatted with petroleum ether (60-80°C) by percolation method and the percentage extractive values were determined. The defatted powder materials thus obtained were further extracted with chloroform and methanol for 72 hrs in a percolator. The solvents were distilled off in reduced pressure and resulting semisolid mass were vacuum dried using rotary flash evaporator to yield a solid residue and the percentage extractive values were recorded (Table 1). The preliminary phytochemical screening was performed for all the extracts to identify the phytoconstituents present in the extracts [16].

Chemicals: 1, 1-diphenyl-2- picryl-hydrazyl (DPPH) was purchased from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). All other chemicals and reagent were of analytical grade.

Free-radical-scavenging activity measured by 1, 1-diphenyl- 2-picryl-hydrazil: The free-radical-scavenging activity of all of the extracts from the three plants, were measured by 1, 1-diphenyl-2-picryl-hydrazil (DPPH). [13] Briefly, an 0.1 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of the all of the extracts solution respectively in petroleum ether, chloroform and methanol at different concentrations (10, 20, 40, 80, 160 and 320 g/ml). The mixture were shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm by using a UV visible spectrophotometer (Genesys 10 UV: The location of Thermo Electron Corporation is USA). Lower absorbance values of reaction mixture indicate higher free radical- scavenging activity.

The capability to scavenge the DPPH radical was calculated using the following equation: DPPH scavenging effect (%) = $[(A_0\text{-}A_1)/A0) \times 100]$, where A_0 is the absorbance of the control reaction and A_1 is the absorbance of presence of all of the extract samples and standard.

RESULTS AND DISCUSSIONS

The stable DPPH radical model is a widely used, relatively quick method for the evaluation of free radical scavenging activity. The effects of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The absorption maximum of a stable DPPH radical in methanol was 517 nm.

Table 1: Extractive values of each plant in different solvents

Plant Species	Pet ether extract, extractive value(% w/w)	Chloroform extract extractive value(% w/w)	Methanol extract extractive value(% w/w)
Eupatorium triplinerve Vahl.	3.92	7.69	10
Hygrophila triflora (Roxb.) Fosberg	2.25	1.05	5.18
Pterocarpus marsupium Roxb.	0.59	1.18	10.05

Table 2: DPPH scavenging effect of the different extracts of *Eupatorium triplinerve* Vahl. and vitamin C.

Extracts	Concentrations(µg/ml)	% of DPPH scavenging activity respectively	Mean ± SEM
Petroleum ether	2, 4, 6, 8, 10, 15	27.18, 33.29, 38.91, 59.51, 66.89 and 75.67	50.24 ± 5.56
Chloroform	2, 4, 6, 8, 10, 15	29.59, 34.52, 45.68, 51.62, 67.27 and 78.45	51.19 ± 4.29
Methanol	2, 4, 6, 8, 10, 15	36.73, 45.29, 51.45, 69.22, 78.24 and 81.41	60.39 ± 4.27
Vitamin C	2, 4, 6, 8, 10, 15	25.70, 32.10, 42.50, 87.54, 92.20 and 97.23	62.87 ± 5.29

Table 3: DPPH scavenging effect of the different extracts of *Hygrophila triflora* (Roxb.) Fosberg and vitamin C.

Extracts	Concentrations (µg/ml)	% of DPPH scavenging activity respectively	Mean ± SEM
Petroleum ether	2, 4, 6, 8, 10, 15	27.13, 29.24, 35.94, 54.56, 63.81 and 80.63	48.55 ± 4.75
Chloroform	2, 4, 6, 8, 10, 15	32.61, 37.82, 58.17, 61.42, 72.27 and 81.85	57.35 ± 5.24
Methanol	2, 4, 6, 8, 10, 15	37.19, 46.29, 61.55, 78.42, 81.24 and 87.61	65.38 ± 4.27
Vitamin C	2, 4, 6, 8, 10, 15	25.70, 32.10, 42.50, 87.54, 92.20 and 97.23	62.87 ± 5.29

Table 4: DPPH scavenging effect of the different extracts of *Pterocarpus marsupium* Roxb. and vitamin C.

Extracts	Concentrations (µg/ml)	% of DPPH scavenging activity respectively	Mean ± SEM
Petroleum ether	2, 4, 6, 8, 10, 15	28.63, 30.58, 36.29, 57.61, 68.47 and 86.83	51.40 ± 4.57
Chloroform	2, 4, 6, 8, 10, 15	33.52, 40.69, 52.28, 68.52, 73.81 and 89.54	59.72 ± 4.27
Methanol	2, 4, 6, 8, 10, 15	45.72, 59.29, 76.25, 81.91, 89.24 and 92.42	74.19 ± 5.15
Vitamin C	2, 4, 6, 8, 10, 15	25.70, 32.10, 42.50, 87.54, 92.20 and 97.23	62.87 ± 5.29

The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical progressed, results in the scavenging of the radicals by hydrogen donation. It is visually noticeable that as a change in colour from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidant activity of antioxidants [17]. It has been reported that oxidative stress, which occurs when free radical formation exceeds the body's ability to protect itself, forms the biological basis of chronic conditions such as arteriosclerosis [18,19].

Based on the data obtained from this study, all the extracts prepared from the three plants, were effective free radical inhibitor or scavenger, as well as a primary antioxidant that reacts with free radicals, which may limit free radical damage occurring in the human body. Tables 2-4 shows a significant ($P < 0.001$) decrease in the concentration of DPPH radicals due to the scavenging ability of the extracts and standard. Free radical scavenging activity also increased with increasing concentration in the range of 2- 15 µg/ml. The methanol extracts of *Hygrophila triflora* and *Pterocarpus marsupium* were found to be produced more significant result than the standard drug Vitamin C. However, *P. marsupium* showed most potent antioxidant activity and high percentage of free radical scavenging activity among the three plants.

The result of phytochemical screening confirms the presence of steroid and terpenoid in pet ether extracts of all the three plants. The positive result for flavonoids, steroid, terpenoids and glycosides in chloroform and methanol extracts were also found. The presence of those chemical constituents in the extracts determines the cause of antioxidant activity effectively shown by all the extracts.

CONCLUSIONS

Based on the results of this study, it is clear that all of the extracts have powerful *in vitro* antioxidants capacity against various antioxidant systems. From the results, it can be concluded that the antioxidant activity of all the extracts were concentration dependent. The possible mechanism of antioxidant activity of all the extracts include hydrogen-donating ability and scavenging of the free radicals, which may be due to the presence of phytoconstituents such as flavonoids, polyphenols, terpenoids and glysoside present in the petroleum ether, chloroform and methanol extracts accordingly of *Eupatorium triplinerve* Vahl., *Hygrophila triflora* (Roxb.) Fosberg *and Pterocarpus marsupium* Roxb. *P. marsupium* showed the most potent antioxidant activity among the three plants.

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