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Antioxidant Potential of Extracts of Leaves of *Tabernaemontana divaricata*

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ABSTRACT

Tabernaemontana divaricata (L) R. Br. (Apocynaceae) commonly known as Tagar in Bengali. It is a garden plant in tropical countries and found throughout the Indian subcontinent. The present investigation assessed the scavenging potential by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay. All of the extracts exhibited potent *in vitro* free radical scavenging activity that increased with extract concentrations. The methanol extract was found to be the most potent in this regard, followed by the benzene and ethyl acetate extracts. Therefore, the present study confirms marked in vitro free radical scavenging activity *Tabernaemontana divaricata* leaves.

Introduction

Antioxidants are our first line of defense against free radical damage and critical for maintaining optimum health and well-being. 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 free radical induced lipid peroxidation, which is responsible for the development of off-flavours 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 DNA and other macromolecules. Although normal cells possess antioxidant defence systems against ROS in the cells induces diseases such as cancer and aging [4].

Antioxidant supplements or foods containing antioxidants may be used to help the human body reduce oxidative damage. The most commonly used antioxidants are BHA, BHT, propyl gallate and tetra-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 is 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. Therefore, search for natural antioxidant has greatly been increased in the recent scenario.

Tabernaemontana divaricata (L) R. Br. (synonym: *Tabernaemontana coronaria*, *Ervatamia coronaria*) (Family: Apocynaceae) is commonly known as Tagar. It is an evergreen shrub to 6 feet (1.8 m) with large shiny leaves distributed in Coast forests of Bengal, Myanmar, mangrove forests of China and Japan [6]. It is a garden plant in tropical countries, is a rich source of alkaloids with various pharmacological properties. Plant used in Thai traditional rejuvenating and neurotonic remedies [7]. Extract inhibits neuronal acetyl-cholinesterase activity in rats [8]. Although, a number of chemical investigations have been performed and some constituents have been reported as alkaloids, carotenoids, flavonoids, glycosides, lipids, triterpines, polyphenols, saponins etc [9, 10]. It has been used in the Indian subcontinent as a folk medicine for anti-infection, anti-inflammation, analgesic, anti-tumour, anti-oxidative effect and the effect in neuronal activity [11]. Two bisindole alkaloids has isolated from the plant, 19,20-dihydrotaberamine and 19,20-dihydroervahanine A and this alkaloids are well-known acetyl-cholinesterase inhibitor. Again new alkaloids (voaharine 1 and conophyline 2) have isolated from the leaves of *Tabernaemontana divaricata* [12, 13].

In the present study, we have aimed to evaluate *in vitro* free radical scavenging activity of different extracts from *T. divaricata* leaves against 1,1-diphenyl-2-picryl-hydrazyl radical.

Materials and Methods

Plant Material

The mature leaves of *Tabernaemontana divaricata* (L) R. Br. (Family: Apocynaceae) were collected in the month of December-January, 2014 from the fields around the area of Kalyani, district Nadia, West Bengal, India. The plant material (Figure 1) was taxonomically identified by Dr. V. P. Prasad of the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen

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(CNH/Tech.II/2015/13/269) was maintained in our research laboratory for future reference. The plant material was shade-dried 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 material (350 gm) was first defatted with benzene (60-80°C) and the defatted powdered material thus obtained was further extracted (percolation) successively with ethyl acetate and methanol for 72 hrs in a cone shaped percolator. The extracts were filtered and their solvents were distilled off in reduced pressure and resulting semisolid masses were vacuum dried using rotary flash evaporator to yield the solid dry extracts and the percentage extractive values were accordingly 2.19% w/w, 1.91% w/w and 11.76% w/w respectively. The preliminary phytochemical analysis was performed on these three extracts to identify the phytoconstituents present in the extracts [14].

Reagents and chemicals

L ascorbic acid (vitamin C) and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade obtained commercially. Doubled distilled water was used throughout the study.

Thin layer chromatographic study

Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel G as stationary phase.

Free radical scavenging activity measured by 1,1-diphenyl-2-picryl-hydrazyl

The free radical scavenging activity of different extracts from the plants were measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) using the reported method [15]. 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 benzene, ethyl acetate and methanol at different concentrations (2,4,6,8,10,15 µg/ml). The mixture were shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using a UV-Visible spectrophotometer (Genesys 10 UV: Thermo Electron Corporation). Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. Ascorbic acid at same concentrations was used as reference. The capability to scavenge the DPPH radical was calculated by using the following equation:

DPPH scavenging effect (%) = $[(A_0 – A_1) / A_0) \times 100]$

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. The results have been stated in Table 2.

Results

Preliminary phytochemical screening on the test extracts revealed the presence of terpenoids, steroids, saponins, fixed oils, carbohydrate and glycoside in benzene extracts; terpenoids, saponins, carbohydrate, glycoside and phenolic compounds in the ethyl acetate extracts; terpenoids, carbohydrates, phenolic compounds, saponins, glycosides, tannins and alkaloids in the methanol extracts of leaves of *Tabernaemontana divaricata* (Table 1.)

Among the various methods for separating plant constituents, the thin layer chromatographic procedure is the one of the most commonly used techniques of general application. Thin layer chromatography (TLC) involves the separation of mixtures of organic compounds on thin layers of adsorbents that are usually coated on glass, plastic, or aluminium sheets; and this particular technique is the easiest, cheapest and most widely used method for the characterization of natural products and their preparations. All of these TLC profiles may serve as characteristic fingerprint of *Tabernaemontana divaricata* leaves. These data would therefore be suitable for monitoring the identity and purity of the plant material and for detecting adulterations and substitutions.

Table 1. Phytochemical test of different extracts of leaves of *Tabernaemontana divaricata*

Sl. no.	Phytoconstituents	Benzene extract	Ethyl acetate extract	Methanol extract
1	Steroids	+	-	+
2	Terpenoids	+	+	+
3	Alkaloids	-	-	+
4	Carbohydrates	+	+	+
5	Tannins	-	-	+
6	Flavonoids	-	+	+
7	Saponins	+	+	+
8	Proteins	-	-	-
9	Fixed oils	+	-	-
10	Glycosides	+	+	+
11	pH	4.5	6.5	7.0

(+) Presence of constituents; (-) Absence of constituents

Discussion

The stable DPPH radical model is a widely used, relatively quick and precise 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. 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 color from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidant activity of different antioxidants [16]. 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 disease conditions such as arteriosclerosis [17].

Based on the data obtained from the present study, all the extracts 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.

Table 2. DPPH scavenging power of the different extracts of leaves of *T. divaricata and* Ascorbic acid

Sl. No.	Conc. (µg/ml)	% of DPPH scavenging activity			
		Benzene Extract	Ethyl acetate Extract	Methanol Extract	Ascorbic acid
1	2	25.81	28.29	37.78	32.18
2	4	27.27	34.62	45.29	43.18
3	6	34.85	46.28	55.24	60.45
4	8	49.44	54.63	63.29	77.54
5	10	57.73	63.25	78.23	82.20
6	15	65.03	71.15	85.33	90.23
Mean ± SEM		43.35 ± 5.16	49.70 ± 3.17	60.86 ± 5.63	64.29 ± 5.19

Figure 2-4 illustrates a significant 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.

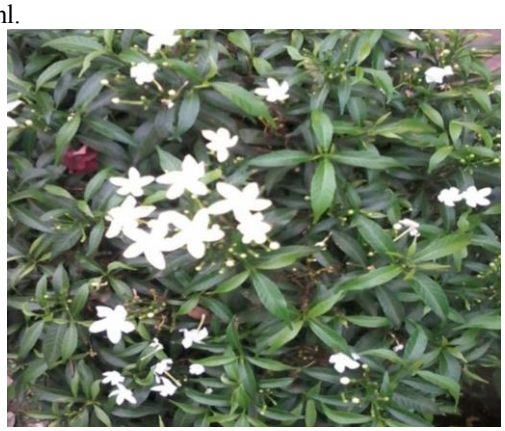

Figure 1. Photodocumentation of *Tabernaemontana divaricata* plant with leaves and flowers

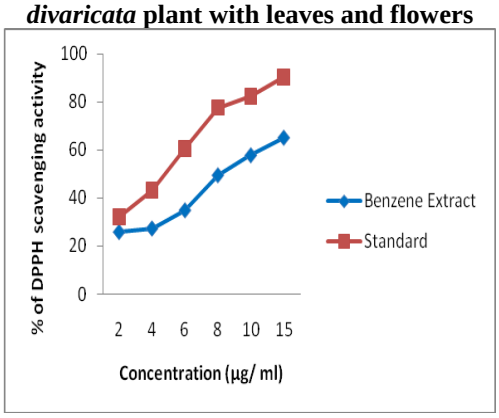

Figure 2. Graphical repesentation of DPPH scavenging activity of Benzene extracts of *T. divaricata* leaves

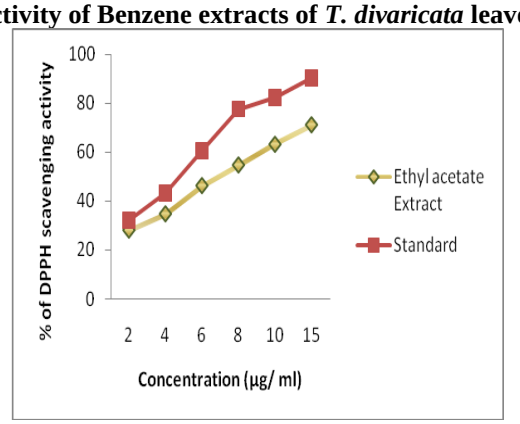

Figure 3. Graphical repesentation of DPPH scavenging activity of Ethyl acetate extracts of *T. divaricata* leaves

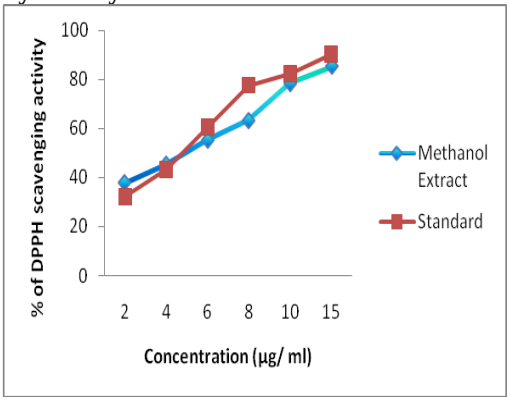

Figure 4. Graphical repesentation of DPPH scavenging activity of Methanol extracts of *T. divaricata* leaves

Conclusion

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 glycoside present in the benzene, ethyl acetate and methanol extracts of the leaves of *T. divaricata* showed the antioxidant activity of the plants. The methanol extract was found to demonstrate the most active free radical scavenging potential even more than that of ascorbic acid at the test concentration, followed by the ethyl acetate and benzene extracts. The present preliminary study confirmed remarkable *in vitro* free radical scavenging activity of *T. divaricata* leaf against DPPH.

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References

[1] Ajitha M, and Rajnarayan K. Role of Oxygen free Radicals in human disease. Indian Drug.2001; 38: 545-554

[2] Gupta Sweta, Mediratta. P.K., Singh S., Sharma K.K., and Shukla R. Antidiabetic, antihypercholesterolemic and antioxidant effect of *Ocimum sanctum Linn*. Seed oil, Ind. Jr. Exp. Biol.2006; 44(4): 300-303

[3] Halliwell B, Aeschbach R, Loliger J, Aruom O.I. The characterization of antioxidants. Food Chem Toxicol.1995; 33: 601-617

[4] Mates J.M, Sanchez- Jimenez FM. Role of reactive oxygen species in apoptosis: Implications for cancer therapy. Int. J. Biochem. Cell Biol.2000; 32: 57-170

[5] Gulcin I, Beydemir S, Ahmet AH, Mahfuz E, Emin BM. *In vitro* antioxidant properties of morphine. Pharmacol Res.2004; 49: 59-66

[6] Sharker Md. S., Chakma S. and Rahman A.A. Phytochemical and antinociceptive study of leaves of *Tabernaemontana divaricata* (L). Journal of Medicinal Plants Research.2011; 5(2): 245-247

[7] Ingkaninan K., Temkitthawon P., Chuenchom K., Yuyaem T., Thongnoi W. Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. J. Ethnopharmacol.2003; 89(2-3): 261-264.

[8] Chattipakorn S., Pongpanparadom A., Pratchayasakul W. *Tabernaemontana divaricata* Extract inhibits neuronal acetylcholinesterase activity in rats J. Ethnopharmacol.2007; 110(1):61-68

[9] Basak U.C., Das A.B., Das P., Chlorophyll, carotenoids, proteins and secondary metabolites in leaves of 14 species of mangroves. Bull. Mar. Sci.1996; 58: 654-659

[10] Ghosh A., Mishra S., Dutta A.K., Choudhury A.,(1985). Pentacyclic triterpenoids and sterols from seven species of mangrove. Phytochem.1996; 24: 1725-1727

[11] Pratchayasakul W., Pongchaidecha A., Chattipakorn N., Chattipakorn S., Ethnobotany and ethnopharmacology of *Tabernaemontana divaricata*. Indian J Med Res.2008; 127: 317-335

[12] Ingkaninan K., Changwijit K., Suwanborirux K., Vobasinyl-iboga bisindole alkaloids, potent

acetylcholinesterase inhibitors from *Tabernaemontana divaricata* root. J Pharm Pharmacol.2006; 58(6): 847-852
[13] Toh-Seok K, Kah-Yeng L, Lay-Har L, Wai-Ling L, Cheng-Hock Chuah and Chen Wei. New alkaloids from the leaves of *Tabernaemontana divaricata*. Tetrahedron Letters.1992; 33(7): 969-972
[14] Harborne J.B., Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis. New Delhi: Springer (India) Pvt. Ltd.; 2010
[15] Blois M.S (1958): Antioxidant determinations by the use of stable free radical. Nature 26: 1199-1200
[16] Chang L.W, Yen W.J, Huang S.C,Duh P.D (2002): Antioxidant activity of sesame coat. Food Chem 78: 347-354
[17] Fatimah Z.I, Zaiton Z, Zamaludin M, Gaptor M.T, Nafeeza M.L, Khairul O. Effect on estrogen and palm vitamin F on malonaldehyde levels toward the development of arteriosclerosis in the New Zealand white rabbit. In: Packer L Ong SH. Eds. Biological Oxidants and antioxidants: Molecular Mechanism and Health Effects. Champaign. IL. AOCS Press.1998; 22.