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Phytochemical investigation and evaluation of *in vitro* free radical scavenging activity of *Tabernaemontana divaricata* Linn.

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We evaluate the *in vitro* free radical scavenging activity of the leaves of *Tabernaemontana divaricata* Linn. Petroleum ether, ethanol and aqueous extracts of *T. divaricata* were prepared with successive extraction in a soxhlet apparatus. Each extract was selected to study the free radical scavenging activity by superoxide scavenging assay method. It was found that the aqueous extract contained carbohydrates, glycosides, amino acids, flavonoids, tannins, alkaloids, and steroids, and the ethanolic extract contained glycosides, amino acids, flavonoids, tannins, alkaloids and steroids. The ethanolic extract of *T. divaricata* showed $58.7 \pm 0.62\%$ inhibition in the superoxide scavenging model. The aqueous extract also showed almost similar activity ($54.9 \pm 0.53\%$ compared to the ethanolic extract), while petroleum ether extract showed poor inhibition of superoxide scavenging activity. All extracts showed the dose- and time-dependent inhibition of the superoxide scavenging activity.

Keywords: *Tabernaemontana divaricata*; superoxide scavenging; antioxidant activity

1. Introduction

Tabernaemontana divaricata, a common garden plant in tropical countries, is used as a traditional medicine. These plants are well known as a major source of modern medicines. From ancient times, people have been utilising plants for the treatment or prevention of diseases, leading to the dawn of traditional medicine. *Tabernaemontana* is one of the genera that is used in Chinese, Ayurvedic and Thai traditional medicine for the treatment of fever, pain and dysentery (Geronikaki, Litina, Chatziopoulos, & Soloupis, 2003; Wasana, Anchalee, Nipon, & Siriporn, 2008).

Literature reveals that the carbonyl groups are responsible for free radical scavenging activity (Nicholls & Budd, 2000). Free radicals are atoms or groups of atoms with an odd number of electrons and can be formed when oxygen interacts with certain molecules. Once highly reactive free radicals are formed, they can start chain reactions. Their major threat comes from the damage they can do when they react with important cellular components such as DNA or cell membranes. Cells may function poorly or die if this occurs. To prevent free radical damage, the body has a defence system of antioxidants

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(Thomas, 2000). Antioxidants can give free radicals, which become companions to their unpaired electrons, thus eliminating the threat of gene alteration which can lead to cancer (Patil, Jolly, & Narayanan, 2003; Sharma, Yelane, & Dennis, 2002). Medicinal plants have attracted the attention of not only professionals from various systems of medicine, but also the scientific community belonging to different disciplines. Herbal drugs, being generally harmless in prescribed doses, are becoming popular all over the world and the WHO currently encourages, recommends and promotes inclusion of these drugs in national health care programmes (Chhajed, Khedekar, & Mundhey, 2007; Khandelwal, 2000). In recent years, these have been of great interest in herbal remedies for the treatment of a number of ailments. Plants are a promising source of drugs. In continuing the search for potential free radical scavenging agents (Kokate, 1999), the present investigation is aimed at determining the free radical scavenging activity of *T. divaricata* (Linn.) R.Br. leaves. Free radical scavenging properties help in strengthening the immune system of the body, which helps to overcome cancer.

2. Materials and methods

2.1. *Collection and identification of the extract*

The leaves of *T. divaricata* (Linn.) R.Br. were collected from Mandsaur, MP, India. The authentication was done by Prof. H.S. Chattri (Principal of Govt. PG College Mandsaur, MP, India). A voucher specimen (BRNCP/T/002/2006) has been deposited at the museum of the college.

2.2. *Preparation of the extracts*

The leaves of *T. divaricata* were collected and shade dried. The dried leaves were coarse powdered and the powder was packed into a soxhlet column and extracted successively with petroleum ether (60–80°C), ethanol (64.5–65.5°C) and distilled water. The extracts were concentrated under reduced pressure (bath temp. 50°C). The dried extracts were stored in an airtight container in a refrigerator below 10–20°C.

2.3. *Preliminary phytochemical screening*

The preliminary phytochemical screening was carried out on petroleum ether, ethanol and aqueous extracts of *T. divaricata* leaves for the detection of various phytochemicals. Tests for common phytochemicals were carried out by standard methods (Bagul, Kanaki, & Rajani, 2005; Richards & Sharma, 1991).

2.4. *Superoxide scavenging activity*

Petroleum ether, aqueous and ethanolic extracts were screened for anti-oxidant activity using superoxide free radical scavenging activity in a dose- and time-dependent manner (Matill, 1947).

The assay was based on the capacity of the samples to inhibit blue formazan formation by scavenging the superoxide radicals generated in a riboflavin-light-NBT system. The reaction mixture contained 50 mM phosphate buffer, pH 7.6, 20 μg riboflavin, 12 mM

EDTA, and 0.1 mg/3 mL NBT, added in that sequence. The reaction was started by illuminating the reaction mixture with different concentrations (5–100 $\mu g\,mL^{-1}$) of samples for 15, 30 and 45 min. Immediately after illumination, the absorbance was measured at 590 nm (Bagul et al., 2005). Ascorbic acid was used as a standard drug. Percentage inhibition and IC_{50} values were calculated (results are shown in Table 1).

3. Results

3.1. *Phytochemical investigation*

It was found that the petroleum ether extract contained steroids, fat and fixed oils; the aqueous extract contained carbohydrates, amino acids, steroids, flavonoids, alkaloids, glycosides and tannins; and the ethanolic extract showed similar phytochemicals to the aqueous extract.

3.2. *Free radical scavenging activity*

The ethanolic extract of *T. divaricata* showed $58.7 \pm 0.62\%$ inhibition in the superoxide scavenging model. The aqueous extract showed similar activity ($54.9 \pm 0.53\%$ compared to the ethanolic extract), while the petroleum ether extract showed poor inhibition of the superoxide scavenging activity. All extracts showed the dose- and time-dependent inhibition of the superoxide scavenging activity. The results are reported in Table 1.

3.3. *Statistical analyses*

Data are the mean $\pm$ SD of three measurements. Statistical analysis was performed by the Student's *t*-test and by ANOVA.

4. Discussion

Nowadays, traditional medicine all over the world is being revalued by extensive research into different plant species and their therapeutic principles. Experimental evidence suggests that free radicals and reactive oxygen species can be involved in a high number of diseases (D'Mello, Jadhav, & Jolly, 2000). As plants produce a lot of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity.

In this study, aqueous and ethanolic extracts were selected as they contain alkaloids, glycosides, saponins, tannins, flavonoids and phenolic compounds. They may have active constituents for producing the free radical scavenging effect.

Free radicals are produced under certain environmental conditions and during normal cellular function in the body. These molecules lose an electron when an electric charge is given to them. To neutralise this charge, free radicals try to withdraw an electron from, or donate an electron to, a neighbouring molecule. The newly created free radical, in turn, looks out for another molecule and withdraws or donates an electron, setting off a chain reaction that can damage hundreds of molecules. Antioxidants halt this chain reaction. Some antioxidants are free radicals themselves, donating electrons to stabilise the dangerous free radicals. Other antioxidants work against the molecules that form free radicals,

Table 1. Percentage inhibition of petroleum, ethanolic and aqueous extracts in superoxide.

		% Inhibition								
		15 min			30 min			45 min		
No.	Concentrations ($\mu g\,mL^{-1}$)	Petroleum ether	Ethanolic	Aqueous	Petroleum ether	Ethanolic	Aqueous	Petroleum ether	Ethanolic	Aqueous
1.	5	26.8 ± 0.28	37.0 ± 0.32	33.6 ± 0.22	32.9 ± 0.34	40.4 ± 0.38	38.7 ± 0.39	39.4 ± 0.44	51.4 ± 0.49	43.1 ± 0.33
2.	10	30.8 ± 0.31	46.0 ± 0.49	39.6 ± 0.27	40.8 ± 0.39	52.5 ± 0.45	42.4 ± 0.47	48.7 ± 0.45	56.3 ± 0.58	48.2 ± 0.51
3.	25	38.6 ± 0.32	50.9 ± 0.53	42.7 ± 0.39	49.4 ± 0.54	57.5 ± 0.55	48.3 ± 0.49	53.6 ± 0.57	61.8 ± 0.66	52.8 ± 0.57
4.	50	43.0 ± 0.52	58.7 ± 0.62	50.0 ± 0.48	54.9 ± 0.53	61.6 ± 0.59	50.9 ± 0.52	60.4 ± 0.63	68.4 ± 0.65	60.0 ± 0.63
5.	100	50.6 ± 0.47	61.2 ± 0.51	57.6 ± 0.53	58.8 ± 0.59	68.6 ± 0.61	60.5 ± 0.64	68.5 ± 0.67	70.8 ± 0.69	67.8 ± 0.58

Note: Data are the mean $\pm$ SD of three measurements. Statistical analysis was performed by the Student's *t*-test and by ANOVA.

destroying them before they can begin the domino effect that leads to oxidative damage (Matill, 1947).

Antioxidants work to control the levels of free radicals before they do oxidative damage to the body. For example, certain enzymes in the body, such as superoxide dimutase, work with other chemicals to transfer free radicals into harmless molecules. Vitamin C is an antioxidant that may prevent cataracts and cancers of the stomach, throat, mouth and pancreas (D'Mello et al., 2000). It may also prevent the oxidation of LDL cholesterol, lowering the risk of heart disease. Scientific literature reveals that the carbonyl groups present in the flavonoids and phenolic compounds were responsible for the free radical scavenging activity (Nicholls & Budd, 2000). This investigation revealed that *T. divaricata* Linn. contains pharmacologically active substances such as alkaloids, glycosides, saponins, tannins, flavonoids and phenolic compounds which are responsible for the superoxide scavenging activity.

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