Research Article

In vitro Antioxidant Activity of Different Extract of *Callistiemon viminalis*.

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ABSTRACT

Free radicals are implicated for many diseases including diabetes mellitus, arthritis, cancer, ageing. etc. In the treatment of these diseases, antioxidant therapy has gained a great importance. Ideally an antioxidant agent should have high percentage of cure with a single therapeutic dose, free from toxicity to the host and cost effective. None of the synthetic drugs available meets there requirements. The origin of many effective drugs is found in traditional medicine and in view of this several researchers have undertaken studies to evaluate folklore medicinal plants for their proclaimed antioxidant activity. Currently much interest has been paid in the searching of medicinal plants with antioxidant activity which may lead to the discovery of new therapeutic agent that is not only used to suppress the free radicals but also used in diverse disease conditions. Different extract (water, alcoholic, chloroform and petroleum ether) of *Callistiemon viminalis* was studied for its in vitro antioxidant activity using Nitric Oxide Radical Inhibition Assay (NORIA)**.** The results were analyzed statistically by the regression method. Its antioxidant activity was estimated by IC50 value. The results showed that the water and alcoholic extracts of *Callistiemon viminalis* showed potent antioxidant activity than the chloroform and petroleum ether extracts. The antioxidant potency of different extracts has been expressed as in order of Water>Alcohol>Chloroform>Petroleum ether. In all the testing, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. The antioxidant property may be related to the polyphenols and flavonoids present in the extract. These results clearly indicate that *Callistiemon viminalis* is effective against free radical mediated disease.

Keywords: *Callistiemon viminalis,* Flavanoids, NORIA, Superoxide.

Received 21 May 2012 Received in revised form 29 May 2012 Accepted 10 June 2012

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INTRODUCTION

Free radicals [reactive oxygen species (ROS)] are an entire class of highly reactive molecules derived from the metabolism of oxygen. Moreover, these radicals can cause extensive damage to cells and tissues, during infections and various degenerative disorders, such as cardiovascular disease, aging, and neurodegenerative diseases like Alzheimer's disease, mutations and cancer [1-3]. Although many anti-oxidant defence systems consisting of enzymatic (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and nonenzymatic (ascorbic acid, glutathione and α-tocopherol) compounds can maintain the balance between free redical generation and protection from damage by these redicals but these anti-oxidant systems do not provide complete protection from against ROS under conditions of severe oxidative stress [4,5]. Bottlebrushes are members of the genus *Callistemon* and belong to the family Myrtaceae. They are closely related to paperbark melaleucas, which also have 'bottlebrush' shaped flower spikes. A study in India showed the leaves of *Callistemon viminalis* yielded an oil: 1,8 –Cineole, pentene, pinene, flavones and methyl acetate were the major components [6,7]. Many studies have been done on medical properties of different species of *Callistemon.* Antibacterial and antifungal activities of methanolic extract obtained

from *Callistemon viminalis* leaf have been studied. This extract also shows good antioxidant activity (comparable with standard Ascorbic acid) which is concentration dependent [8,9].

Callistemon vaminalies has a great medicinal importance. In our previous work, fruits of Callistemon citrinus were reported to have relaxant (antispasmodic) activity. Acute toxicity and brine shrimp cytotoxicity of crude methanol extract are also performed to standardize it.

MATERIALS & METHODS

Preparation of extracts

Leaves of *Callistiemon viminalis* was collected from the district. Forest Research Institute, Dehradun, UK & identified by Department of Botany, FRI, Dehradun. The freshly cut leaves of *Callistemon viminalis* was dried in the drying room with active ventilation at ambient temperature. 500 g of powdered of *Callistiemon viminalis* has been taken. Fractionation has been done according to increasing polarity as Petroleum ether, Chloroform, alcoholic and water. The obtained extract was filtered and evaporated by using vacuum evaporator under 40C to give different % yield [PEt: 12.82 gm, CH: 17.48 gm, AL: 32.6 gm, WA: 43.32 gm. of dried crude extract.

Nitric Oxide Radical Inhibition Assay

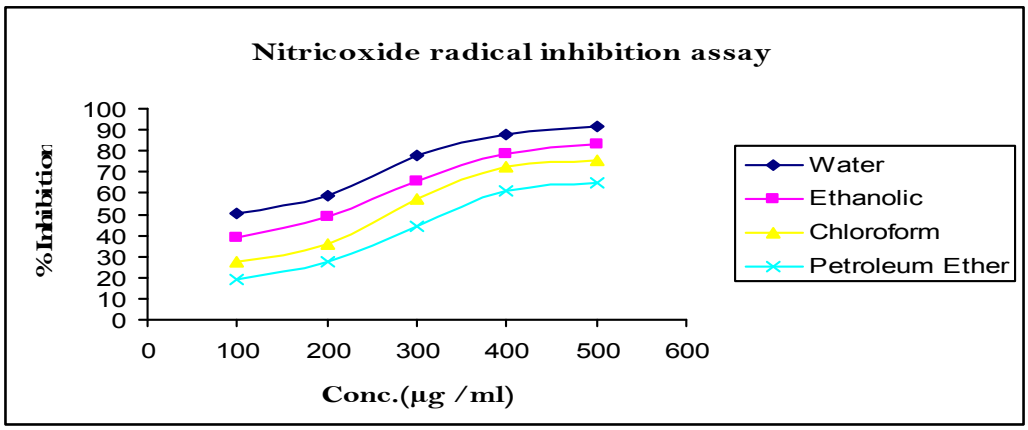
Nitric oxide radical inhibition can be estimated by the use of Griess Illosvoy reaction. In this assay, Griess Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1 % w/v) instead of 1-napthylamine (5 %). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and *Callistiemon viminalis* extracts (100 to 600 µg/ml) or standard solution (ascorbic acid, 300 (µg /ml) was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25°C. A pink coloured chromophore is formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. Ascorbic acid was used as a standard [10].

RESULTS AND DISCUSSION

Five of each concentrations ranging from 100–600 µg/ml of all four extract of *Callistiemon viminalis* were tested for their antioxidant activity in Nitric oxide radical inhibition assay models. It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner up to the given concentration in all the extracts (**Table 1**). Absorbance of control 0.5210 was found. The IC50 values of Water, Alcohol, Chloroform and Petroleum ether were calculated as 336.3, 288.6, 204.3 and 98.8 µg. The scavenging of nitric oxide by plant extract was increased in a dose dependent manner as illustrated in (**Fig. 1**).

Table 1: Absorbance and % inhibition of different extracts of *Callistiemon viminalis.*

Conc (µg /ml)	Water Extract		Ethanolic Extract		Chloroform Extract		Petroleum Ether Extract	
	Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition
Ascorbic acid								
300	0.4026	100	0.4026	100	0.4026	100	0.4026	100
500	0.2021	91.7	0.1572	83.55	0.0986	75.78	0.0875	64.8
400	0.2363	87.5	0.1981	78.68	0.1451	72.4	0.1387	60.8
300	0.3206	77.6	0.2645	65.69	0.2297	57.05	0.1782	44.26
200	0.3468	58.69	0.3168	49.2	0.2721	36.04	0.2068	27.2
100	0.3692	50.19	0.3364	39.04	0.3051	27.2	0.2732	19



CONCLUSION

The results of the present study show that the extract of *Callistiemon viminalis* exhibits the greatest antioxidant activity through the scavenging of free radicals which participate in various pathophysiology of diseases including ageing [11]. This observation also shows presence of a great amount of polyphenols [12,13].

ACKNOWLEDGEMENT

The authors sincerely thank to Chairman of Dev Bhoomi Group of Institution, Dehradun, U.K. India for providing the necessary facilities to carry out this research work.

REFERENCES

1. Ames B. Micronutrients prevent cancer and delay aging. Toxicol. Lett. 1998;102:5-18.
2. Cox DA and Cohen ML. Effects of oxidized low density lipoprotein on vascular contraction and relaxation. Pharmacol. 1996;48:3-9.
3. Cesaratto L, Vascotto C, Calligaris S and Tell G. The importance of redox state in liver damage. Ann. Hepatol. 2004;3:86-92.
4. Evans C, Miller NJ. Factors affecting the antioxidant activity determined by the ABTS radical cation assay. Free Radic. Res. 1997;195:26-27.
5. Finkel T and Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000;408:239-247.
6. Wojdylo, J. Oszmianski and R. Czemerys, Antioxi-dant Activity and Phenolic Compounds in 32 Selected Herbs. Food Chemistry. 2007;105(3):940- 949.
7. Sharma R. K. Kotoky R. and Bhattacharya P. R.. Vola-tile Oil from the Leaves of Callistemon lanceolatus D.C. Grown in Northeastern India. Flavonoid and Fragrance Journal. 2006;21(2): 239-240.
8. Dlugosz A. Lembas-Bogaczyk J. and Lamer-Zaraw- ska E. Antoxid Increases Ferric Reducing Antioxidant Po- wer (FRAP) even Stronger than Vitamin C. Acta Poloniae Pharmaceutica. 2006;63: 446-448.
9. Harish R. and Shivanandappa T. Antioxidant Activity and Hepatoprotective Potential of Phyllanthus niruri. Food Chemistry. 2006;95(2):180-185.
10. Temraz A, Tantawy HE. Characterization of antioxidant activity of extract from Artemisia vulgaris. Pak. J. Pharm. Sci. 2008;21(4):321-326.
11. Ganapaty S, Chandrashekhar VM, Chitme HR, Lakshmi NM. Free radical scavengingactivity of gossypin and nevadensin: An in vitro relation. Indian Journal of Pharmacology. 2007;39:281-283.
12. Shirwaiar, A. Shirwaikar, I.S.R. Punitha. Antioxidant studies on the methanol stem extract of Coscinium fenestratum. Natural Product Sciences. 2007;13:40-45.
13. Spiteller G. Peroxidation of linoleic acid and its relation to aging and age dependent diseases. Mech Ageing Dev. 2001;122:617–657.