Journal of Medicinal Chemistry and Drug Discovery
ISSN: 2347-9027
Special Issue
Analytical Chemistry Teachers And Researchers Association
National Convention/Seminar
18 January 2015

"*In vitro* free radical scavenging activity of aqueous juice of the root of wound healing plant *Ziziphus jujuba* Mill. and *Vitex nigundo* Linn"

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Abstract

A number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Various herbs and spices have been reported to exhibit antioxidant activity. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins, hence, the synthetic ones can replace with that of plant products. It is a current trend in different ground, especially food production and medicine, to search for natural products. The present work is carried out to investigate antioxidant activity of fresh aqueous juice of the root of *Ziziphus jujuba* Linn and the fresh leaves of *Vitex nigundo* Linn, maintained in a refrigerator, since both plants are rich in flavonoids. The fresh aqueous juice of the root of *Z. jujuba* showed maximum peroxidase (2.01 U/mg protein), Superoxide dismutase (81.09 U/mg protein) and Catalase (59.98 U/mg protein) respectively. However, the aqueous juice of *V. nigundo* showed moderate scavenging activity. Relatively *Z. Jujuba* is more potent than *V. nigundo*. The antioxidant potential of *Z. jujuba* may be attributed to the presence of flavonoids and the other constituents present therein. The findings substantiate the therapeutic applications of the plant in the indigenous system of medicine.

Key words: *Ziziphus jujuba* Mill., Aqueous juice, Antioxidant,

Introduction

The role of free radicals and reactive oxygen species (ROS) has gained much attention in recent years because they are closely associated with many pathological conditions such as

cancer, arthritis, cardiovascular diseases, liver diseases (Fejes et al., 2000) and wound (Houghton et al., 2005). When wounding occurs, it is accompanied within quite a short time by pain, and reddening and edema of the surrounding tissue. These are all classical symptoms of inflammation and are caused by the release of the eicosanoids, prostaglandins and ROS. Thus inhibitions of eicosanoid synthesis and antioxidant activity are both properties of extracts and their constituents which can be tested *in vitro*. In living organisms the activity of ROS are counteracted by antioxidants. Many antioxidant compounds occurring naturally in plant sources have been identified and proved as free radical scavenger both *in vitro* and *in vivo.* The antioxidant potential of the root of *Ziziphus jujuba* and *Vitex nigundo* has not been evaluated so far. Thus, this supportive parameter is evaluated in the present investigation.

Ziziphus jujuba Mill. (Rhamnaceae), Common jujube, a spiny deciduous shrub or small tree and commonly cultivated in India. The Chemical constituents like Carbohydrates, fat protein, amino acids, anthocyanins from fruit, seeds and leaves were reported. Leucocyanidin from bark, Leucopelargonidin, betulinic and ceabothic acids from wood, Rutin from leaves, Mauritines A,B,C,D,E and F, frangufoline and amphbines B,D and F. Ziziphine A,B,C,D,E,------Q from stem and root bark were documented by various researchers. The roots are useful in wounds and ulcers. The leaves are bitter and are useful in wounds, syphilitic ulcers. Fruits are useful in leprosy, skin diseases, pruritus, wounds and ulcers, haemorrhages and general debility. The seeds are acrid and are useful in wounds.

Ziziphus jujuba is being used by tribal Adivasies in eastern parts of Jalgaon District (Maharashatra State) influencing injuries small cuts and or animals bite, attack and wounds. Various activities like anti-inflammatory (Adzu and Haruna, 2007); sedative and hypnotic (Gong et al., 2000); anticancer, antiretroviral (Mukharjee et al., 2003); anticomplementary (Sang et al., 2004) and antioxidant (Seong et al., 2008) has been reported.

Vitex negundo *Linn* (Fam. Verbenaceae) is a large shrub available throughout India (Gupta et al., 2005). Leaves of this plant have been studied thoroughly for their varied therapeutic activities, such as CNS depressant activity (Gupta et al., 1999) inhibition of rat peritoneal cavity mast cell degranulation (Nair et al., 1995), and prevention of genotoxicity (Balboa and Lim - Sylianco, 1993). Mosquito repellent effects (Hebbalkar et al., 1992),

antiulcerogenic (Sahni et al., 2001), antiparasitic (Parveen, 1991), antimicrobial (Rusia and Srivastava, 1998), and hepatoprotective (De et al., 1993) potential of the leaves have also been studied.

Vitex negundo Linn has been investigated extensively for its anti-inflammatory (Ravishankar et al., 1985; Jana et al., 1999; Telang et al., 1999; Valamathi et al., 2000) and analgesic (Ravishankar et al., 1985; Telang et al., 1999) activities, but it was Telang et al., (1999) who noticed the inhibitory activity of the extract on PG biosynthesis and confirmed NSAID-like activity with selective COX-2 inhibition activity (Ravishankar et al., 1985; Telang et al., 1999). Thus, it was hypothesized that a plant possessing a significant anti–inflammatory activity may also demonstrate a potential anti-implantation activity.

It has been noted that the dynamic oxyradical-antioxidant balance serves as a good marker for biophysical and biochemical changes occurring in any biological system (Tiwari, 2001). Hence, superoxide anion radical and superoxide dismutase have been used previously to study any physiological changes occurring in the uterine milieu (Nivsarkar et al., 2001, 2002, 2005, 2006).

The objective of the present study was to investigate antioxidant potential of the root of *Z.jujuba* and *V. nigundo*

Methodology

Plant sample extraction – selected wound healing plants taken for the study collected freshly from the North Maharashatra Region and stored under refrigerated condition till use. The sample were prepared by grinding one gram each of the plant part in 2 ml of 50% ethanol separately in a pre-chilled mortar and pestle and the extract were centrifuged at 10000 g at 4^0c for ten minutes. The supernatant thus obtain were used within four hours for various enzymatic antioxidant assay.

MATERIALS AND METHODS

Collection of plant material

The plant is collected from North Maharashtra Region in the period of September 2007 to January 2008.

Plant sample extraction

Each of the plants taken for the study are collected freshly from the local area and stored under refrigerated condition till use. The samples were prepared by grinding one gram each of the plant parts in 2 ml of 50% ethanol, separately, in a pre-chilled mortar and pestle and the extracts were centrifuged at 10,000 g at 4ºC for 10 minutes. The supernatants thus obtained were used within four hours for various enzymatic and non-enzymatic antioxidants assays.

Assay of superoxide dismutase (SOD) activity (Das, et. al., 2000)

The assay of superoxide dismutase was done according to the procedure of Das *et al*. (16). In this method, 1.4ml aliquots of the reaction mixture (comprising 1.11 ml of 50 mM phosphate buffer of pH 7.4, 0.075 ml of 20 mM L-Methionine, 0.04ml of 1% (v/v) Triton X-100, 0.075 ml of 10 mM Hydroxylamine hydrochloride and 0.1ml of 50 mM EDTA) was added to 100 _l of the sample extract and incubated at 30ºC for 5 minutes. 80 _l of 50 _M riboflavin was then added and the tubes were exposed for 10 min to 200 W-philips fluorescent lamps. After the exposure time, 1ml of Greiss reagent (mixture of equal volume of 1% sulphanilamide in 5% phosphoric acid) was added and the absorbance of the color formed was measured at 543 nm. One unit of enzyme activity was measured as the amount of SOD capable of inhibiting 50% of nitrite formation under assay conditions.

Assay of catalase activity (Sinha, 1972)

Catalase activity was assayed by the method of Sinha (17). The enzyme extract (0.5 ml) was added to the reaction mixture containing 1ml of 0.01 M phosphate buffer (pH 7.0), 0.5 ml of 0.2 M H_2O_2, 0.4 ml H_2O and incubated for different time period. The reaction was terminated by the addition of 2 ml of acid reagent (dichromate/acetic acid mixture) which was prepared by mixing 5% potassium dichromate with glacial acetic acid (1:3 by volume). To the control, the enzyme was added after the addition of acid reagent. All the tubes were heated for 10 minutes and the absorbance was read at 610 nm. Catalase activity was expressed in terms of _moles of H2O2 consumed/min/mg protein.

Assay of peroxidase activity (Addy et.al., 1972)

The assay was carried out by the method of Addy and Goodman (18). The reaction mixture consisted of 3ml of buffered pyrogallol [0.05 M pyrogallol in 0.1 M phosphate buffer (pH 7.0)] and 0.5 ml of 1% H2O2. To this added 0.1 ml enzyme extract and O.D. change was measured at 430 nm for every 30 seconds for 2 minutes. The peroxidase activity was calculated using an extinction coefficient of oxidized pyrogallol (4.5 litres/mol).

Assay of glutathione peroxidase (GPx) activity (Rotruck et.al., 1973)

Glutathione peroxidase was assayed according to the procedure of Rotruck *et al*. (19) with some modifications. The reaction mixture consisting of 0.4 ml of 0.4 M sodium phosphate buffer (pH 7.0), 0.1 ml of 10mM sodium azide, 0.2 ml of 4 mM reduced glutathione, 0.1 ml of 2.5 mM H2O2, 0.2 ml of water and 0.5 ml of enzyme was incubated at 0, 30, 60, 90 seconds respectively. The reaction was terminated with 0.5 ml of 10% TCA and after centrifugation; 2 ml of the supernatant was added to 3 ml of phosphate buffer and 1ml of DTNB reagent (0.04% DTNB in 1% sodium citrate). The color developed was read at 412 nm and the enzyme activity is expressed in terms of µg of glutathione utilized/min/mg protein.

Estimation of reduced glutathione (Boyne et.al., 1972)

The amount of reduced glutathione in the samples was estimated by the method of Boyne and Ellman (22). 1ml of the sample extracts were treated with 4.0 ml of metaphosphoric acid precipitating solution (1.67 g of glacial metaphosphoric acid, 0.2 g EDTA and 30 g NaCl dissolved in 100ml water). After centrifugation, 2.0 ml of the protein-free supernatant was mixed with 0.2 ml of 0.4 M Na2HPO4 and 1.0 ml of DTNB reagent (40 mg DTNB in 100 ml of aqueous 1% tri sodium citrate). Absorbance was read at 412 nm within 2 minutes. GSH concentration was expressed as n mol/mg protein.

Table 1 Effect of fresh juice of *Ziziphus jujuba* and *Vitex nigunda* for enzymatic antioxidant profile

Plants	Peroxidase U/mg protein	Catalase U/mg protein	SOD U/mg protein	GPx U/mg protein
Ziziphus jujuba	2.01 ± 0.007	59.98 ± 0.27	81.09 ± 0.85	17.72 ± 4.07
Vitex nigunda	1.94 ± 0.008	39.56 ± 0.13	50.75 ± 0.36	23.11 ± 3.79

Results and Discussion

In our laborotary, we have investigated wound healing properties of four plants namely *Cynodon dactylon, Sphaeranthus indicus, Ziziphus jujuba* and *Vitex nigundo*. One of the major factor attributed for healing potentials is antioxidant property. Therefore, we thought to investigate them in detail. Presence of enzyme like Catalase, Glutathione peroxidase (GPX). Superoxide dismutase (SOD) and Peroxidase in the targate tissue is attempted as they play an important role in healing. Among the two plants, the enzymetic antioxidant activities of *Ziziphus jujuba* are greater than *Vitex nigundo* except GPx. The order of antioxidant properties of *Z. jujuba* and *V. nigundo* were SOD > Catalase > GPx > Peroxidase.

Non-enzymatic antioxidants The concentration of Reduced glutathione was found to be maximum in *Vitex nigundo.* The antioxidant potential of *Z. jujuba* may be attributed to the presence of flavonoids and the other constituents present therein. The findings substantiate the therapeutic applications of the plant in the indigenous system of medicine.

Acknowledgement

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