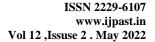


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# Vitex lacton Linn. bark's antioxidant activity: a comparative in vitro evaluation

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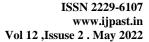
#### **ABSTRACT:**

Several human neurological disorders, including diabetes, inflammation, Alzheimer's disease, autoimmune diseases, and gastrointestinal system disorders, include free radical damage as a basic cause. Because of this, antioxidants are crucial in the fight against this illness. The current investigation seeks to compare the antioxidant activity of three different extracts of Vitex leucoxylon Linn. bark: one in ethyl acetate, one in hexane, and one in methanol. A member of the verbenaceae family, Vitex leucoxylon Linn is a traditional remedy for catarrh and headaches. After 48 hours of incubation, HIME reached 1.8964  $\mu$ g/ml. The results showed that after 48 hours of incubation, HIME inhibits HT29 cells at a concentration-dependent inhibitory effect, with an IC50 value of 1.8964  $\mu$ g/ml.

# INTRODUCTION:

Vitex leucoxylon Linn. (Verbenaceae) commonly known as Songarbhi (Marathi) an excellent herbal crude drug in the nature which has composition of the entire essential constituent that are required for normal and good health of human. It is small to large tree with a sort thick trunk and a spreading crown and almost throughout the Deccan peninsula of India up to an altitude 900 metres, it extends northwards up to Jhansi and part of Bihar. The trees are generally found on the river bank, stream & ponds. The root and the bark are astringent and roots are used as a febrifuge. The leaves are smoked for reliving headache and catarrh and are also used for medicinal baths in fever and, anti-depressant, antiinflammatory anaemia General pharmacological studies revealed

anti-psychotic, anti-parkinsonian and antimicrobial activities of aqueous and ethanolic extracts of leaves of V. Leucoxylon 2. Sarma et al 3 have studied the anti-inflammatory and wound healing properties of the crude alcoholic extract of the leaves in acute inflammation model 3. The roots and bark are astringent and the roots are reported to be used as a febrifuge. β- Sitosterol, dimethyl terphthalate, vitexin, isovitexin, agnuside and aucubin were isolated from the leaves or barks of V. Leucoxylon4. Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals5. Free radicals are fundamental to any biochemical process and antirepresent an essential part of aerobic life and metabolism 6. The most common reactive oxygen





species (ROS) include superoxide (O2) anion, hydrogen peroxide (H2O2), peroxyl (ROO) radicals and reactive hydroxyl (OH) radicals. The nitrogen derived free radicals are nitric oxide (NO) and peroxynitrite anion. ROS have been implicated in over a hundreds of diseases states which range from arthritis and connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome 7.

In treatment of these diseases, antioxidant therapy has gained an immense importance. Current research is now directed towards finding naturally occurring antioxidants of plant origin. Antioxidants have been reported to prevent oxidative damage by free radical and ROS, and may prevent the occurrence of disease, cancer and aging. It can interfere with the oxidation process by reacting with free radicals, chelating, and catalytic metals and also by acting as oxygen scavengers 8-9. Plant and plant products are being used as a source of medicine since long. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability 10. Flavonoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, anti inflammatory, anticarcinogenic etc11. They were also suggested to be a potential iron chelator 12-13. Novel natural antioxidants from some plants have been extensively studied in the past few years for their antioxidant and radical scavenging properties. In view of this and the present understanding about ROSinduced multiple diseases, we have selected one of such ayurvedic herb Vitex leucoxylon Linn. The objective of this investigation was to ascertain the scientific basis for the use of this

plant in the treatment of antioxidant, using different antioxidant models.

#### **MATERIALS AND METHODS:**

Vitex leucoxylon Linn. (Verbenaceae) were collected in flowering stage during late September from the natural population of Jhansi (U.P.) and authenticated by Dr. P.B Singh, Head of regional Research Institute Jhansi, shade dried and powdered then passed from 40# mesh size.

# PREPARATION OF VARIOUS EXTRACTS OF VITEX LEUCOXYLON:

Powdered material (750 g) of *V. leucoxylon* bark, was extracted with hexane (2 L), ethyl acetate (1.75 L) and methanol (1.75 L) using a Soxhlet apparatus and the spent material was then successively extracted with aqueous methanol (80%, 2 L) and water (2 L). The extract was concentrated in a rotary flash evaporator and dried in desiccators.

# **Hydroxyl Radical Scavenging Activity:**

The scavenging capacity for hydroxyl radical was determined according to the modified method **14**. The assay was performed by adding 0.1 ml of EDTA, 0.01 ml of ferric chloride, 0.1 ml of hydrogen peroxide, 0.36 ml of deoxyribose, 1.0 ml of test solutions (5-100 µg/ml) in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid were dissolved in sequence. The mixture was then incubated at 37°C for 1 hr and 1.0 ml portion of the incubated mixture was mixed with 10% TCA and 1.0 ml of 0.5% TBA to develop the pink chromogen and measured at 532 nm.

# **DPPH Radical Scavenging Activity:**

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability, using the stable



radical, DPPH **14**. A 0.1 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of control i.e. standard butylated hydroxyl toluene (BHT) at different concentration (25-100  $\mu$ g/ml) and test solutions at different concentrations (5-100 $\mu$ g/ml) in different test tubes. Thirty minutes later, the absorbances were measured at 517 nm.

# **Nitric Oxide Scavenging Activity:**

Nitric oxide scavenging activity was measured by the spectrophotometric method 15. Sodium nitroprusside (5 mM) in phosphatebuffer saline was mixed with a control without the test compound, but with an equivalent amount of methanol. solutions at different concentrations (5-100 µg/ml) were dissolved in methanol and incubated at 25°C for 30 min. After 30 min, to 1.5 ml of the incubated solution was diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyl ethylenediamine dichloride). The absorbance of the chromophore formed during the diazotization of the nitrile with sulphanilamide and the subsequent coupling naphthyethylene with diamine dihydrochloride was measured at 546 nm.

Superoxide Scavenging: Superoxide scavenging was carried out using the alkaline dimethyl sulfoxide (DMSO) method **16**. Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 hrs and the solution was filtered immediately before use; the filtrate (200  $\mu$ l) was added to 2.8 ml of an aquous solution containing nitroblue tetrazolium (56  $\mu$ M), EDTA (10  $\mu$ M) and potassium phosphate buffer (10  $\mu$ M, pH 7.4). Test solutions at different concentrations (5-100  $\mu$ g/ml) were added and absorbances were recorded at 560 nm against the control.

# **Statistical Analysis:**

The results are presented as mean ± SEM. All parameters were analysed using Student's *t*-test. P<0.05 was considered as significant.

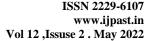
#### **RESULTS:**

Inhibition of DPPH Radical: The potential decrease in the concentration of DPPH radial due to scavenging property of ethyl acetate extract of *Vitex leucoxylon* Linn and BHT showed significant free radical scavenging activity viz. 88.52 and 86.73 %, respectively at  $100 \mu g/ml$ , whereas Hexane and Methanol extract of *Vitex leucoxylon* Linn. did not show any significant activity (Table 1).

Nitric Oxide Scavenging Activity: The scavenging of nitric oxide by ethyl acetate extract of *Vitex leucoxylon* Linn and BHT was concentration dependent. There was a moderate inhibition of nitric oxide formation with the maximum inhibition being 74.00 and 82.24% respectively at 100µg/ml ethyl acetate extract of *Vitex leucoxylon* Linn and BHT. Similar results were not found in case of Hexane and Methanol extract of *Vitex leucoxylon* Linn (Table 1).

Table 1: Free radical scavenging activity of various extracts of *Vitex leucoxylon* Linn.

Drug	Concentration (µg/ml)	DPPH radical inhibition (%)	Nitric oxide
Ethyl	1		1
acetate	5	10.60±0.2698	42.70±0.5411
extract of	10	17.24±1.596	51.67±0.5457*
Virex	25	54.21±2.191**	62.29±1.0380**
leucoxylon	50	84.29±0.1402***	71.00±0.9290**
(ECVL)	100	88.5240.3861***	74.00±1.7698**
Hexane	5	09.53±0.5543	37.57±0.6910
extract of	10	10.02±1.029	41.28±0.5382
Vitex	25	17.74±0.4495	44.18±0.4970
leucoxylon	50	20,99±0.5698	47.24±0.6458+
(HEVL)	100	26.74±1.6920	49.11±0.2250*
Methanol	s	06.24+0.109	02 54+0 103
extract of	10	12.45±0.122	06.88±0.142
Vitex	25	20.26±0.002	13.99±0.005
leucoxylon	50	22.26±0.009	26.28±0.008
Linn. (MEVL)	100	25.59±0.004	33.81±0.029
Butylated	25	86.73±0.3915 88.47±0.1520	77.13±0.6458
hydroxyl	50		79.23±1.7770
toluene (BHT)	100	91.45±0.1782	82,24±0,4976





Values are mean± SEM, 6 independent analysis, P<0.05\*, P<0.01\*\*, P<0.001\*\*\* as compared to standard (Student's *t*-test).

#### **Superoxide Radical Scavenging:**

The ethyl acetate extract of Vitex leucoxylon Linn and BHT showed a moderate inhibition of the superoxide radical 74.22 and 81.76% respectively at 100  $\mu g/ml$ . There was no significant inhibition of superoxide radical by Hexane and Methanol extract of *Vitex leucoxylon* Linn. (Table 2).

# **Hydroxyl Radical Activity:**

The effect of ethyl acetate extract of *Vitex leucoxylon* Linn and BHT on hydroxyl radical and iron (II)-dependent deoxyribose damage was protected significantly at all concentrations; the percentage of inhibition of hydroxyl radical being 79.04 and 73.03 % respectively at 100  $\mu$ g/ml. No significant inhibition of superoxide radical by Hexane and Methanol extract of *Vitex leucoxylon* Linn. (Table 2)

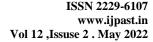
#### **DISCUSSION:**

The antioxidative system protects the organism against ROS-induced oxidative damage. There are restrictions on the use of synthetic antioxidants such as BHT, as they are suspected to be carcinogenic . Natural antioxidants therefore have gained importance. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to form a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. .The ethyl acetate extract of Vitex leucoxylon Linn has potent antioxidant and free radical scavenging effects in different in-vitro systems, but Hexane and Methanol extract of Vitex leucoxylon Linn. showed no

significant effects as compared to standard BHT.

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