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IN VITRO DETERMINATION OF ANTI-OXIDANT AND ANTI-BACTERIAL ACTIVITIES OF *VITEX NEGUNDO* LINN.

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ABSTRACT

The investigations were carried out to evaluate anti-oxidant and anti-bacterial activities, of stem methanol extract of *vitex negundo* at various concentrations. The methanol stem extract noticed significant scavenging activity on DPPH, superoxide, nitric oxide and hydrogen peroxide. The anti-bacterial activity was determined by agar diffusion well method using *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli* and *Salmonella paratyphi.* The methanol stem extract exhibited significant inhibitory effect on all tested organisms. The highest susceptibility was noticed with *bacillus subtilis*. The phytochemical analysis revealed the presence of various phytochemicals *viz*., alkaloids, saponins, cardic glycosides etc., which play a vital role as anti-microbial agents.

KEY WORDS: anti-oxidant, anti-bacterial, scavenging activity, susceptibility, anti-microbial agents

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INTRODUCTION

Reactive Oxygen Species (ROS) are the reactive molecules and free radicals which are derived from molecular oxygen and produced during the mitochondrial electron transport chain in aerobic species are potential to cause deleterious effects[1]. However, formation of ROS and RNS (Reactive nitrogen species e.g. nitric oxide, NO) is necessary intermediates of metal catalyzed oxidation reactions [2]. The excess production of reactive nitrogen species leads to nitrosative stress [3,4] The condition when the system exceeds generation of reactive nitrogen species and difficult to neutralize and eliminate them. In general there are many anti-oxidant defense mechanisms are involved either enzymatically or non-enzymatically to protect and balance the system from severe oxidative stress.[5] It is preferred to use natural antioxidants because to minimize the side effects which are associated with the synthetic antioxidants[6] (Plant polyphenolic compounds, such as flavonoids are described as scavengers of free radicals[7]. Medicinal plants are considered as the source for phyto medicine that acts as natural anti-oxidant and as well as anti-microbial agents[8]. It is also necessary to develop natural anti-microbial drugs as an alternative source for the synthetic drugs which has numerous side effects. It can be possible by only investigation on medicinal plants which are considered as factories of natural drugs [9,10]. *Vitex negundo* Linn belongs to family verbenaceae is commonly known as five leaved chaste tree (English), Nirgudi (Marathi), Nirgundi (Hindi), Indrani (Sanskrit)[11]. It is large, aromatic, shrub grow up to 4.5 m in height. It is found abundantly near moist places, most parts of India [12]. All parts of v*itex negundo* is used as indigenous medicine, the leaves are reported to be more use for medicinal use viz., brain tonic and improve memory[13,14], anti-cancer[15], anti-inflammatory[16], gastroprotective[17], antioxidant[18,19] central nervous system depressant[20], anti-convulsant[21] etc,. *Ascof*® commercially available in the market as Lagundi tablets used for relief of mild and to moderate bronchile asthma and cough are prepared from leaves and of *Vitex negundo* [22]. In the context with above information cited about the medicinal uses of this plant, here we investigated methanol extract of stem of *Vitex negundo* for the evaluation of anti-oxidant and anti-bacterial activities.

MATERIALS AND METHODS

Collection of plant material

The stems of *vitex negundo* were collected from local areas of Warangal, Andhra Pradesh, India. The plant was authenticated by the Prof. Thirupatiah taxonomist, Department of Biotechnology, Chaityanya Postgraduate College, Kishanpura, Hanamkonda.

Chemicals

L-Ascorbic acid, 1, 1 -diphenyl-2-picrylhydrazyl (DPPH), Ethylene diamine tetra acetic acid (EDTA), Nitro blue tetrazolium (NBT), were purchased from Sigma Aldrich, Mumbai, India and all other chemicals and reagents used were of analytical grade.

Preparation of extract

The stem of *vitex negundo* were dried under shade and made to a fine powder. The powder (100 grams) were Soxhlet-extracted with methanol and dried under rotavapor at 40-50^{o}C for 3 hours.

Phytochemical Analysis

Preliminary phytochemical investigation of stem methanol extract of *vitex negundo* was carried out for qualitative

determination of the groups of organic compounds present in them, by using various methods.[23]

Anti-oxidant Activity

DPPH radical scavenging activity

The total anti-oxidant potential of samples was determined by using the procedure described by Brand-Williams et al[24] and Parejo et al[25]. Various concentrations of test sample were prepared by serial dilution and 0.1 ml of each dilution was added to 3.9 ml of a 6.0×10−5 M methanol solution of $DPPH^{\cdot}$, followed by vortexing. The reaction was allowed to take place in the dark at room temperature to reach a plateau. The decrease in the absorbance at 517 nm was determined by using a Pharmacia Biotec Novaspec II spectrophotometer. The concentration of remaining $DPPH^{\cdot}$ in the reaction medium was calculated from the calibration curve, as follows:

Scavenging effect (%) =

$$(1 - \frac{\text{A Sample(517nm)}}{\text{A Control (517nm)}}) \times 100$$

Super oxide free radical scavenging activity

Different concentrations of 50, 100, and 150 µg/ml (10, 20, 25 µl) of plant extract were taken and volume was made up to 150µl with methanol, to each of this, 100µl riboflavin, 200µl EDTA, 200µl methanol and 100µl NBT was mixed in test tubes and further diluted up to 3ml with phosphate buffer and absorbance was measured after illumination for 5 min. at 590 nm on UV visible spectrometer Shimadzu, UV-1601, Japan and results were compared with ascorbic acid.

Scavenging of nitric oxide

Sodium nitroprusside (5µM) in std. phosphate buffer solution was incubated with different concentration of the test extracts dissolved in standard phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25 °C for 5 h. After 5 h, 0.5 ml of incubation solution was removed and diluted with 0.5 ml Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in water). The absorbance of chromophore formed was read at 546 nm. The control experiment was also carried out in similar manner, using distilled water in the place of extracts. The activity was compared with ascorbic acid [26,27].

Scavenging of hydrogen peroxide

A solution of hydrogen peroxide (20mM) was prepared in phosphate buffered saline (PBS, pH 7.4). Various concentrations of 1ml of the extracts or standards in methanol were added to 2 ml of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm, after 10 min against a blank solution that contained extracts in PBS without hydrogen peroxide [28]. IC_{50} value is the concentration of the sample required to scavenge 50 % free radical. The percentage inhibition was calculated by using the following formula.

Scavenging activity (%) = [{OD control - OD sample}/ OD control] x 100

Anti-bacterial activity

The anti-bacterial activity of methanol stem extract of *vitex negundo* was carried out according to the method described by elsewhere with slight modifications. Each selective medium was inoculated with the test organism suspended in nutritive broth. Once the agar was solidified, it was punched with a six millimeters diameter wells and filled with 25 µL of the plants extracts of various concentrations and corresponding wells with positive and negative control. The concentration of the methanol extracts employed at concentrations 50, 100 and 150 µg/ml

simultaneously, gentamycin sulfate (10 µg/ml) is used as positive control. The test was carried out in triplicate. The plates were incubated at 35 ± 2°C for 24 h. The anti-bacterial activity was calculated in terms of inhibition Zone diameter mm.

RESULT AND DISCUSSION

The preliminary phytochemical analysis of methanol stem extract of *vitex negundo* revealed the presence of cardic glycosides, tannins, alkaloids, saponins, flavanoids and steroids. The present investigation on DPPH scavenging activity revels that the plant extract of *vitex negundo* exhibited high scavenging activity at all concentrations tested (Fig.1). DPPH is a free radical which accepts an electron or hydrogen to become diamagnetic. These unpaired electrons are paired off by interacting with an anti-oxidant by accepting electron, thus free radical is neutralized and convert it to 1 – 1 diphenyl – 2 – picryl hydrazine as a result the purple color solution turns to color less. The decrease in DPPH radical is measured at 517 nm. Fig.1. DPPH, Superoxide, Nitric Oxide, Hydrogen Peroxide scavenging activity of *Vitex negundo* methanol stem extract at various concentrations measured and compared with ascorbic acid used as standard reference.

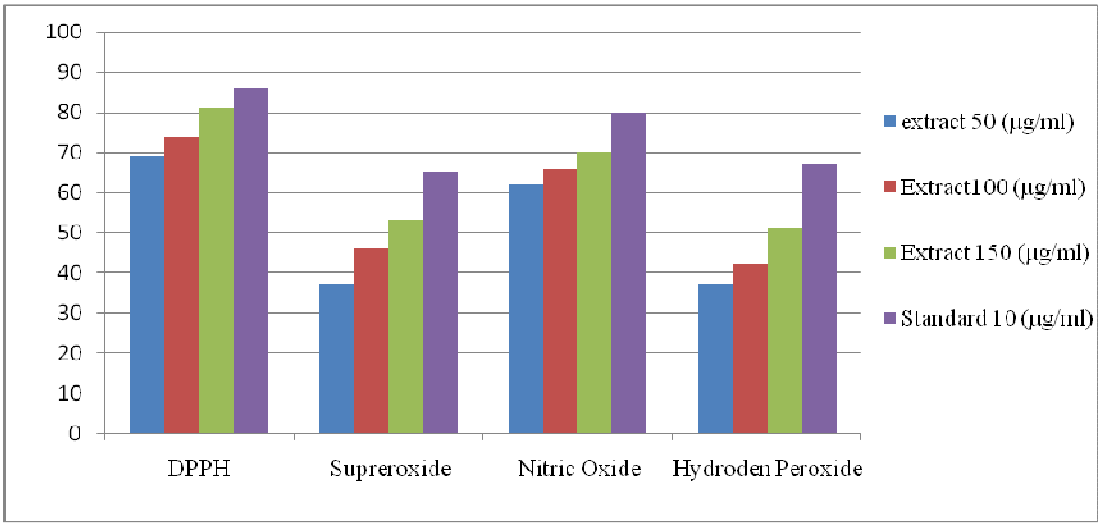

Figure.1.
DPPH, Superoxide, Nitric Oxide, Hydrogen Peroxide scavenging activity of Vitex negundo methanol stem extract at various concentrations and measured and compared with ascorbic acid.

Legends

Table.1
Anti-bacterial activity of methanol stem extract of Vitex negundo and compared with known standard.

Test organisms	Concentration µg/ml			
	Plant extract			Gentamycin
	50	100	150	10
S.aureus	8.0	10.0	12.6	19
B. subtilis	13.6	15.9	17.5	19
E.coli	12.0	14.03	15.7	19
S. typhi	11.4	13.0	15.0	19

Table.1. Anti-bacterial activity of methanolic stems extract of *Vitex negundo* and compared with gentamycin sulphate which is used as standard reference.

Super oxides are generated from molecular oxygen of oxidative enzymes and as well as non-enzymatic reactions such as autoxidation by catecholamines [29, 30]. Superoxide free radical inreacts with Nitro blue tetrazolium (NBT) and produce blue colored diformazan which is measured at 590 nm. The studies on super oxide free radical scavenging activity revels that the methanol stem extract of *vitex negundo* was noticed significant reduction of the super oxide anions (Fig. 1). Stem of *Vitex negundo* scavenge superoxide radical and thus inhibit foamazan formation. The nitric oxides which are commonly generated in adequate amounts by macrophages, neurons and endothelial cells are commonly generates which play vital role in various physiological activities whereas, the excess concentration of nitric oxide leads to several diseases [31, 32, 33]. This excess nitric oxide reacts with oxygen and generates nitrites and peroxynitrites which are well known free radicals [34]. Sodium nitroprusside at suitable pH naturally produces nitric oxide which in turn interacts with oxygen to generate nitrate ions which are estimated by use of greiss reagent. The methanol stem extract of *vitex negundo* proved as good scavengers of nitric oxide that compete with oxygen which is inhibited the production of nitric oxide (Fig. 1). H_2O_2 is a weak oxidizing agent and have immense potency of oxidation of essential thiol (-SH) groups leads to inactivation of some enzymes. The cytotoxicity character of H_2O_2 is because of hydroxyl radical which is generated by H_2O_2 interaction with Fe^{+2} and Cu^{+2} which occurs inside the cell. The methanol stem extract of *vitex negundo* noticed significant inhibitory activity of H_2O_2 as a result of its antioxidant and free radical scavenging activity (Fig. 1).

CONCLUSION

The methanol stem extract of *vitex negundo* produced significant results of anti-oxidant and anti-microbial activities at various tested concentrations. Therefore, based on this studies *vitex negundo* can be used as folklore for treatment of various ailments.

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