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Vesicular Arbuscular Mycorrhiza Association Augments Antioxidant Activity of Vitex negundo Leaves

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ABSTRACT

The study was conducted to evaluate the effect of Vesicular Arbuscular Mycorrhiza treatment on antioxidant activity of leaves of *Vitex negundo*. Mycorrhiza spores were added to the plants at different time intervals the plants were catergoried as T1, T2, T3 and T4 according to treatments. T1 plants are control while T2, T3 and T4 plants received treatment of spores for one time, twice at intervals of 30 days and every day for 15 days till harvesting. After harvesting and processing of the leaves, methanolic extracts were prepared by maceration and designated as MEVNT1, MEVNT2, MEVNT3 and MEVNT4. The extracts were studied for quantitative analysis of antioxidant phytoconstituents (total phenolic, flavonoid and ascorbic acid contents) and in vitro antioxidant property in 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, hydrogen peroxide scavenging assay, reducing power assay, anti-lipid peroxidation assay. Methanol extract of *V. negundo* leaves and ascorbic acid showed significant scavenging of ($p < 0.05$) DPPH free radicals, reducing potential, hydroxyl radical scavenging and anti-lipid peroxidation effect. Among these parameters, IC_{50} value of MEVNT4 was lowest among all extracts in almost all parameters studied besides its high content of antioxidant phytochemicals. The results indicated that Mycorrhiza treatment caused marked elevation on antioxidant status of the plant. It further states that single treatment or treatment of long intermittent duration may have lesser potential to affect the antioxidant profile that the continuous treatment period. This suggested that the treatment have tendency to augment the concentration or effect of antioxidant present in the leaves.

Keywords-- Oxidative stress, Nirgundi, Flavonoids, Phenolic content, Ascorbic acid

I. INTRODUCTION

Vitex negundo (family: Verbenaceae) is an important plant of Indian system of medicine commonly known as Sambhalu, Nirgundi and leaved chaste tree. It is a woody, aromatic, deciduous shrub growing as a small tree up to the height of 3 m. It is an erect tree with quadrangular branches, its most striking feature centers on a cluster of five pointed leaves resembling a palm. Flowers are bluish purple in color while the fruit is succulent and turns black when ripe [Meena et al., 2011]. The plant thrives in humid lands and along water courses in wastelands. It occurs adequately in India, Afghanistan, Malaysia, Pakistan, Eastern Africa and Madagascar [Khare et al., 2004]. All parts of the plants have medicinal value. The plant is used in reducing pains and swellings of joints in arthritis [Chopra et al., 1986]. The whole plant is used in curing various ailments, the plant also possess anticonvulsant, antimicrobial, analgesic, antiparasitic, drug potentiating, hepatoprotective, anticancer and enzyme inhibitory activity [Aswar et al., 2009]. The leaves have potent antioxidant, anti-inflammatory, analgesic, antihistaminic, snake venom neutralizing, hepatoprotective and CNS depressant activities [Muthuswamy et. al., 2012]. The flowers are astringent, febrifuge, anti-dirrhoeal and prescribed in liver complaints. The oil extracted from seeds has application in treating sinus and scrofulous sores. Fruits are nervine stimulant, emmenagogue and vermifuge. Roots are used as tonic, febrifuge, expectorant and diuretic. All parts of Vitex negundo, from root to fruits, possess a multitude of phytochemicals as secondary metabolites which impart a variety of medicinal uses to the plant. Nishindaside, mussaenosidic acids, vitedoin, negundin and vitexin are some important bioactive agents found in the plant. Besides above,

Vitex negundo is a source of natural antioxidants [Rabeta et. al., 2013]. Vitedoin A, one of the phytochemical present in the plant acts as a strong antioxidant [Ono et. al., 2004)]. Its antioxidant activity is reported to be even more than that of vitamin E and L-cysteine.

Figure 1.(a) and (b) showing nursery of vitex. 1(c) showing flowering twig of vitex

Mycorrhiza is the intricate associations in roots with fungal groups. They represent the underground absorbing organs in most plants in nature (Gianinazzi pearson et al., 1984). One of the most important attributes of VAM is their exploitation of mineral resources from soil efficiently. It also plays a key role in water uptake, thereby protecting the plants under drought stress, moreover it also enhances for phosphate nutrition of host plants [Bolan 1991]. Occurrence of arbuscular mycorrhizal fungi in medicinal plants several plants were noticed by [Rao *et.,al*, 1989]. The VAM association to different plants has been demonstrated to modulate the medicinal activities of the respective plants and significantly alter their therapeutic potential. VAM association was found to modulate the levels of antioxidant enzymes in certain plants. [Thapa et al., 2015] studied association and colonization of arbuscular mycorrhizal fungi in vitex negundo , studies reported 75% colonization of VAM with vitex, mycorrhizal association and spore density in vitex was studied by Zhao et al.,2004, findings show a strong association of mycorrhizal ststus in vitex and spore density was found to be 1395, which evidently supports the intimate and healthy VAM associations in vitex, the occurrence of VAM symbiotic association with Vitex negundo is very common, which supports the proposed study and favours the research. Despite the strong association of VAM with Vitex, no studies have been conducted to delineate its influence on antioxidant status of Vitex negundo. Hence, it was though worthwhile to investigate the influence of VAM treatment on antioxidant status of Vitex negundo. The present study carried out estimation of antioxidant phytochemicals and in vitro antioxidant activity in vitro assays.

II. MATERIALS AND METHODS

2.1 Collection and identification of the plant material

Vitex negundo plants were grown as small plantlets in an open area to develop a nursery, later they were transferred in pots till maturity. The plant was identified by a Dr. N.K. Pandey, Research Officer (Botany), National Research Institute of Ayurveda-Siddha Human Resource Development, Gwalior (Madhya Pradesh). The voucher specimen was submitted to the Herbarium and Museum Section of the Institute.

2.2 Mycorrhiza treatments

The plants grown were subjected to Vesicular Arbuscular Mycorrhiza (VAM) treatments by adding VAM spores at different time intervals. Treatments groups were divided four types depending upon the time interval [Chikkaswamy et al., 2014]

- T1-control- no spores were given to plant.
- T2- one time addition of spores.
- T3- addition of spores twice, at an interval of 30 days.
- T4- addition of spores after every 15 days, till the plants were harvested.

2.3 Processing of the Plant Material

Leaves of Vitex negundo plant that received different treatments were collected and shade dried at room temperature. The dried leaves were grinded into coarse powder and used for further investigations.

2.4 Preparation of Extract and preliminary phytochemical screening

The methanolic extract of leaves of Vitex negundo was prepared by cold maceration as MEVNT1, MEVNT2, MEVNT3 and MEVNT4. Briefly, the coarse powder was soaked in methanol for 48 hours with intermittent stirring. After 48 hours, the extract was filtered by Whatman filter

paper No.1 and then dried in rotary evaporator to get a dark green color extract with percent yields of MEVNT1 7.6%w/w, MEVNT2 8.5% w/w, MEVNT3 10.6%w/w, MEVNT4 11.4% w/w. All extracts were stored in desiccator for further use.

Preliminary phytochemical screening (Khandelwal, 2006) of each extract was carried out to identify the presence of various phytoconstiuents.

2.5 Quantitative Estimation of Phytoconstituents

2.5.1 Total phenolic content

The total phenolic content of the extract was determined spectrometrically (Singleton et al., 1965; 1999). One milliliter of Folin-Ciocalteu's reagent, previously diluted (1:20), was added to 1 ml of samples (1000µg/ml) and mixed thoroughly. To the mixture, 4 ml of sodium carbonate (75 g/L) and 10 ml of distilled water were added and mixed well. The mixture was allowed to stand for 2h at room temperature. Contents were then centrifuged at 2000g for 5 min and the absorbance of the supernatant was taken at 760 nm. A standard curve was obtained using various concentrations of tannic acid. Results were expressed as mg of tannic acid equivalents (TAE) per gram of extract.

2.5.2 Total flavonoid content

Total flavonoid content was measured by aluminum chloride colorimetric assay [Marinova et al., 2005]. One milliliter of extract (1000µg/ml) or standard solution of different concentrations quercetin was added to 10 ml volumetric flask containing 4 ml of distilled water. To the above mixture, 0.3 ml of 5% $NaNO_2$ was added. After 5 minutes, 0.3 ml of 10% $AlCl_3$ was added. At 6th min, 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. A standard curve was obtained using various concentrations of quercetin. Total flavonoid content of the extracts was expressed as mg of quercetin equivalent per g of extract.

2.5.3 Ascorbic acid content (Vitamin C)

Ascorbic acid content was assayed as described previously by [Omaye et al. 1979] with some modifications. One gram of extract was ground in a pestle and mortar with 5 ml of 10% TCA, the extract was centrifuged at 3500 rpm for 20 minutes. The pellet was re-extracted twice with 10% TCA and supernatant was increased 10 mL and used for estimation. To 0.5 ml of the extract, 1 ml of DTC reagent (2,4-Dinitrophenyl hydrazine-Thiourea-CuSO4 reagent) was added and mixed thoroughly. The tubes were incubated at 37°C for 3 hours and to this a solution of 0.75 ml of ice cold 65% H_2SO_4 was added. The tubes were then allowed to stand at 30°C for 30 min. The resulting color was read at 520 nm in a spectrophotometer (UV 1800, Shimadzu, Japan). The ascorbic acid content was determined using a standard curve prepared with ascorbic acid and the results were expressed in mg per g extract.

2.6 Assessment of In Vitro Antioxidant Activity

2.6.1 DPPH (1,1-Diphenyl-2-picryl-hydrazyl) free radical scavenging activity

The free radical scavenging activity of extract was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH·) using the method described by [Brand-Williams et al. 1995]. Briefly, 0.1 mM solution of DPPH· in ethanol was prepared. 3.5 ml of the solution was added to 0.5 ml of extract solution of different concentrations in water. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using a spectrophotometer (UV 1800, Shimadzu Corporation, Japan). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Ascorbic acid was used as standard antioxidant. The percent DPPH scavenging effect was calculated using the following equation:

$$\text{DPPH} \bullet \text{scavanging effect (\%)} = 100 \times \frac{A_1}{A_0}$$

Where, A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the test.

2.6.2 Reducing power assay

The reducing power of the extract was determined as per previously described method [Oyaizu, 1986]. Different concentrations of extracts were prepared in suitable solvent. Each concentration (0.5 ml) was mixed with phosphate buffer (1.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (1.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (1.5 ml) was diluted with distilled water (1.5 ml). Finally, $FeCl_3$ (300µl, 0.1%) was added and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The experiment was performed in triplicate.

2.6.3 Scavenging of hydrogen peroxide

The ability of the extract to scavenge H_2O_2 was determined according to the method of [Ruch et al. 1989] with slight modification. The molarity of H_2O_2 to be taken is 4 mM, however, this molar solutions do not work in the samples like plant extract and absorbance goes in negative direction and therefore, higher molar solution need to be taken for feasible measurement of absorbance. In present study, a solution of H_2O_2 (40mM) was prepared in phosphate buffer saline (pH 7.4). H_2O_2 concentration was determined spectrophotometrically from absorption at 230 nm by using a spectrophotometer (UV 1800, Shimadzu, Japan). Extract (1.5ml) in different concentrations in distilled water was added to a H_2O_2 solution (1.5 ml, 40mM). The ratio of amount of extract/standard solution and H_2O_2 solution need to be fixed from preliminary experiments. Absorbance of H_2O_2 at 230 nm was determined within 10 min. against a blank solution containing phosphate buffer saline H_2O_2. Ascorbic acid was used as standard antioxidant compound. The percentage of scavenging of hydrogen peroxide radicals

by the extract and standard (ascorbic acid) was calculated as below.

$$\%\ \text{Scavanged}\ H_2O_2 = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the test.

2.6.4 Anti–lipid peroxidation assay

Lipid peroxidation in flex (*Linum usistatissium*) seed homogenate was evaluated by the TBARS method on the basis of principle described earlier [Ohkawa et al., 1979], [Pande et al., 2017]. The reaction mixture containing 1.0 ml (10%) flex seed homogenate prepared in 0.15M KCl, and 1.0 ml of different concentrations of drug extract were prepared. Lipid peroxidation was initiated by adding 100 µl of 15mM ferrous sulphate solution. The reaction mixtures were incubated for 30 min at 37°C. After incubation, the reaction was stopped by adding 1 ml 0.67% ice-cold thiobarbituric acid (TBA) solution. The reaction mixtures were heated for 60 min at 80°C, cooled, and centrifuged at 5000 rpm for 15 min. The absorbance of supernatant was measured at 532nm against a blank, which contained all reagents except homogenate and drug. $FeSO_4$ induced control contained homogenate plus $FeSO_4$ while Sample contained extract/BHT in various concentrations in addition. The percentage OD of different test/standard was subtracted to get sample OD reading at different concentrations. The percentage of anti-lipid peroxidation (ALP %) was calculated by following formula:

$$\text{ALP}\ (\%) = \frac{FeSO_4\ \text{induced O.D.} - \text{Sample O.D.}}{FeSO_4\ \text{induced O.D.}} \times 100$$

III. RESULTS

3.1 Preliminary phytochemical screening

All the four extracts (MEVNT1 to MEVNT4) showed identical presence of phytoconstituents. The extracts revealed the presence of alkaloids, glycosides, phenolic compounds and flavonoid (Table 1).

Table 1: Phytochemical screening

Phytoconstituents	Phytochemical Test	MEVN
Carbohydrates	Molish's test	Present
Proteins	Biuret test	Present
	Xanthoprotein test	Absent
Alkaloids	Hager's Test	Present
	Wagner's Test	Present
	Tannic Acid Test	Present
Glycosides	Keller-killiani test	Present
	Conc. Sulphuric acid test	Present
Saponins	Haemolytic Test	Present
	Froth Test	Present
Sterols	Salkowski test	Present
Tannins and Phenolic compounds	Ferric chloride Test	Present
	Alkaline reagent test	Present
Flavonoids	Shinoda Test	Present
	Lead acetate test	Present
	Alkaline reagent test	Present

3.2 Quantitative estimation of antioxidant phytochemicals

All the four extracts (MEVNT1 to MEVNT4) revealed the fair amount of total phenolic, total flavonoid and ascorbic acid content. The extract from T4 treatment (MEVNT4) showed highest amount of all the three antioxidant class of compounds compared to other treatment groups (Table 2).

Extracts	Antioxidant Phytoconstituents		
	Total Flavonoid Content (mg Quercetin	**Total Phenolic Content** (mg Tannic acid	**Ascorbic Acid Content** (mg /g of extract)

	equivalents/ g of extract)	equivalents/g of extract)	
MEVNT 1	31.19±16.6	97.16±12.2	100.1±35.0
MEVNT 2	187.2±11.0	157.2±13.9	113.4±47.6
MEVNT 3	290.8±10.6	283.4±10.6	144.3±47.4
MEVNT 4	420.4±15.9	442.1±21.5	149.4±35.6

3.3 Assessment of Antioxidant Activity

3.3.1 DPPH Scavenging Assay

MEVN in concentration range of 500-3000 µg/ml inhibited DPPH radical formation as indicated by concentration dependent decrease in the purple color of the solution. Similar effect was obtained with standard antioxidant ascorbic acid in the concentration range of 20-100 µg/ml. In linear regression analysis of concentration versus percent DPPH inhibition (Figure 2), the linear regression coefficient of all four methanolic extracts and ascorbic acid were MEVNT1 r^2= 0.9564, MEVNT2 r^2= 0.977, MEVNT3 r^2= 0.9103, and MEVNT4 r^2=0.9689 and ascorbic acid r^2=0.9227, respectively. The IC_{50} value are MEVNT1= 2317.15, MEVNT2 = 1964.806, MEVNT3 = 1770.122, and MEVNT4 = 1586.983 and ascorbic acid=51.263 obtained from regression analysis. MEVNT4 showed lowest IC_{50} among all the extracts.

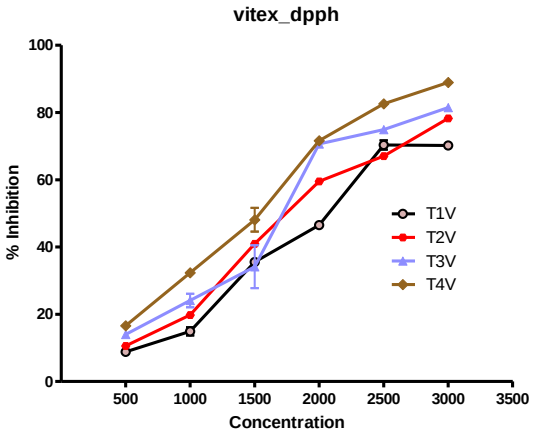

Figure 2. Linear regression analysis of DPPH scavenging activity of MEVN

3.3.2 Reducing Power Assay

MEVN in the test concentration range of 100-1000 µg/ml showed concentration related reduction of ferricyanide to ferrocyanide as indicated by increase in the green color absorbance measured at 700 nm. Similar effect was obtained with standard antioxidant- ascorbic acid in the concentration range of 100-1000µg/ml. In linear regression analysis of concentration versus absorbance at 700 nm was carried out (Figure 3). The linear regression coefficient of all four *Vitex* extracts and ascorbic acid were T_1 r^2= T_2 r^2= T_3 r^2= T_4 r^2= 0.9816 and r^2 = 0.9164, respectively, suggesting that the reducing potential was concentration dependent (Figure 3).

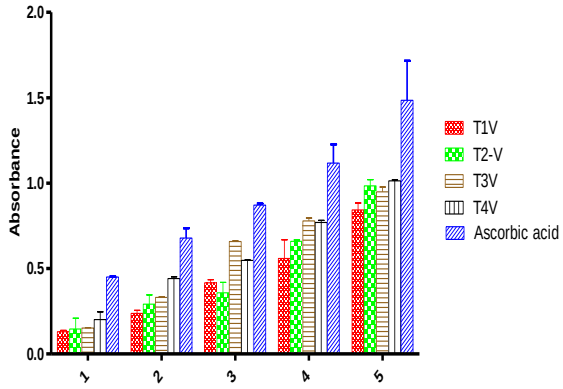

Figure 3. Effect of VAM treatment on leaf extracts on reducing potential of MEVN

3.3.3 H_2O_2 Scavenging Assay

MEVN in concentration range of 100-1000 µg/ml showed H_2O_2 scavenging as indicated by concentration dependent decrease in the absorbance of H_2O_2 solution. Similar effect was observed with standard antioxidant-ascorbic acid in the concentration range of 20-100 µg/ml. In linear regression analysis of concentration versus percent H_2O_2 inhibition was carried out. The linear regression coefficient of MEVN and ascorbic acid were MEVNT1 r^2 = 0.9124, MEVNT2 r^2 = 0.9272, MEVNT3 r^2 = 0.9421, and MEVNT4 r^2= 0.9129 and Ascorbic acid r^2 = 0.9798, respectively, suggesting that the scavenging of H_2O_2 was concentration dependent. The IC_{50} values are MEVNT1 = 1072.642, MEVNT2 = 730.7048, MEVNT3 = 624.1844, and MEVNT4 =490.0845 obtained from linear regression analysis (figure4). Among extracts, MEVNT4 showed lowest IC_{50} value which was nearly ten times higher than ascorbic acid (51.263).

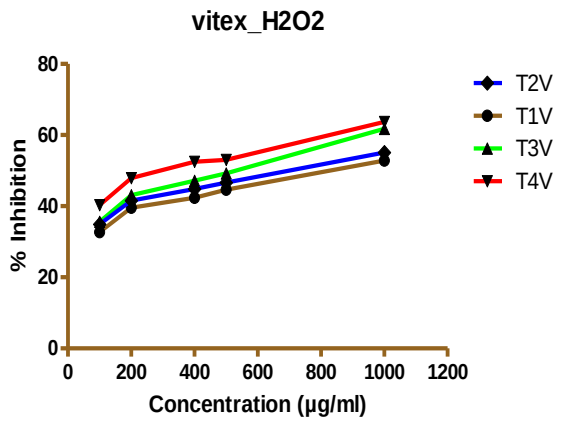

Figure 4. Linear regression analysis of H_2O_2 scavenging activity of MEVN

3.3.4. Anti–lipid Peroxidation (LPO) Assay in Flex Seed Homogenate

MEVN in concentration range of 100-1000 µg/ml showed inhibition of lipid peroxidation as indicated by concentration dependent decrease in the absorbance of TBARS. Similar effect was obtained with standard antioxidant, ascorbic acid in the concentration range of 20-100 µg/ml. In linear regression analysis of concentration versus percent anti-LPO was carried out. The linear regression coefficient of extracts and ascorbic acid were MEVNT1 r^2 = 0.9169, MEVNT2 r^2 = 0.9102, MEVNT3 r^2 = 0.9099, and MEVNT4 r^2= 0.9183 and Ascorbic acid r^2 = 0.9555, respectively, suggesting that the inhibition of LPO was concentration dependent. The IC_{50} value of MEVNT4 was lowest (631.874) as compared to other extracts MEVNT1 = 721.7629, MEVNT2 = 694.3217 and MEVNT3 = 665.8132. This value was higher than IC_{50} value of ascorbic acid (45.28).

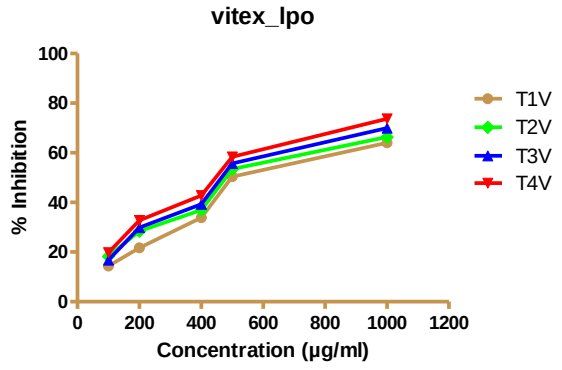

Figure 5.Linear regression analysis of anti-lipid peroxidation activity of MEVN

V. DISCUSSION

In the present study, the effect of VAM treatment on antioxidant effect of leaf extract of Vitex negundo was studied. The VAM treatment was found to augment the antioxidant capacity of the leaf extracts. The antioxidant activity of compound is generally attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging. In the present study, the antioxidant activity of the extracts (MEVNT1 to MEVNT4) was evaluated by studying its DPPH free radical scavenging, reducing potential, hydroxyl radical scavenging and effect on lipid peroxidation.

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Gulcin et al., 2003). The % DPPH radical scavenging activity is presented in figure 2. The DPPH scavenging effect of extracts from four treatment groups was compared among themselves and also with ascorbic acid as standard antioxidant. The regression analysis indicated that all extracts exhibited concentration dependent inhibition of DPPH radicals. The minimum IC_{50} value of MEVNT4 indicated its highest antioxidant potency and suggested that daily VAM treatment for 15 days was more effective to augment the DPPH scavenging action compared to other two VAM treatment options. MEVNT4 has showed lowest IC_{50} value which indicates that intermittent addition of VAM spores at regular time intervals of 15 days have brought a upgradation in the antioxidant levels of plant. In the ferric reducing assay method, VAM treatment was found increase the reducing potential of leaves of Vitex negundo and extracts showed concentration dependent increase in reducing potential (Figure 3). Among all MEVNT4 exhibited highest reducing potential compared to other extracts. The reducing potential showed significant antioxidant activity when compared with ascorbic acid which was used as standard antioxidant. Looking at the results, the absorbance of 100 µg/ml concentration of ascorbic acid was found equivalent with that of 1000 µg/ml. Here again the T4 extract of vitex has shown a remarkable increase in the reducing potential, mycorrhiza may have interfered in the phenol and flavonoid synthesis which inturn might have lead to elevate the reducing potential of plant inoculated with VAM spores. The reducing power and DPPH radical-scavenging activity of a substance is an indicator of its antioxidant activity (Hall and Cuppet, 1997) and thus supports the antioxidant action of vitex.

H_2O_2 is highly important because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. Thus, removal of H_2O_2 is very important for protection from oxidative damage. In the present study, VAM treatment increased the H_2O_2 scavenging activity of Vitex negundo leaf extracts compared to MEVNT1 (extract from untreated plant). The IC_{50} value of MEVNT4 was found lowest (490.60) indicating highest antioxidant power of MEVNT4 compared to other extracts of plant including normal plant which was uninoculated with VAM. The standard antioxidant ascorbic

acid (IC_{50}= 52.75) was found to be much potent than the extracts. This suggests that VAM treatment augmented H_2O_2 scavenging potential and enhanced the antioxidant action of the plant.

In the present study, VAM treatment increased the anti-lipid peroxidation effect of the Vitex negundo leaf extracts as indicated by inhibition of TBARS formation in the lipid peroxidation induced by ferrous sulphate in flax seeds . The maximum anti-lipid peroxidation effect was found with MEVNT4 as indicated by lowest IC_{50} among all the extracts, suggests its maximum antioxidant action. Similar effect was also observed with standard antioxidant-ascorbic acid. Lipid peroxidation is the oxidative degeneration of polyunsaturated fatty acids (PUFA) and involves lipid radicals leading to membrane damage. Free radicals have ability to induce lipid peroxidation in PUFA rich tissues or biomaterials (Coyle and Puttfarcken, 2003). The initiation of lipid peroxidation by ferrous sulphate takes place through ferryl-perferryl complex or through hydroxyl radicals by Fenton's reaction thereby initiating a cascade of oxidative reactions. The anti-lipid peroxidation effect may be due to several reasons such as inhibition of ferryl-perferry complex formation, scavanging of superoxide radical or chelation of iron itself. It is possible that VAM treatment might have augmented either of these properties of the extracts.

In above-mentioned models for screening of antioxidant assessment, it is clear that VAM treatment has markedly influenced antioxidant action of Vitex negundo leaf extracts, although lesser in potency than that of the standard antioxidants. Previously, VAM symbiotic association has been shown to modulate antioxidant response in salt-stressed Trigonella foenum-graecum plants (Evelin and Kapoor, 2014) and supports the present finding. Antioxidant potential of a plant is ascribed to its strong phenolic and flavonoid presence. Previously it has also been claimed that the composition of extract to be the ruling factor behind a plant's potential and its medicinal use (Zargar et al., 2011). Presence of phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes contributes to the antioxidant character of plant [Preethi et. al., 2010]. Particularly flavonoids are the potent antioxidants and possess free radical scavenging activity [Narayana et. al., 2001]. Vitex negundo also contains a number of polyphenolic compounds, terpenoids, glycosides and alkaloids and are responsible for antioxidant effect [Rabeta et. al., 2013].

It is very difficult to elucidate the exact mechanism of augmenting the antioxidant capacity of the Vitex negundo leaves. The augmentation of antioxidant potential of Vitex negundo leaves due to VAM treatment may be attributed to increase in flavonoid and phenolic content present in leaves though enhanced biosynthesis of the same. The estimation of phytoconstituents revealed the presence of higher amount of flavonoid and phenolic content and ascorbic acid content compared to extract from untreated plant (MEVNT1). It indicated that mycorrhiza may have some effect on the de novo synthesis of these constituent in *Vitex negundo* leaves. However, in the present study mycorrhiza is playing a vital role in elevating the levels of phytoconstituents that are in turn responsible for potency of plant and to be of great medicinal value. Therefore the study holds a strong vision that mycorrhizas can alter the medicinal efficiency of plants and the probable reason behind this elevation can be elucidated through studies at molecular level.

The extracts have been found potent in decreasing the superoxide dismutase scavenging activity; H_2O_2 levels [Vishal et al., 2005].

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