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Original Article

In vitro antioxidant activity on roots of *Limnophila heterophylla*

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ABSTRACT

Objective: The present study was investigating the antioxidant activity of methanol extract of roots of *Limnophila heterophylla* (Plantaginaceae). **Methods:** Roots of methanol extract of *Limnophila heterophylla* were tested for *in vitro* free radical scavenging assays, such as hydroxyl radical, antioxidant assay by thiocyanate method, inhibition of superoxide anion radical, 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH), lipid peroxidation assay, scavenging of 2, 2′- azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt radical cation assay (ABTS), total antioxidant activity (phosphomolybdic acid method) and reducing ability. **Results:** *Limnophila heterophylla* roots extract effectively scavenged free radicals at all different concentrations and showed its potent antioxidant activity. Further, these effects were in a dose dependent manner. Results were compared to standard antioxidants such as vitamin E, α-tocopherol, curcumin, quercetin, rutin, butylated hydroxytoluene (BHT), and ascorbic acid. **Conclusion:** *Limnophila heterophylla* enclose strong antioxidant potential. Further the study validates the therapeutic benefits of the Indian system of medicine.

Key words: *Limnophila heterophylla*, ABTS, Phosphomolybdic acid, Plantaginaceae, Reducing ability.

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INTRODUCTION

Over production of reactive oxygen species (ROS) in human beings, by endogenous or external sources, e.g. Tobacco smoke, certain pollutants, organic solvents or pesticides, leads to oxidative stress.[1] Oxygen is an essential for survival however, its univalent reduction generates several harmful reactive oxygen species (ROS), inevitable to living cells and highly associated with the wide range of pathogenesis such as diabetes, liver damage, inflammation, aging, neurological disorders and cancer.[2] In spite of comprehensive network of cellular defensive antioxidants, many ROS still escape this surveillance inflicting serious anomalies favoring such diseases states.[3] Though synthetic antioxidants, butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA) and radio protector, war far in are being used widely, however, due to their potential health hazards, they are under strict regulation.[4] Antioxidant principles from natural resources are multifaceted in their multitude/magnitude of activities and provide enormous scope in correcting the imbalance through regular intake of proper diet. Therefore, in the recent years, the interest is centered on antioxidants derived from herbal medicine in view of their medicinal benefits.[5] Phyto antioxidants, commonly available, less toxic, serving food and medicinal components have been suggested to reduce threat of wide range of ROS.[6] Recently, interest has increased in naturally-occurring antioxidants that can be used to protect human beings from oxidative stress damage.[7] Many plants contain natural antioxidants that act in metabolic response to endogenous production of free radicals and other oxidant species. These responses are due to ecological stress or promoted by toxins produced by pathogenic fungi and bacteria.[8]

More recent ethno pharmacological studies show that *Limnophila heterophylla* is used in many parts of the world for the treatment of a number of disease e.g. foul ulcers, agalactia, galactic impurities, anorexia, dyspepsia, helminthiasis, constipation, inflammations and strangury.[9] Various studies this plant indicates that, it possess various pharmacological activities such as COX-Inhibitor activity,[10] antibacterial, antifungal.[11] Some of the countries with a long history of traditional medicinal use of *Limnophila* include south East Asia. A part from this it shows presence of various phytoconstituents such as nevadensin, hymenoxin, katonic acid, p-cymene, ursolic acid.[12] The objective of the present study was to investigate the antioxidant activity of the methanol extract of roots of *Limnophila heterophylla* by using *in-vitro* models.

MATERIALS AND METHODS

Collection of plant material

The roots of *Limnophila heterophylla* were collected from Tirumala Hills of Chittoor district, Andhra Pradesh, India and the plant material was taxonomically identified and authenticated by the Dr. Madhava chetty (Research Officer) botany, Andhra Pradesh. Voucher specimen (GIP-006/2013-2014) of this plant has been retained in the GITAM Institute of pharmacy, GITAM University, Visakhapatnam, Andhra Pradesh, India.

Extraction of plant material

The roots of *Limnophila heterophylla* were dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with methanol in soxhlet apparatus at 60°C. The solvent was completely removed by rotary vacuum evaporator and concentrated. The extract was freeze dried and stored in a vacuum desiccators for further phytochemical and antioxidant studies.

In-vitro antioxidant assay

DPPH radical scavenging assay

The free radical scavenging capacity of the methanol extract of *Limnophila heterophylla* was determined using DPPH assay. A methanol DPPH

(2, 2-diphenyl-1-picrylhydrazyl) solution (0.15%) was mixed with serial dilutions (5 μg/ml to 80 μg/ml) of the methanol extract of *Limnophila heterophylla*, and after 10 min, the absorbance was read at 515 nm using a spectrophotometer. Quercetin used as a standard. The inhibition curve was plotted and IC_{50} values were obtained.[13]

Nitric oxide radical scavenging assay

Nitric oxide radical inhibition was calculable by the employment of Griess Illosvoy reaction.[14] During this investigation, Griess Illosvoy chemical agent was changed by victimization naphthyl ethylene diamine dihydrochloride (0.1% w/v) rather than 1-napthylamine (5%). The reaction mixture (3 mL) containing sodium nitroprusside (10 mM, 2 mL), phosphate buffer saline (0.5 mL) and therefore the methanol extract of *Limnophila heterophylla* (10 μg to 320 μg) or standard solution (rutin) was incubated at 25^{0}C for a 150 min. After incubation, 0.5 mL of the reaction mixture mixed with 1 mL of sulfanilic acid chemical agent (0.33% in 20% glacial carboxylic acid acid) and allowed to face for five minutes for finishing diazotization. Then, 1 mL of naphthyl ethylene diamine dihydrochloride was additional, mixed and allowed to face for thirty minutes at 25^{0}C. A pink colored chemical group was shaped in diffused light. The absorbance of those solutions was measured at 540 nm against the corresponding blank solutions. Rutin was used as a customary.

Superoxide anion assay

Measurement of superoxide anion scavenging activity of methanol extract of *Limnophila heterophylla* was done based on the Nishimiki method.[15] About 1 mL of nitroblue tetrazolium (NBT) solution (156 μM NBT in 100 mM phosphate buffer, pH 7.4), 1 mL NADH solution (468 μM in 100 mM phosphate buffer, pH 7.4) and 0.1 mL of the methanol extract of *Limnophila heterophylla* were mixed separately (5 μg to 160 μg) in water. The reaction was started by adding 100 μL of phenazine methosulphate (PMS) solution (60 μM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25^{o}C for 5 min, and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. Curcumin was used as a positive control.

Lipid peroxidation assay

The rat liver microsomal fraction was prepared by the Bouchet *et al.*[16] The reaction mixture contained five hundred μL of liver microsomal fraction, 300 μL buffer containing the methanol extract of *Limnophila heterophylla* (12.5–200 μg), a 100 μL of $FeCl_3$ (1 mM) and 100μL vitamin C (1 mM) during a final volume of 1.0 cubic centimetre to start peroxidation. Samples were incubated at 37^{0}C for 1 h and lipid peroxidation was measured using the reaction with thiobarbituric acid (TBA). Thiobarbituric acid reactive substances were determined by the strategies of Houghton[17] and Aruoma.[18] The absorbance of the organic layer was measured at 532 nm. All reactions were applied in triplicate. Vitamin E was used as a regular standard.

Antioxidant assay by thiocyanate method

The antioxidant activity of methanol extract of *Limnophila heterophylla* decided in step with the thiocyante methodology.[19,20] Briefly, five hundred μl of methanol extract of *Limnophila heterophylla* at totally different concentrations (25 μg to 200 μg) were mixed with 2.5 mL of 0.02 M linolic acid emulsion (contains equal weight of tween-20 in phosphate buffered saline, pH 7.4) and also the final volume was adjusted to 5 ml with phosphate buffered saline in a test-tube and incubated at 37^{o}C in dark place. The quantity of peroxide decided by measuring absorbance at 500 nm after coloring with 0.1 ml of $FeCl_2$ (0.02 M) and 0.1 ml salt (30%) at intervals throughout incubation. The solutions while not the plant extract was taken as blank for the study. α-tocopherol was used as a reference compound.

Hydroxyl radical scavenging assay

The assay was performed as represented by Halliwell[21] with minor changes. All solutions were ready freshly. 1.0 ml of the reaction mixture contained one hundred μL of twenty eight mM 2-deoxy-2-ribose (dissolved in phosphate buffer pH 7.4), 500 μL resolution of assorted concentrations of the methanol extract of *Limnophila heterophylla* (10 to 320 μg), 200 μL of 200 μM $FeCl_3$ and 1.04 mM ethylenediamine tetracetic acid (EDTA, 1:1 v/v), 100 μL H_2O_2 (1.0 mM) and 100 μL ascorbic acid (1.0 mM). Once associate degree period of time of 1 hr at 37^{o}C, the extent of deoxyribose degradation was measured by the TBA reaction.[15,16] The absorbance was measured at concerning 532 nm against the blank solution. Vitamin E was used as a positive management.

Reducing power activity

The reducing power of methanol extract of *Limnophila heterophylla* was determined according to the method of Oliveira *et al.*,.[22] Various concentrations of the extract (125-1000 μg/ mL) in 1.0 mL of deionized water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). The mixture was incubated at 500^{o}C for 20 min; aliquots of trichloroacetic acid (2.5 mL, 10%) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared $FeCl_3$ solution (0.5 mL, 1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Scavenging of 2, 2′- azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) radical cation

To 0.2 ml of various concentrations (5, 10, 20, 40, 80 μg/ml) of the methanol extract of *Limnophila heterophylla* or standard solution, 1.0 ml of distilled DMSO and 0.16 ml of ABTS solution (2 mM) were added and incubated for 20 min. absorbance of these solutions was measured spectrophometrically at 734 nm.[23] Butylated hydroxytoluene was used as a positive control.

Total antioxidant activity (Phosphomolybdic acid method)

The antioxidant activity of the sample was evaluated by the transformation of molybdenum (VI) to molybdenum (V) to form phosphomolybdenum complex.[24] An aliquot of 0.4 ml of sample solution was (125, 250, 500, 1000 μg/ml) combined in a vial with 4 ml of reagent solution (0.6 M sulfuric acid, 28 milimole sodium phosphate and 4 milimole ammonium molybdate). These vials were capped and incubated in a water bath at 95^{o}C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expressed relative to that of ascorbic acid.

RESULTS

Percentage yield

The percentage yield (Table 1) of the methanol extract of *Limnophila heterophylla* was found to be 24 gm.

Table 1: Percentage yield of methanol extract of *Limnophila heterophylla*

Extract name	Yield (gm)
Methanol extract of *Limnophila heterophylla*	24.0

In-vitro antioxidant studies

DPPH radical scavenging activity

The methanol extract of *Limnophila heterophylla* of decreased the concentration of DPPH radical due to free radical scavenging ability. The

result was shown in Figure 1. The methanol extract of *Limnophila heterophylla* had a well hydrogen donating ability with an IC_{50} value of 18.02 μg/mL and the value was found to be higher than that of standard, Quercetin (IC_{50} value of 9.5 μg/mL).

Nitric oxide radical inhibition assay

The scavenging of nitric oxide by the methanol extract of *Limnophila heterophylla* was increased in a dose-dependent manner as illustrated in Figure 2. At concentration of 192.04 μg/mL methanol extract of *Limnophila heterophylla* 50% of nitric oxide generated by incubation was scavenged. This IC_{50} value (IC_{50} 97.06 μg/mL) of methanol extract of *Limnophila heterophylla* was found to be more than that of standard, Rutin.

Superoxide anion scavenging activity

The superoxide anion derived from dissolved oxygen by phenazine methosulphate/NADH coupling reaction reduces nitro blue tetrazolium. The decrease in the absorbance at 560 nm with the methanol extract of *Limnophila heterophylla* indicated the consumption of superoxide anion in the reaction mixture. As mentioned in Figure 3, the extract of plant as well as Curcumin showed the scavenging activity with the IC_{50} values, 30.04 μg/mL and 18.06 μg/mL, respectively.

Lipid peroxidation assay

The activity of methanol extract of *Limnophila heterophylla* against non-enzymatic lipid peroxidation in rat liver microsomes was shown in Figure 4. Addition of Fe^{2+}/ascorbate to the liver microsomes caused increase in lipid peroxidation. The extract of *Limnophila heterophylla* showed inhibition of peroxidation effect in all concentrations, which showed 50% inhibition effect at 196.2 μg/mL. The extract inhibition value was found to be more than the standard, vitamin E (IC_{50} value 93.6 μg/mL).

Antioxidant assay by thiocyanate method

The effects of methanol extract of *Limnophila heterophylla* on peroxidation of linoleic acid emulsion were shown in Figure 5. From this method, the amount of peroxides formed in emulsion during incubation was determined spectrophotometrically by measuring absorbance at 500 nm. High absorption is an indication of high concentration of peroxides formed. Therefore, low absorbance indicates high antioxidant activity. The IC_{50} values were found to be 99.2 μg/ml and 26.4 μg/ml for methanol extract of *Limnophila heterophylla* and standard, α- tocopherol, respectively.

Hydroxyl radical scavenging assay

To attack the substrate deoxyribose hydroxyl radicals were generated by reaction of Ferric-EDTA together with H_2O_2 and ascorbic acid. When the methanol extract of *Limnophila heterophylla* was incubated with the above reaction mixture, it could prevent the damage against sugar. The results was shown in Figure 6, the concentrations of 50% inhibition were found to be 50.2 μg/mL and 34.2 μg/mL for the methanol extract of *Limnophila heterophylla* and standard compound, vitamin-E, respectively. The IC_{50} value (42.4 μg/mL) of the extract *Limnophila heterophylla* extract was found to be more than the standard.

Reducing power ability

Figure 7 shows the reductive capability of methanol extract *Limnophila heterophylla* when compared to the standard, butylated hydroxytoluene (BHT). Like the antioxidant activity, the reducing power increased with increasing amount of the fractions. The methanol extract *Limnophila heterophylla* (1000 μg/ml) of *Limnophila heterophylla* showed the highest reducing ability than all the other fractions tested. However, the activity was less than the standard BHT.

Total antioxidant activity-ABTS radical cation decolourization assay

Total antioxidant activity of *Limnophila heterophylla* was assessed by measuring the reduction of the ABTS radical cation as the percentage of inhibition at 734 nm. The effect of various concentrations of extract (from 5 to 80 μg/ml) on ABTS radical was shown in Figure 8. *Limnophila heterophylla* exhibited effective antioxidant activity. The inhibition was found to be concentration dependent and the IC_{50} values were found to be 15.4 μg/ml and 9.2 μg/ml for methanol extract of *Limnophila heterophylla* and standard BHT, respectively.

Total antioxidant activity (phosphomolybdic acid method)

The total antioxidant activity of methanol extract of *Limnophila heterophylla* was determined by phosphomolybdate method. Maximum free radical scavenging potential activity was observed at 1000 μg/ml (Figure 9). The IC_{50} of the methanol extract of *Limnophila heterophylla* and standard (ascorbate) was found to be 438.5 μg/ml and 375.4 μg/ml.

DISCUSSION

Free radicals, species with one (or) additional unpaired electrons, are a unit made in traditional (or) pathological cell metabolism from xenobiotics, (or) through radiation. Electron acceptors like molecular chemical element react simply with free radicals to become radicals themselves ROS (Reactive chemical element species). The first derivatives of chemical element (O_2, H_2O_2, ·OH, O_2) play a vital role in mediating ROS connected effects. Short lived reactive species generated in state of affairs will react with non radicals and produce chain reaction.[25] Free radicals have aroused important interest among scientists within the past decade. Their broad vary of effects in biological systems has drawn the eye of the many experimental works. There are units several reports that support the employment of antioxidant supplementation in reducing the extent of aerobic stress and in retardation or preventing the event of complications related to diseases.[26] Many manmade inhibitor parts have shown hepatotoxic and/or agent effects that have shifted the attention towards the present antioxidants. Varied plant constituents have tested to indicate radical scavenging or antioxidants activity.[27] There's increasing evidences that recommend that consumption of natural inhibitor contained in vegetables, fruits and medicative herbs area unit helpful in preventing the hurtful consequences of oxidative stress.[28]

The present investigation demonstrated that, DPPH, a radical, stable at temperature that produces a purple color solution in methanol. It's reduced within the presence of an antioxidant inhibitor molecule, giving rise to achromatic methanolic solutions. Figure 1 illustrates the decrease within the concentration of DPPH radical as a result of radical scavenging ability of methanol extract of *Limnophila heterophylla* and quercetin, which is comparable to the reported value of Thabrew *et al*.,.[29]

Nitric oxide radical inhibition study proved that methanol extract of *Limnophila heterophylla* was a potent scavenger of Nitric oxide. This nitric oxide generated from sodium nitroprusside reacts with oxygen to nitrate group. The extract of plant inhibits nitrate formation by competitive with oxygen to react with nitric oxide directly and additionally to inhibit its synthesis. Scavengers of nitric oxide contend with oxygen resulting in reduced production of nitric oxide.[30] From the nitric oxide take a look at, rutin was used as a typical. The IC_{50} worth of the rutin is of the same kind to the reportable worth of Badami *et al*.[31]

In the PMS/NADH–NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants therefore indicates the consumption of superoxide anion within the reaction mixture. Addition of varied concentrations of methanol extract of *Limnophila heterophylla* yet as curcumin (standard) in higher than coupling reaction showed decrease in absorbance.

The liver granule fraction undergoes speedy non-enzymatic peroxidation once incubated with $FeCl_3$ and ascorbic acid. The employment Fe (III) within the presence of a reductant like ascorbate produces ·OH[32]

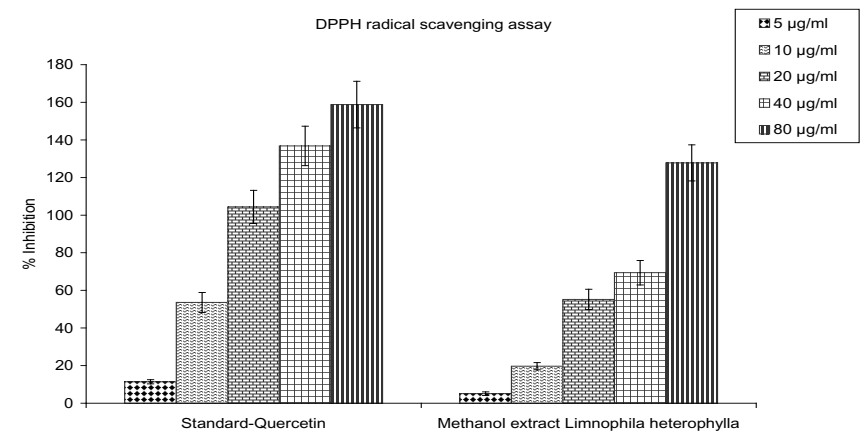

Figure 1: Scavenging effect of methanol extract of *Limnophila heterophylla* and standard quercetin on 1, 1'-Diphenyl-2-picryl hydrazyl (DPPH) radical. Results are mean ± S.D of five parallel measurements.

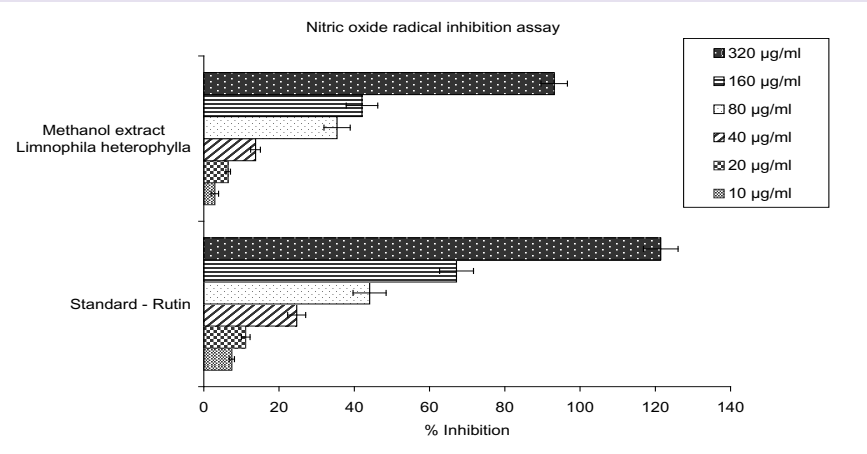

Figure 2: Scavenging effect of methanol extract of *Limnophila heterophylla* and standard rutin on Nitric oxide radical. Results are mean ± S.D of five parallel measurements.

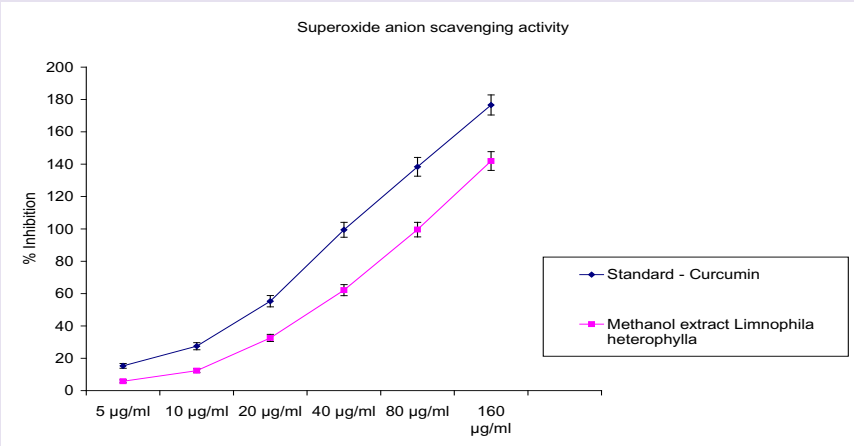

Figure 3: Effect of methanol extract of Limnophila heterophylla and curcumin on scavenging of superoxide anion radical formation. Results are mean ± S.D of five parallel measurements.

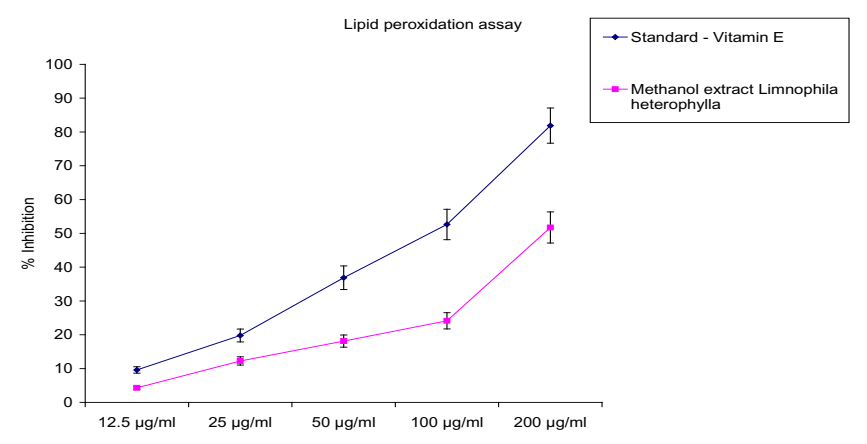

Figure 4: Effect of methanol extract of *Limnophila heterophylla* and vitamin E on lipid peroxidation of liver microsome induced by Fe^{2+}/ascorbate. Results are mean ± S.D of five parallel measurements.

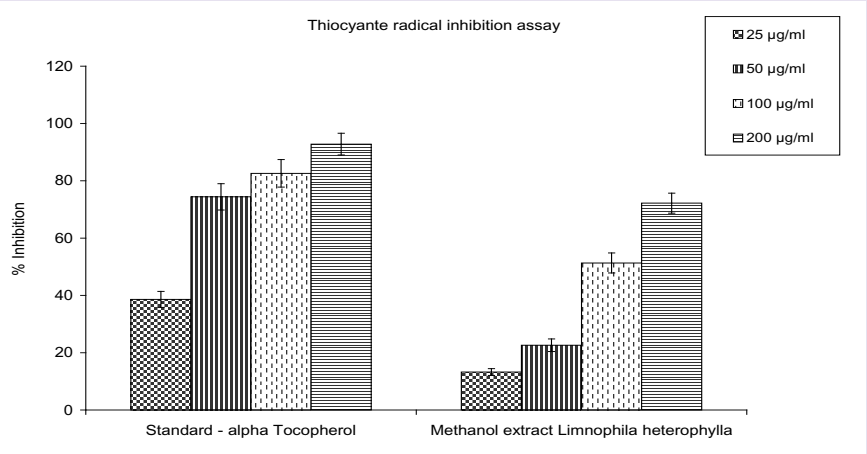

Figure 5: Percentage inhibition of lipid peroxidation using the standard (α- tocopherol) and methanol extract of *Limnophila heterophylla* in linoleic acid emulsion. Results are mean ± S.D of five parallel measurements.

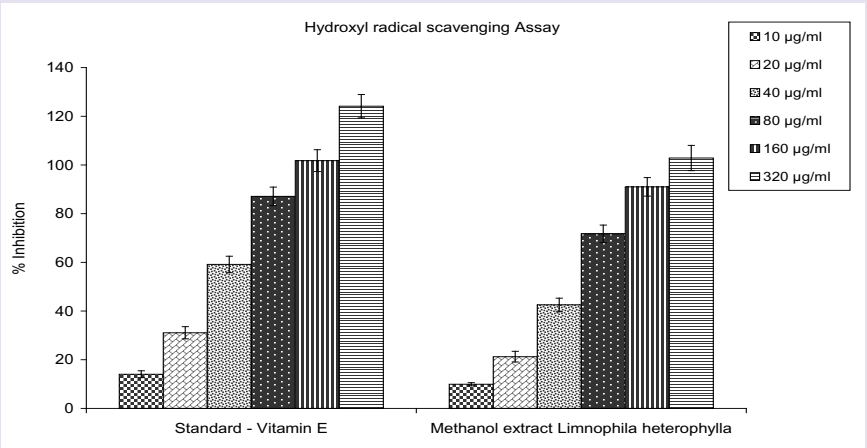

Figure 6: Effect of methanol extract of *Limnophila heterophylla* and vitamin E on deoxyribose degradation assay. Results are mean ± S.D of five parallel measurements.

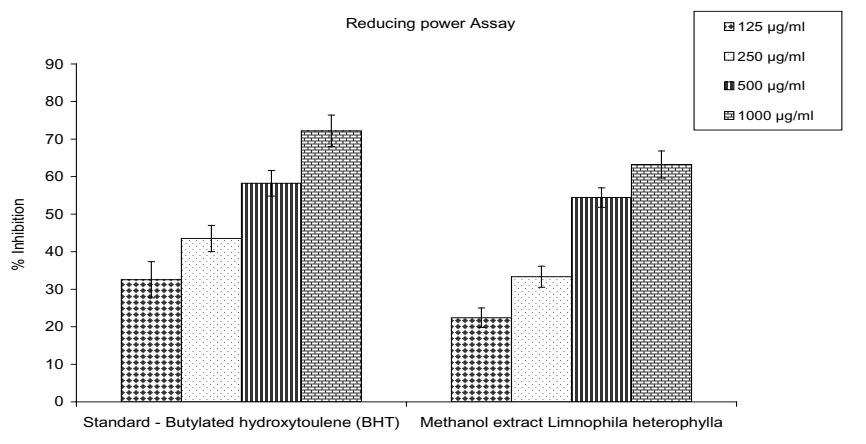

Figure 7: Effect of methanol extract of *Limnophila heterophylla* and BHT on reducing power ability assay. Results are mean ± S.D of five parallel measurements.

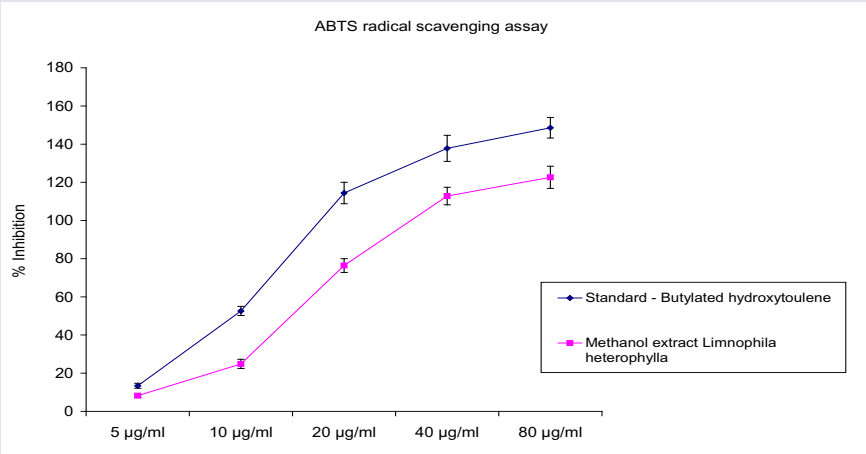

Figure 8: Effect of methanol extract of *Limnophila heterophylla* and BHT on reducing power ability assay. Results are mean ± S.D of five parallel measurements.

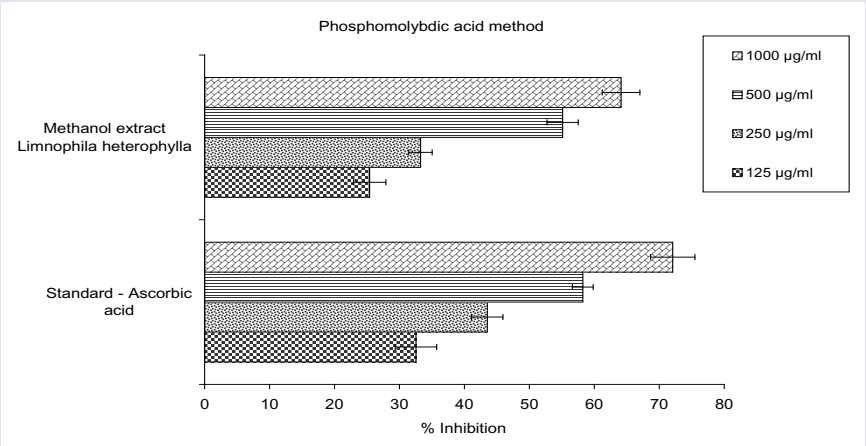

Figure 9: Effect of methanol extract of *Limnophila heterophylla* and ascorbic acid by phosphomolybdate method assay. Results are mean ± S.D of five parallel measurements.

and that they attack the biological material. This ends up in the formation of MDA (malonodialdehyde) and alternative aldehydes that forms a pink compound with TBA, engrossing at 532 nm.[33] The methanol extract of *Limnophila heterophylla* and Vitamin E exhibited sturdy scavenging impact of hydroxyl group that may inhibit macromolecule injury at totally different concentration. The scavenging impact of Vitamin E is in accordance with the report of Hemanth *et al*.,.[34] The extract inhibits macromolecule injury caused by hydroxyl group radicals and also the inhibition values mentioned in Figure 4.

Further, *in-vitro* antioxidant activity of methanol extract of *Limnophila heterophylla* was determined by exploitation the thiocyanate technique. From this technique, the number of peroxides shaped in emulsion throughout incubation was determined spectrophotometrically by measuring absorbance at 500 nm. High absorption is a sign of high concentration of peroxides formed. Therefore, low absorbance indicates high antioxidant activity.[19] The methanol extract of *Limnophila heterophylla* and customary of α–tocopherol were shown to inhibit the formation of peroxides at varied concentrations.

The extract was examined for its ability to act as ·OH radical scavenging agent. Ferric EDTA was incubated with H_2O_2 and ascorbic acid at pH -7.4; hydroxyl group radicals were shaped in free solution and were detected by their ability to degrade 2-deoxy-2-sugar into fragments that on heating with TBA at low hydrogen ion concentration form a pink compound.[18,21] When methanol extract of *Limnophila heterophylla* and vitamin E were additional to the reaction mixture, they removed hydroxyl group radicals and prevented the degradation of 2-deoxy-2-sugar as mentioned higher than the IC_{50} observed values of each plant extract and Vitamin E were analogous to the reportable values of Sen *et al*.,.[35]

Total anti oxidant activity of *Limnophila heterophylla* was determined by ABTS radical cation decolourization assay[23] by measuring the reduction of the radical cation as the percentage inhibition. *Limnophila heterophylla* exhibited effective antioxidant activity at all doses. The scavenging effect of *Limnophila heterophylla* and BHT was observed to be linear increase in ABTS radical scavenging activity with increasing concentration. The inhibition was found to be concentration dependent and BHT.

The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity.[22] In the reducing power increased with the increasing amount of extract. The reducing capacity of plant may serve as a significant indicator of its potential antioxidant activity. The reducing power of *Limnophila heterophylla* increased with increasing amount of sample. The results obtained from phosphomolybdic acid assay show that methanol extract has strong ability to reduce molybdenum (VI) to molybdenum (V) by donating electron.

CONCLUSION

The present study proved promising antioxidant potential of methanol extract of roots of *Limnophila heterophylla* against a variety of free radicals. It is reported that phenolics and flavonoids are natural products which have been shown to possess various biological properties related to antioxidant mechanisms. Thus the antioxidant activity of *Limnophila heterophylla* may be attributed to the presence of these compounds as confirmed by qualitative phytochemical analysis. Hence these results support the view that some traditionally used Indian medicinal plants are promising source of potential antioxidants.

ACKNOWLEGMENT

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CONFLICT OF INTEREST

Authors have no conflict of interest.

ABBREVIATION USED

ROS: Reactive oxygen species; **BHT:** Butylated hydroxytoluene; **BHA:** Butylated hydroxyanisole; **DPPH:** 2, 2-diphenyl-1-picrylhydrazyl; **NADH:** Nicotinamide adenine dinucleotide; **NBT:** Nitro blue tetrazolium; **PMS:** Phenazine methosulphate; **TBA:** thiobarbituric acid; **EDTA:** Ethylenediamine tetracetic acid; **ABTS:** 2, 2'- azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); **MDA:** malonodialdehyde.

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SUMMARY

- Roots of methanol extract of *Limnophila heterophylla* effectively scavenged free radicals in different models like *hydroxyl radical, antioxidant assay by thiocyanate method, inhibition of superoxide anion radical, 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH), lipid peroxidation assay, scavenging of 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt radical cation assay (ABTS), total antioxidant activity (phosphomolybdic acid method)* and reducing ability at different concentrations and showed its potent antioxidant activity.
- Further, these effects were in a dose dependent manner. Results were compared to standard antioxidants such as vitamin E, α-tocopherol, curcumin, quercetin, rutin, butylated hydroxytoluene (BHT) and ascorbic acid. *Limnophila heterophylla* enclose strong antioxidant potential.

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