International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 11
November 2017

# Phytochemical Analysis of Field Grown and Tissue Culture Derived *Mentha arvensis* L. Plants with special reference to Antioxidative Potentials

**Debleena Roy* and Bidisha Mallick**
Department of Botany, Lady Brabourne College, P 1/2 Suhrawardy Avenue, Kolkata-700017, India

## ABSTRACT

*Mints, belong to the family lamiaceae, were aromatic plants of high importance. Mentha arvensis was one of the most important herbs of this family exploited widely for medicinal purpose making the natural resource threatened. High costs and high demand of this plant for manual labor disallowed large scale propagation with green cuttings. Due to presence of high amount of secondary metabolites, in vitro rapid propagation for production of improved clones was desirable for conservation and commercial exploitation. The present investigation deals with theconservation this medicinal plant for future exploitation through in vitro propagation to evaluate the antioxidative potential of the tissue culture derived clonesand to compare it with the in vivo field grown plants so that tissue culture derived plants can be taken in future as an alternative to the naturally grown threatened plants.It was observed that the antioxidant property of the field grown plants were well maintained in the tissue culture derived plant suggesting (total phenol 2.36±0.44mgGAE/g, rosmarinic acid 3.5±0.98mg/g, total flavonoid content 22.18±0.54 mgQE/g) in vitro regeneration as an alternative for sustainable use of this medicinally important threatened plant. The tissue culture derived plants when compared to the field grown plants have also shown the capacity to scavenge the free radicals and inhibit the formation of lipid peroxides.*

***Keywords: Mentha arvensis, micropropagation, total phenolic content, total antioxidant activity, DPPH, Lipid peroxidation***

## 1. INTRODUCTION

The principal importance of medicinal plants is highlighted as a source of natural antioxidants and functional foods. Various chemical compounds are found in medicinal plants of which polyphenols have received much attention because of their role in several degenerative and ageing related diseases [1, 2]. Epidemiological and experimental studies reveal a negative correlation between the consumption of diets rich in fruits and vegetables and the risks for chronic angiogenic diseases, such as cardiovascular diseases, arthritis, chronic inflammation and cancer[3]. Radicals of oxygen can cause damage to cell. Phenolic compounds safely interact with free radicals and terminate the chain reaction before the vital molecules are damaged. The phenolic compounds are of increasing interest in the food and drug industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [4, 5].

*Mentha arvensis* L. the aromatic culinary herb of the family lamiaceae has been identified as a source of food, flavoring agent and various phytochemicals, including polyphenols which are highly effective antioxidants and less toxic than the well-known synthetic antioxidants as BHA and BHT[6].In rural areas utilization of wild populations of different common medicinal plants including *Mentha* as crude drugs is a regular practice among the poor people for treatment against the life threatening diseases. In addition the pharmaceutical companies also make use of wild populations of different medicinal plants for their own interest. All these have created an increased demand of many medicinal plants including *Mentha*.As all species of the genus contain high amounts of secondary metabolites, this genus is getting over exploited by the food and drug industries making the natural resource threatened.*In vitro* rapid propagation for the production of improved clones is desirable for rapid conservationand commercial exploitation of this economically important threatened plant[7].To preserve these medicinal plants from future extinction, cultivation is necessary.

International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 11
November 2017

Extraction of secondary metabolites (which is responsible for its medicinal properties) from naturally grown whole plants on a commercial basis involves large scale crop cultivation. Hence the application of plant tissue culture has gained major industrial importance in three main areas:1. In breeding and genetics - a way to conservation [8]. 2. As model systems for plant biochemistry and pathology- ways to produce disease free crops. 3. Production of secondary metabolites - for exploitation in food, drug and pharmaceutical industries [9]. So the application of the tissue culture technique will be very useful in order to conserve and future augmentation of the metabolites present in these very useful medicinal plants.

The aim of the present report is to conserve this medicinal plant for future exploitation through *in vitro* propagation and to evaluate the antioxidative potential of the tissue culture derived clones and to compare it with the *in vivo* field grown plants so that tissue culture derived plants can be taken in future as an alternative source of food and drug in comparison with the naturally grown threatened plants.

## 2. MATERIALS AND METHODS

### 2.1Collection of plant material*:*

*Mentha arvensis* L. was collected from NBPGR, New Delhi bearing strain no IC:54538. Field grown plantswere maintained in the medicinal garden of Lady Brabourne College. The antioxidant potential of the field grown plants in 4 seasons (summer, monsoon, autumn and winter) were taken into account and an average was calculated and the average annual productivity was compared with the *in vitro* regenerates.

*In vitro culture*—Apical and nodal portions with axillary buds of 2-3 cm length from field grown plants were taken as explants. Media used for *in vitro* culture was Murashige and Skoog (MS) modified basal medium supplemented with different concentrations of BAP: 6-benzylaminopurine. All the cultures were maintained in a culture room at 24±1 °C in a light intensity of 48 µmol $m^{-2}$ $s^{-1}$ photosynthetic photon fluxes. Cultures were maintained by sub-culturing the material after every 4 weeks under aseptic condition.Shoots collected from plants regenerated *in vitro* in a medium with best response were considered as control. Antioxidative potentials were analyzed from control tissues and compared with *in vivo* plants.

### 2.2Preparation of the plant extract [5]:

The fresh leaves(5g) was crushed with 50 ml 80% methanol in a homogenizer(REMI) and filtered. The content was decanted through a filter paper and the residue was re-extractedagain with 80%methanol. The extracts were lyophilized (Hahntec Lyophilizer) till free of solvents and crude extract was prepared. A comparative study was done using ethanol as solvent.

### 2.3 Estimation of phenolics:

The amount of total phenolics in extracts was determined with the Folin ciocalteau reagent. Gallic acid was used as a standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE). 1 mg/ml of plant extract were prepared in methanol and 0.5 ml of each sample was introduced into test tubes. It is mixed with 2.5 ml of 10 fold dilute Folin ciocalteau reagent and 2 ml of 7.5% sodium carbonate. The reaction mixture is allowed to stand for 30 minutes at room temperature and the absorbance was read at 760 nm in UV-Vis spectrophotometer. The concentration was calculated using gallic acid as the standard and the results were expressed as mg gallic acid equivalents/g of extract. The standard curve was drawn using gallic acid as standard. The total phenolic content was determined as GAE(gallic acid equivalent) using an equation (y=a*x, a= 9.909+/-0.3133) from the calibration curve of gallic acid standard solution(covering the concentration range between 20µg to 100µg).

### 2.4 High performance liquid chromatography (HPLC) analysis of rosmarinic acid for quantification—

High Performance Liquid Chromatography (HPLC) (Shimadzu, SPD-10A UV-Vis detector, LC-10 AD Liquid chromatography) separation was achieved by using a C18 column, (150 × 4.6mm, Hypersil) with a particle size 5.0µm and the temperature was set at 30ºC. The flow rate was 0.5 ml/min. The mobile phase for chromatographic analysis was with water: acetonitrile (83:17 v/v). Ultraviolet detection was set at 330 nm[7, 5]

International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 11
November 2017

**2.5 Estimation of flavonoids**:

Total flavonoid content was measured by the aluminium chloride colorimetric assay [10]. In this method quercetin was used as standard and flavonoid contents were measured as quercetin equivalent from the standard curve (y=a*x, a= 0.001339+/-0.3133). 1 ml of extract (500µg/ml) was added to a 10ml volumetric flask containing 4 ml of distilled water.0.3 ml 5% $NaNO_2$ was added.After 5min, 0.3 ml10% $AlCl_3$ was added. At $6^{th}$ min, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with water.The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm by UV-Vis spectrophotometer.

**2.6 Total antioxidant activity**:

The total antioxidative activity was measured by using the method described by[11].Plant extracts were dissolved in methanol to obtain a concentration of 500µg/ml.0.3ml of extract was placed in a test tube; 3 ml of reagent solution (0.6M $H_2SO_4$ +28mM Sodium phosphate+ 4mM Ammonium molybdate) was added. The reaction mixture was incubated at $95^0$ C for 90 minutes. The mixture was cooled to room temperature; the absorbance of each solution was measured by using Systronics UV-Vis spectrophotometer at 695nm against blank. The experiment was performed in triplicate. A calibration curve was constructed, using Ascorbic acid(100-500µg/ml) as standard and total antioxidant activity of extract (µg/ml)expressed as ascorbic acid equivalents.

**2.7 Free radical scavenging activity**:

The antioxidant activity of the extracts was assessed by their ability to scavenge 2-2 diphenyl-1picryl hydrazyl stable radicals (DPPH) by using the methods of Shimada et al 1992[12.]1 ml of methanolic extract and 5ml of freshly prepared 0.1mM DPPH methanolic solution were thoroughly mixed and kept in the dark for 60 minutes. The absorbance of the reaction mixture at 517 nm was measured by spectrophotometer. The blank set was prepared by replacing the extract with methanol (1 ml).The percentage of free radical scavenging activity was calculated as follows:

Free radical scavenging (%) =100-(1-$A_{sample}/A_{blank}$),

**2.8 Lipid peroxidation activity**:

The antioxidant potential of *Mentha* was measured by inhibition of lipid peroxidation of Linoleic acid[13]. 0.5 ml (1 mg/ml) of mint extract was taken and mixed with 2.0 ml of Linoleic acid emulsion (pH 7.0) and the final volume was made upto 3ml by adding 0.5ml of Phosphate Buffer (0.2M, pH 7.0). The Linoleic acid emulsion was prepared by mixing 0.28gm of Linoleic acid, 0.28gm of Tween-20 as emulsifier and 50ml of Phosphate Buffer. The mixture was then homogenized. The reaction mixture was incubated at room temperature. Aliquots (0.1%) was drawn after every 30 minutes upto 4 hours to access peroxidation of linoleic acid using Thiocyanate method by sequentially adding Ethanol (4.7ml, 75%) , Ammonium thiocyanate (0.1ml, 30%) , sample solution (0.1ml) and Ferric chloride (0.1ml , 0.02M in 3.5% HCl). After 30 minutes the mixture was diluted using 1ml of extract and 4ml of distilled water. The mixture was allowed to stand for 3 minutes and absorbance was recorded by double beam spectrophotometer at 500nm. A negative control was run in an identical manner where extract is replaced by water. Ascorbic acid was used as a positive control.

**2.9 Statistical analysis:**

Data were analyzed statistically following one way Anova assay to determine the least significant difference. Three replications of each set of experiment were taken for study and an average was calculated.

## 3. RESULTS AND DISCUSSION

Axillary buds were most suitable explant sources for multiplication of fieldmint. The bud break was observed mostly after 9 days after culture in all cases. All the cultures were maintained up to twelve weeks. For *Mentha arvensis* L. various combinations of different cytokinins on shoot bud multiplication were used. BAP showed the highest potentiality for shoot bud multiplication than the other two cytokinins tested[7]. Root formation started after fifteen days of culture. Presence of auxin only gave a better response in root production. After

complete regeneration they were transferred to the pre acclimation chamber (PAC). The plants were maintained there up to 3 weeks. The upper half of the PAC was removed after 3 weeks and kept in outer environment. It was seen that plants survived with a good growth. Somatic chromosome analysis of regenerated plants showed chromosome number stability with 2n=36 in *M. arvensis*). Chromosome analysis revealed chromosome number stability in the regenerates. This may indicate the efficacy of BAP and the culture conditions in maintaining the genome stability of the regenerates. Such chromosome number stability in the regenerates has also been observed using BAP as the only cytokinin in culture medium[7].The eight week old plants are taken for further tests.

**3.1 Total phenol, rosmarinic acid and flavonoids:**

The methanolic extracts of plant samples of different season were analyzed for total phenol and flavonoids (Table 1).The results showed that among the two solvents used methanol proves to be more efficient than the other (Data not shown). The amount of total phenol varies in the four seasons for field grown plants with an average of 2.19±0.32 mg GAE/g of methanolic extracts where tissue culture derived plants showed continuous and constant production of total phenolics (2.36±0.44 mg GAE/g) which is 7.76% more than the field grown plants. Rosmarinic acid content also showed slight increased (9.375%) in tissue culture derived plants[7]. In case of total flavonoid content, it also varies through the season with an average production of 22.18±0.54 mg QE /g of the extracts where tissue culture derived plants showed 9.25% increased production of total flavonoid content. Tissue culture derived plant had a higher content of phenol as compared to the field grown plants[15].

Seasonal variation of phenol and rosmarinic content had been reported earlier for other *Mentha* species also [7, 5].The total phenolic content of *Mentha* varies in each season. It was observed by many researchers that the physiological and biochemical changes are synergistic effects of different biotic and abiotic parameters. It is difficult to determine which environmental factor is mainly responsible for the variations. In laboratory experiments factors can be set and regulated[16]. Tissue culture approach may establish a shoot based clonal line experimental purpose and it was observed that a continuous stable production of total phenolics, total flavonoids and rosmarinic acid content was synthesized in shoot based clonal lines.

**3.2 Total antioxidant capacity:**

The total antioxidant capacity of extracts of field grown *Mentha* plants was found to be 7.49±0.29µg/ml (expressed as ascorbic acid equivalents)that of tissue cultured plants was 9.52± 0.36 µg/ml (expressed as ascorbic acid equivalents)at the same concentration(Fig 1).

The results were not significantly different from each other($p<0.05$).Our results are similar to the results obtained from the extracts of *Phyllanthus niruri* and*Mentha piperita* L., where the *in vitro* plants had higher TAC value than the samples obtained from the market[16, 5].

**3.3 DPPH Radical scavenging activity:**

The DPPH radical scavenging activities of the extracts were given in Figure 2.The activity increased by increasing the concentration of the sample extract. This assay has been largely used as a quick, reliable and reproducible parameter to search for the *in vitro* antioxidant activity of pure compounds as well as plant extracts. The percentage inhibition of the field grown plant extract is 59.18±0.46, while that of the tissue cultured plants is 61.59±0.16. Ascorbic acid the well-known antioxidant has a percentage inhibition of 92.97±0.11.The present results suggests that the extracts are apparently good free radical scavenger and probably have the ability to inhibit auto oxidation of lipids and could thus be beneficial in the treatment of various diseases where lipid peroxidation is an important mechanism for pathogenesis [18]. Our observation also supported the fact that *M.arvensis* showed higher DPPH activity when compared to other plant sample including *M. piperita*[19, 5].

**3.4 Lipid peroxidation**:

The FTC method measures the amount of peroxidases in the beginning of lipid peroxidation where ferric ion was formed upon reaction of peroxide with ferrous chloride. The ferric ion will then unite with ammonium thiocyanate producing ferric thiocyanide, a red coloured substance. The absorbance data of linoleic acid

peroxidation after adding plant sample with positive control ascorbic acid is shown in Table 2. Thus a high absorbance value was an indication of high peroxide formation during incubation. After the completion of the second hour all the samples have shown a lower absorbance value indicating inhibition of lipid peroxidation. The positive control, ascorbic acid have also shown similar results. The phenolic components present in the tissue cultured derived plants(control set)may donate hydrogen and can terminate the free radical reaction chain by changing into stable compounds. In case of water(the positive control), the formation of peroxides goes on whereas in case of plant samples at the end of second hour, the process of formation of peroxides is lowered due to the presence of phenolic compounds. The leaves of the plants of lamiaceae, have high antioxidative activity.[20]The hydrophyllic fractions of the plants of lamiaceae may be useful as food stuffs for liver protection.[21]

## 4.CONCLUSION:

In the present investigation a simple and rapid *in vitro* propagation protocol has been established. Both the field grown and tissue culture derived plants were shown to have significant amount of polyphenolic components, expressed as gallic acid equivalents. The results of antioxidant evaluation based on two models, DPPH and TAC used in this study revealed that the methanolic extract of both field grown and tissue cultured *Mentha arvensis* possess interesting antioxidant activity. It was observed that the antioxidative properties of the field grown plants were well maintained in the tissue culture derived plants suggesting*in vitro* regeneration as an alternative for sustainable use of this medicinally important threatened plant. These observations enhance potential interest in the culinary herbs for improving the efficacy of different products as neutraceutical and pharmacological products.

## 5. ACKNOWLEDGEMENT:

The authors are thankful for financial assistance from DBT, under the DBT Star college scheme, Department of Botany, Lady Brabourne College, Kolkata.

**Table 1: Determination of total phenol, rosmarinic acid and total flavonoid**

| Plant sample | Total phenol (mg GAE/g)* | Rosmarinic acid (mg/g)* | Total flavonoid content (mg QE /g)* |
|---|---|---|---|
| Field grown plants | 2.19±0.36 | 3.2±0.32 | 22.18±0.54 |
| Tissue culture derived plants | 2.36±0.44 | 3.5±0.98 | 24.26±0.38 |

*Data expressed as Mean ± S.E. from 3 replicates; $P \leq 0.0001$. Means were compared by one wayAnova and on the basis of analysis it can be said that F values are higher than critical F values at 0.01% level of significance. The data indicates significant differences among different parameters.

**Table 2: Determination of Lipid peroxidation activity**

| sample | Time (1hr) | Time (2hr) | Time (3hr) | Time (4 hr) |
|---|---|---|---|---|
| **AscAcid(+ve control)** | $0.156\pm0.06_a{}^a$ | $0.80\pm0.12_b{}^a$ | $0.120\pm0.12_c{}^a$ | $0.159\pm0.13_d{}^a$ |
| **Field grown plants** | $0.265\pm0.08_a{}^a$ | $0.121\pm0.19_b{}^a$ | $0.165\pm0.11_b{}^a$ | $0.187\pm0.36_b{}^a$ |
| **Tissue cultured derived plants(Control)** | $0.251\pm0.07_a{}^a$ | $0.126\pm0.03_b{}^a$ | $0.140\pm0.09_c{}^a$ | $0.166\pm0.18_d{}^a$ |
| **Water (-ve control)** | $0.430\pm0.22_a{}^b$ | $0.490\pm0.17_b{}^c$ | $0.494\pm0.11_b{}^d$ | $0.503\pm0.12_b{}^d$ |
| **LSD** | 0.13 | 0.071 | 0.082 | 0.15 |

International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 11
November 2017

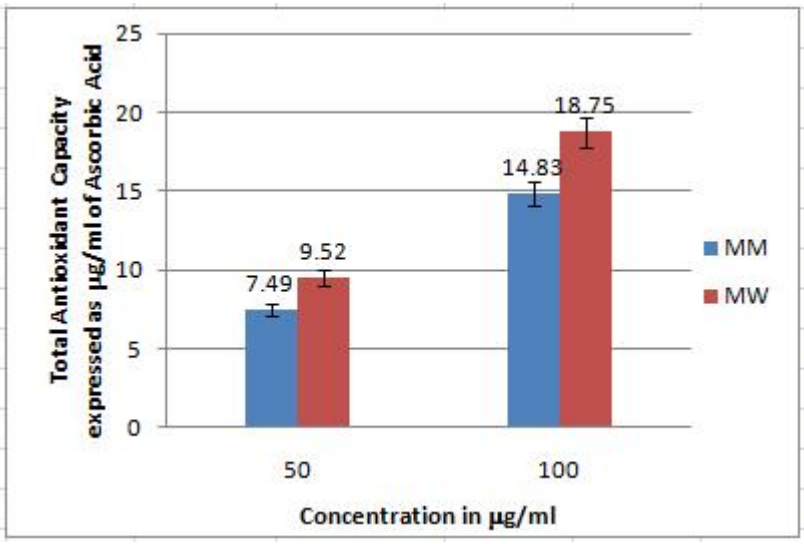


**Fig: 1-Total Antioxidant Capacity of field grown (MM) and tissue cultured (MV) *Mentha arvensis* L.plants expressed as «g/ml of Ascorbic Acid (Values are expressed as mean ± SEM, n=3)**

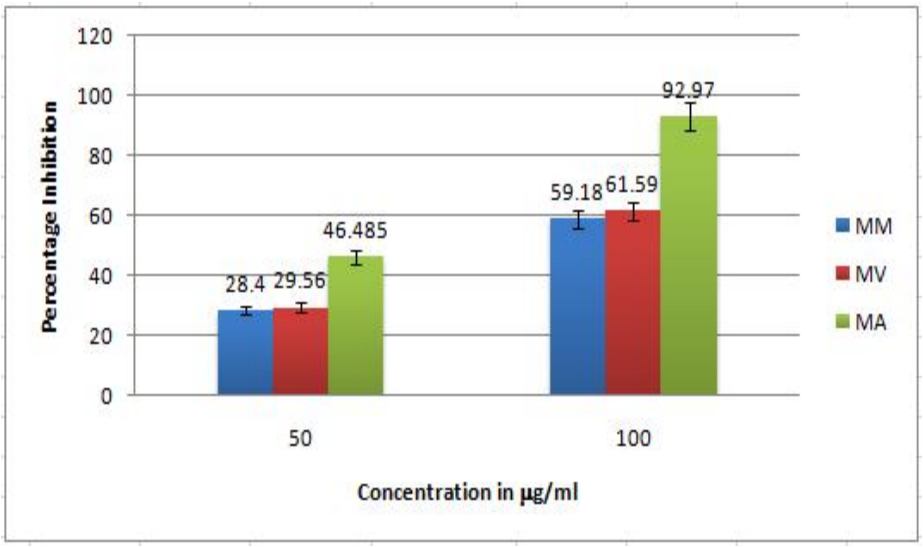


**Fig 2: DPPH radical scavenging activities of extracts of field grown (MM) & tissue cultured (MV) *Mentha arvensis* L. in comparison with Ascorbic Acid (AA) (Values are expressed as mean ± SEM, n=3)**

## 6. REFERENCES:

1. Bhattacharyya, P., Kumaria, S. andTandon. P., 2016.High frequency regeneration protocol for *Dendrobium nubile*: A model tissue culture approach for propagation of medicinally important orchid species. South African J of Botany.104: 232-243.
2. Brewar, M. S., 2011. Natural antioxidants: sources, compounds, mechanism of actions, and potential applications. Comp reviews in food sc and food safety.).
3. Miller, H. E., Rigelhof, F., Marquart, L., Prakash A. andKanter, M. 2000. Antioxidant content of whole grain breakfast cereals, fruits and vegetables. Journal of American College of Nutri.19 (3):312S-319S.
4. Kahkonen, M. P., Hopia, A. I., Vuorela, H. J., Pihlaja, K., Kujala, T. S. and Heinonen, M.1999. J Agric Food Chem. Antioxidant activity of plant extracts containing phenolic compounds.47: 3954-3962.

**5.** Mallick, B., Sinha, S. and Roy, D., Evaluation of antioxidant potential of field grown and tissue culture derived *Mentha piperita* L. plants.2016. Int J of Curr Micro and ApplSc. 5 (3) : 382- 391
6. Sreenivasan, S., Ibrahim, D., MohdJassim, M.J.N.2007. Free Radical scavenging activity and total phenolic compounds of *Gracilaria changii*. International J. Natural Eng.Sci.1:115-117.
7. Roy, D. and Mukhopadhyay, S. 2012. Enhanced rosmarinic acid production in species of *Mentha*. Indian J Exp Biol. 50(11):817-25.
8. Julia, K., Claudia, J. 2012. Draught, salt, temperature stress induced metabolic rearrangements and regulatory networks. J Expert Bot.63:1593-1608.
9. Tisserat B. and Vaughn S., 2008. Growth, morphogenesis and essential oil production in *Mentha spicata* L. shoots *in vitro*. *In vitro* Cell Dev Biol. 44:40-50.
10. Ebrahimzadeh, M. A., Nabavi, S. M. and Nabavi, S. F. 2010. Biological activities of *Mentha spicata*. Pharmacology online. 1:841-848.
11. Prieto, P., Pineda, M. and Aguilar, M.1999. Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to the determination of Vit E. Annal.Biochem. 269:339-341.
12. Shimada K, Fujikaewa K, Yahara K, Nakamura T. 1992. Antioxidative properties of Xanthan on the autooxidation of Soybean oil in cyclodextrin. J Agric Food Chem.40: 945-948
13. Masuda H, Hirunaka S, Matsui Y, Hirooka S, Hirai M, Hirata Y, Akao M, Kumagai H, Comparative study of antioxidative activity of culinary herbs and spices and hepatoprotective effects of three selected lamiaceae plants in cabon tetrachloride induced oxidative stress in rats, *Food sc and tech research*, 21(3) (2015) 407-418.
14. Gordon, M. H.1990.The mechanism of antioxidant action *in vitro*, in food antioxidants..Elsevier Applied Sci London. 1-18.
15. HudaFaujan, N., Noriham, A., Norrakiah, A. S. and Babji, A. S.2009. Antioxidant activity of plant methanolic extracts containing phenolic compounds.African J of Biotech.8 (3): 484-489.
16. AkashDeep, Rana, P., Gosal S. and Soni, G. 2013. Antioxidant potential of tissue cultured *Mentha spicata*. J Pharmacy Res.1: 90-96.
17. Siatka, T. and Kasparova, M. 2010. Seasonal variation in total phenolic and flavonoid contents and DPPH scavenging activity of *Bellis perennis* L. flowers. Molecules. 15: 9450-9461.
18. Gami, B., Kothari, I. L.2011. Antioxidant and antimicrobial activity of *in vivo* and *in vitro* grown plants of *Phyllanthus niruri* L., International J of Pharma and biosciences. 2 (2).
19. Aliyu, A. B., Ibrahim, M. A., Ibrahim, H., Musa, A. M., Lawal, A.Y., Oshanimi, J. A., Usman, M., Abdulkadir, I. E., Oyewate, A. O., Amupitan, J .O.2012. Free radical scavenging and total antioxidant capacity of methanol extract of *Ethuliaconyzoides* growing in Nigeria, *Romanian Biotech letters*,17: 7458-7465.
20. Raghavendra H., Lakshmanashetty, Vijayananda B., Nagaraj, Madhumathi G., Hiremath, Vadlapudi Kumar. 2010. *In vitro* antioxidant activity of *Vitex negundo*.L.leaf extracts.Chiang Mai J Sci.37: 489-497.
21. Takao T, KitataniF, Watanabe N, Yagi A, Sakata K, A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish, *Biosci. Biotechnol,Biochem.* 58(1994) 1780-1783.