Journal of Applied Pharmaceutical Science Vol. 4 (06), pp. 079-083, June, 2014
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2014.40612
ISSN 2231-3354

In vitro lipid peroxidation of tissue cultured and tissue culture derived mentha plant

Akash Deep*, Pooja Rana, S S Gosal and Giridhar Soni

Department of Biochemistry, Punjab Agricultural University, Ludhiana, Punjab, India. [1]School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India.

ARTICLE INFO

Article history:
Received on: 25/12/2013
Revised on: 08/02/2014
Accepted on: 23/05/2014
Available online: 28/06/2014

Key words: Flavonoids, lipid peroxidation, *Mentha*, phenols, tissue culture.

ABSTRACT

The effect of tissue cultured and tissue culture derived *Mentha* species viz. *Mentha piperita*(PPR611), *Mentha arvensis* (SH) and *Mentha spicata* (SPR 8) on antioxidant potential using lipid peroxidation model was studied. The extracts prepared were analyzed for total phenols and flavonoids. Tissue cultured derived plants were found to have higher content of total phenols and flavonoids. Furthermore, *Mentha spicata* (SPR 8) was found to possess higher content of total phenols, however, flavonoid content of tissue cultured samples was more compared to tissue culture derived plants in *Mentha arvensis* (SH) and *Mentha spicata (*SPR 8).Tissue cultured plants of all the three species were more potent in lipid peroxidation inhibition model. Tissue cultured derived plants were less effective in preventing lipid peroxidation and inhibition potential decreased with development period in all the three species.

INTRODUCTION

Plants contain a wide variety of phytochemicals or bioactive molecules, which can neutralize free radicals and thus retard the progress of many chronic diseases associated with oxidative stress and Reactive Oxygen Species (ROS). Studies on dietary free radical scavenging molecules have attracted the attention to characterize phenolic compounds and other naturally occurring phytochemicals as antioxidants (Ani *et al.*, 2006).Herbs have also been identified as source of various phytochemicals, many of which possess important antioxidant activity. Medicinal plants contain large amounts of antioxidants other than vit C, vit E and carotenoids. Flavonoids which are widely distributed in the leaves, seeds, bark and flowers of plants are a broad class of low molecular weight compounds and highly effective antioxidant and less toxic than synthetic antioxidants such as BHA and BHT and have received greater attention and studied extensively (Naczk, 2004). Synthetic antioxidants such as butylatedhydroxytoluene (BHT) and butylated hydroxyanisole (BHA) should be replaced by natural compounds due to their possible toxicity (Sreenivasan *et al.*, 2007). Therefore, by considering adverse effects of synthetic antioxidant on human health, alternative natural and safe sources of food antioxidant should be identified (Goli *et al.*, 2005).Plant extracts due to the presence of secondary metabolites possess similar or even higher antioxidant activity can be natural alternatives to synthetic antioxidants, so they are strongly of interest in the food industry (Le floch *et al.*, 1998). Many herb species, especially those of *Lamiaceae* family, such as sage, orenago, thyme, *Mentha*, show strong antioxidant activity. *Mentha* is a genus of aromatic perennial herbs belonging to the family *Labiateae* (*Lamiaceae)*(Reverchon, 2006).

Three *Mentha* species *M.spicata, M. piperita* and *M.arvensis* are commonly cultivated in the world for essential oil production that is extensively used in the liquor and confectionary industries, flavoring, perfume production and medicinal purposes (Moreno *et al*., 2002). *Mentha spicata* (spearmint) is a fast growing perennial herb in mint family that biosynthesizes significant amounts of rosmarinic and phenolic acids (Choudhary *et al*., 2006). Mint possesses antioxidant properties due to the presence of active constituents like menthone, menthol, rosamarinic acid and carvone (Padmini *et al.*, 2010). The growing conditions could have implications on the metabolic activity for spearmint earmarked as a source of high quality rosamarinic acid and other phenolic compounds of commercial use (Al Aimer *et al.*, 1999).

* *Corresponding Author*
Dr Akash Deep, Department of Biochemistry,
Punjab Agricultural University,Ludhiana, Punjab
***Email.**-Akash_bhagirath@yahoo.in **Tel.no-** 09872100719*

Changing environmental conditions, resulting in an increased occurrence of heat and drought stress and the metabolic effects of stress have become a major focus of plant research (Wang & Zheng, 2001).Antioxidant potential of herbs vary greatly with varietal differences, cultivation conditions and environment (Dragland *et al.,* 2003) .Tissue culture approach is a novel approach to the improvement of genotypes under drought, salinity and extreme temperature and can exploit maximum genetic gain achieved in breeding programmes (Julia & Claudia 2012).Work on the antioxidant potential of *Mentha* is being already carried out in our laboratory (Akashdeep *et al.,* 2011,Akash Deep *et al.,* 2013).Therefore, taking this information into consideration ,present work was planned to study the effect of maturity of tissue cultured (TC) and tissue culture derived(TC_0 andTC_1) *Mentha* species on antioxidant potential using lipid peroxidation inhibition model.

MATERIALS AND METHODS

Mentha species viz. *Mentha piperita* (PPR 611), *Mentha arvensis* (SH), *Mentha spicata* (SPR-8), selected for present studies, were obtained from the School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana.

Micropropagation of *Mentha* species

Preparation of culture medium

The medium used was the Basal medium as described by Murashige and Skoog, 1962.

Inoculation (culturing) and propagation

After surface sterilization with mercuric chloride solution (1%) collected samples of explants (leaves) of *Mentha* species were inoculated aseptically into culture vessel containing Murashige and Skoog medium. These cultures were incubated at a temperature of 25±2°C with relative humidity (60%-80%).Cultures were given florescent light (3000-5000 Lux), 16 h light/8 h dark regimes.

After one month of culturing, tissue cultured samples (TC) of all the three *Mentha* species were shifted to soil in glass house after for further development and were named as TC_0. After 30 days, cutting (3 cm) of few plants of TC_0 was done and these cuttings were replanted in soil in glass house and were called TC_1. Both these generations of tissue cultured derived plants (TC_0 and TC_1) were grown for the three months. Samples were collected from tissue culture (TC) and tissue culture derived plants (TC_0 and TC1).

Preparation of plant extract

The plant material (leaves) 2 g was crushed with methanol (25 ml) and shaken for 12h at room temperature. After filtration, the residues were re-extracted twice under same conditions. Solvents were removed from the combined filtrates under vacuum at 45°C in Buchi rotary evaporator. The yield of crude extracts was determined gravimetrically.

Estimation of phenols and flavonoids

The extracts were analyzed for total phenols (Swain & Hills, 1959) and results were expressed as g gallic acid equivalents per 100 g dry weight and flavonoids (Balabaa *et al.,* 1974) and expressed as g of catechin equivalents per 100 g dryweight.

Lipid peroxidation inhibition potential

The antioxidant activity of various *Mentha* species was determined by measuring inhibition of lipid peroxidation of linoleic acid (Mitsuda *et al.,* 1966). Different concentrations of extracts of *Mentha* were made free of the solvent and mixed with 2.0 ml of linoleic acid emulsion (pH 7.0) and the final volume was adjusted to 3.0 ml with phosphate buffer (0.2 M, pH 7.0). The linoleic acid emulsion was prepared by mixing 0.28g of linoleic acid, 0.28g of tween-20 as emulsifier and 50 ml of phosphate buffer and the mixture was homogenized. The reaction mixture was incubated at 37°C. Aliquots (0.1 ml) were drawn at different time intervals(30mins) upto 4hrs to assess per-oxidation of linoleic acid using thiocyanate method by sequentially adding ethanol (4.7 ml, 75%), ammonium thiocyanate (0.1 ml, 30%), sample solution (0.1 ml) and ferrous chloride (0.1 ml, 0.02M in 3.5% HCl).

The mixture was allowed to stand for 3 min and absorbance was recorded at 500 nm. A control was run in an identical manner but without the extracts. Vitamin C was used as a positive control.

RESULTS AND DISCUSSION

Phenolics and flavonoid content of *Mentha* species

The methanolic extracts of various plant samples of *Mentha* species were analysed for total phenol and flavonoids (Table 1).

Total phenolic content of methanolic extracts of mint plants was evaluated using Folin Ciocalteau reagent and results were expressed as g Gallic Acid (GA) equivalents per 100 g dry weight .It evident from result that total phenol content of TC plantlets was highest in SPR-8 (3.20 g/GA Eq) followed by SH (1.16 g/GA Eq) and PPR 611 (0.67 g/GA Eq). Tissue culture derived plants (TC_0 and TC_1) had substantially higher content of total phenols as compared to TC plantlets. Furthermore, total phenol content increased over the growth period of tissue culture derived (TC_0 and TC_1) plants in all the three species and was found to be highest in TC_1 SPR-8 plants (7.3 g/GA Eq) at 3 months of development period. Phenolic compounds are the main antioxidant ingredient in medicinal plants. These compounds have high antioxidant and free radical scavenging capacity (Gan *et al*., 2010).Increase in phenolic content with time can probably be due to acclimatization stage in plants. Furthermore, it is considered that total phenolic content is due to their high redox potentials, which allows them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Miguel 2010). Total flavonoid content of mint plants was expressed as g catechin (CE) equivalents per 100 g dry weight.

Table. 1: Total phenol and flavonoids content of *Mentha* species at different development periods .

Stage	Development period (month)	Species *Mentha piperita* (PPR 611)	*Mentha arvensis* (SH)	*Mentha spicata* (SPR 8)
Total phenols (g GA eq/100g DW)				
TC		0.67±0.03	1.16±0.03	3.20±0.02
TC_0	1	3.70±0.03	1.50±0.03	4.36±0.02
	2	4.60±0.03	5.20±0.06	4.70±0.03
	3	6.50±0.06	5.58±0.07	6.80±0.08
TC_1	1	2.70±0.06	2.20±0.05	3.90±0.02
	2	4.60±.0.01	5.10±0.06	6.30±0.04
	3	6.15±0.07	5.50±0.06	7.30±0.08
Flavonoids (g CE eq/100gDW)				
TC		1.85±0.01	0.71±0.04	3.80±0.04
TC_0	1	1.70±0.01	0.69±0.03	1.68±0.02
	2	1.50±0.01	0.78±0.04	1.12±0.05
	3	1.30±0.01	0.68±0.03	0.97±0.06
TC_1	1	1.49±0.04	0.68±0.02	1.30±0.02
	2	1.40±0.07	0.61±0.06	0.87±0.06
	3	1.20±0.04	0.60±0.05	0.86±0.02

Values are mean±SD, n=3

Table. 2: Lipid peroxidation inhibition potential (% inhibition) in PPR 611, SH and SPR 8 tissue cultured (TC) *Mentha* species during 1st month.

Concentration(µg/ml)	Time	50	100	200	500
Ascorbic Acid (positive control)	0.5 h	5.00± 0.02	5.00± 0.02	15.00±0.08	25.00±0.20
	1.0 h	14.81±0.24	18.52±0.30	25.93±0.18	37.03±0.35
	1.5 h	22.22±0.30	30.56±0.31	33.33±0.41	36.11±0.27
***Menha piperita* PPR(611)**	0.5 h	10.00±0.08	20.00±0.13	25.00±0.19	50.00±0.62
	1.0 h	18.51±0.12	22.22±0.11	25.92±0.19	51.85±1.14
	1.5 h	16.66±0.10	16.66±0.12	25.00±0.18	33.33±0.17
***Mentha arvensis* (SH)**	0.5 h	10.00±0.11	15.00±0.19	30.00±0.48	35.00±3.10
	1.0 h	17.40±0.19	18.51±0.30	33.33±1.15	44.44±2.28
	1.5 h	30.55±2.11	36.11±2.17	44.42±2.88	55.55±2.61
***Mentha spicata* (SPR11)**	0.5 h	25.00±1.11	40.00±1.27	40.00±1.15	50.00±2.26
	1.0 h	25.90±1.10	40.74±2.27	40.74±3.12	55.55±3.38
	1.5 h	44.44±1.19	50.00±2.22	55.55±4.48	66.60±3.39

Values are mean±s.d,n=3

Tissue culture samples of all the three species of *Mentha* had higher content of flavonoids as well in comparison to TC_0 and TC_1 samples at either of the growth period. Further, there was gradual decrease in flavonol content in TC derived plants (TC_0 and TC_1) over a growth period of three months in all the *Mentha* species. Like total phenols, the flavonol content of SPR-8 was found to be higher than other species. However in TC derived plants (TC_0 and TC_1) PPR 611 had higher content of flavonols as compared to that of SPR-8 and SH.

Lipid peroxidation inhibition potential

Tables 2-4 present the effect of methanolic extracts of different *Mentha* species on the peroxidation of linoleic acid as a function of incubation period. Ascorbic acid was included as a positive control. The methanolic extracts of *Mentha* species exhibited inhibition of the peroxidation of linoleic acid in a concentration dependent manner. Methanolic extract of tissue cultured *Mentha* plantlets of all the three species were found to be more effective in preventing linoleic acid peroxidation, as compared to standard antioxidant ascorbic acid. Ascorbic acid showed maximum inhibition of 37.03% at a concentration of 500 µg/ml upto 1 h of incubation. Methanolic extract of TC PPR 611 showed maximum inhibition of 51.85% at 1h, whereas TC SH and TC SPR 8 were found more effective, showing maximum inhibition of 55.55% and 66.66% respectively upto 1.5 h of incubation (Table 2). Comparison of lipid peroxidation inhibition potential of tissue culture raised PPR 611, SH and SPR 8 *Mentha* species revealed that SPR 8 to be more effective with maximum inhibition of 66.66% upto 1.5 h (Table2). Tissue Cultured plants of all species were also more effective in comparison to TC derived plants (TC_0 and TC_1) . Reported inhibition of lipid peroxidation may be due to presence of flavonoid which has scavenging potential by reducing free radicals. Most studies on antioxidant compounds in *Lamiaceae* family are directed to phenolic diterpenes, flavonoids and phenolic acids(Kivilompo & Hyotylainen 2007). Crude methanolic extracts of *Mentha* has antioxidant potential and presence of flavonoids and fatty acid methyl esters may be a contributing factor for scavenging potential (Rameshwar *et al.*, 2012) . Moreover, this inhibition

Table. 3: Lipid peroxidation inhibition potential (% inhibition) in PPR 611, SH and SPR 8 tissue culture derived (TC_0) *Mentha* species.

Concentration (µg/ml)	Time (h)								
	Mentha piperita (PPR 611)			*Mentha arvensis* (SH)			*Mentha spicata* (SPR 8)		
	0.5h	1.0h	1.5h	0.5h	1.0h	1.5h	0.5h	1.0h	1.5h
					1st Month				
50	0.00±0.0	7.41±0.64	8.33±0.08	10.00±0.10	11.11±0.12	27.78±1.19	25.00±0.19	25.93±1.18	38.89±2.26
100	5.00±0.01	7.41±0.06	8.33±0.04	10.00±0.12	14.81±0.19	33.33±2.19	30.00±1.18	37.03±2.20	44.44±1.19
200	10.00±0.04	14.81±1.29	22.22±0.42	25.00±1.10	25.92±2.21	38.89±1.14	40.00±2.12	40.74±3.31	50.00±4.11
500	40.00±0.03	48.14±2.23	38.88±0.31	35.00±2.11	40.74±3.19	55.56±3.10	55.00±2.20	59.25±4.28	63.88±3.19
					2nd Month				
50	0.00±0.0	7.41±0.15	11.11±0.11	10.00±0.18	11.11±0.11	30.55±2.21	15.00±0.11	22.22±0.28	33.33±2.29
100	5.26±0.01	7.41±0.18	13.89±0.18	10.00±0.16	14.8±0.18	30.55±2.21	15.00±0.45	33.33±3.95	41.67±3.91
200	15.00±0.12	18.52±1.19	19.44±0.56	15.00±0.12	22.22±1.14	36.11±3.39	20.00±0.65	33.33±2.28	47.22±3.62
500	35.00±1.15	37.04±1.16	22.22±1.19	55.00±0.13	40.74±2.65	50.00±2.29	55.00±0.32	62.96±3.54	62.96±4.18
					3rd Month				
50	0.00±0.0	3.70±0.05	5.56±0.02	5.00±0.03	11.11±0.65	19.44±0.75	10.00±0.01	18.52±1.14	30.56±2.59
100	5.00±0.04	7.41±0.04	11.11±0.35	10.00±0.19	11.11±0.35	19.44±0.54	15.00±1.27	25.92±1.73	38.89±2.61
200	10.00±0.54	1.11±0.02	20.00±0.71	10.00±0.98	14.81±1.18	30.55±1.83	30.00±2.65	37.04±1.65	44.44±2.85
500	17.65±0.87	37.03±2.76	22.22±0.91	30.00±1.15	33.33±2.97	36.11±2.92	45.00±3.61	48.15±2.82	50.00±3.84

Values are mean±s.d,n=3

Table. 4: Lipid peroxidation inhibition potential (% inhibition) in PPR 611, SH and SPR 8 tissue culture derived (TC_1) *Mentha* species.

Concentration (µg/ml)	Time (h)								
	Mentha piperita (PPR 611)			*Mentha arvensis* (SH)			*Mentha spicata* (SPR 8)		
	0.5h	1.0h	1.5h	0.5h	1.0h	1.5h	0.5h	1.0h	1.5h
					1st Month				
50	10.00±0.25	25.92±1.63	5.55±0.08	5.00±0.04	18.52±0.64	5.56±0.04	0.00	11.11±1.42	27.77±1.97
100	20.00±0.17	33.33±1.90	11.11±0.71	15.00±0.35	25.93±1.46	11.11±0.56	10.00±0.93	14.81±0.82	41.67±2.75
200	20.00±0.83	33.33±2.83	16.66±1.18	15.00±0.75	29.63±1.72	16.67±0.81	20.00±0.57	29.63±1.58	47.22±2.74
500	30.00±0.42	40.74±1.58	27.87±2.81	30.00±1.98	37.04±2.29	41.66±3.53	50.00±3.82	55.56±3.81	55.56±3.71
					2nd Month				
50	0.00	3.70±0.05	2.77±0.02	0.00	3.70±0.03	16.66±0.82	0.00	7.41±0.05	19.44±0.81
100	5.00±0.42	22.22±1.59	5.56±0.05	10.00±0.73	18.52±0.52	19.44±0.92	0.00	11.11±0.54	22.22±1.10
200	15.00±0.84	29.63±1.94	11.11±0.63	20.00±0.89	22.22±1.64	25.00±1.39	20.00±1.53	29.63±1.93	52.77±3.61
500	25.00±1.86	40.74±2.92	16.67±0.85	30.00±1.97	33.33±2.93	38.88±2.76	50.00±2.86	51.85±2.83	41.67±2.82
					3rd Month				
50	0.00	3.70±0.05	2.78±0.02	0.00	3.70±0.05	8.33±0.02	0.00	7.41±0.02	16.67±1.93
100	0.00	14.81±0.12	2.78±0.05	10.00±0.04	14.81±0.08	11.11±0.09	5.00±0.04	7.41±0.05	19.44±1.16
200	10.00±0.04	25.93±1.61	8.33±0.26	15.00±0.06	18.51±0.02	16.67±0.02	15.00±0.08	25.93±1.94	30.55±2.69
500	20.00±0.92	37.03±1.18	8.33±0.23	25.00±1.96	25.93±1.94	38.88±1.93	40.00±1.92	44.44±2.92	36.11±2.81

Values are mean±s.d,n=3

potential decreased with the development period in TC derived (TC_0 and TC_1) plants. The inhibition potential though decreased over developmental period in tissue culture derived (TC_0 & TC_1) plant but still better in SPR8 (Table4). Being hydrophilic, ascorbic acid may prove less effective antioxidant in oil in water emulsion system. By moving to water phase, the hydrophilic antioxidants become too diluted to adequately protect the oil at oil-water interface. Thus, a poor antioxidant potential of ascorbic acid observed in this system is obvious. On the other hand, the herbal extracts used for in the present study possess some unique structural elements which help them act at an oil-water interface and thus show better activity in linoleic acid system.

The inhibitory activity of clary sage extract (250 µg/ml) towards the peroxidation of linoleic acid through thiocyanate method was reported to be higher than that of α-tocopherol (Gulcin *et al* 2004). In this context, the presence of various flavonoids in herbal extracts might have been involved in inhibition of peroxidation (Ratty *et al.,* 1998). Total phenolic contents or probably some other factors in various fruits and vegetables played major role in the antioxidant potential against linoleic acid peroxidation system (Velioglu *et al.,* 1998).

CONCLUSION

Tissue cultured derived plants were found to have higher content of total phenols. *Mentha spicata* (SPR 8) was found to possess higher content of total phenols compared to the other two species. However, flavonoid content of tissue cultured samples was more compared to tissue culture derived plants in *Mentha arvensis* (SH) and *Mentha spicata (*SPR 8). It was observed that, tissue cultured plants of all the three species were more potent in lipid peroxidation inhibition model compared to tissue cultured derived plants and inhibition potential decreased with development period in all the three species. However, more studies will be needed in comparisons of antioxidant properties of tissue culture and tissue cultured derived plants.

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How to cite this article:

Akash Deep, Pooja Rana, S S Gosal and Giridhar Soni. *In vitro* lipid peroxidation of tissue cultured and tissue culture derived mentha plant. J App Pharm Sci, 2014; 4 (06): 079-083.