Antioxidant and Antibacterial Activities of Extracts from Wild and *in Vitro*-Raised Cultures of *Prunella vulgaris* L.

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ABSTRACT

MeOH, EtOH, CHCl$_3$ and aqueous extracts from the whole plant of wild *Prunella vulgaris*, a Kashmir Himalayan perennial medicinal herb, as well as from *in vitro*-regenerated plants were evaluated and compared for their antioxidant and antimicrobial properties. Antioxidant activity was screened by using various *in vitro* models: scavenging of the free radicals using DPPH, riboflavin photo oxidation, DNA damage, inhibition of lipid oxidation via PMS, FTC and TBA assay. The MeOH and CHCl$_3$ extract from wild and *in vitro*-regenerated plants possessed an almost equal radical scavenging effect. *In vitro* and wild grown plant extracts in different solvent systems were also screened for antimicrobial activity against medically important bacterial strains by the agar well diffusion method. The MeOH extract of both (wild and *in vitro*) plants extracts were almost equally effective against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Kleibsella pneumonae*. Both *in vitro* and wild dried plant extracts showed an almost similar concentration-dependent antioxidant and antimicrobial inhibition. Therefore, the commercial manufacture of active constituents from these improved elite lines would be useful and profitable. The present study provides first evidence that *in vitro* grown *P. vulgaris* has antioxidant and antibacterial activities, suggesting the potential of the tissue culture technique to substitute wild *P. vulgaris* in the pharmaceutical industry.

INTRODUCTION

Prunella vulgaris (Lamiaceae), a Kashmir Himalayan perennial medicinal herb, also known as self-heal, popular in Europe and China, is inching towards extinction due to tremendous medicinal use. In China it has been used as an astringent and an anti-pyretic agent (Pinkas *et al*. 1994). It is used in the treatment of fever, sore throat and ulcers (Markova 1997). In India and China, *P. vulgaris* has been used against pulmonary disease, jaundice and liver inflammations. Moreover, it has been used as a laxative, anticough, antiparasitic, antirheumatic, against vertigo and hemorrhoid as well as for eye and ear diseases (Ahmed *et al*. 2008). The whole herb is used for medicinal purpose (Phytomania French 2000). The organic fraction of *P. vulgaris* was found to exhibit: DPPH scavenging activity, inhibition against *in vitro* human LDL copper mediated oxidation (Psotova *et al*. 2003). The aqueous extract of *P. vulgaris* contains an anti-HIV-1 active compound named Prunellin which is chemically a polysaccharide (Tabba *et al*. 1989). Remarkable anti-HIV-1 activity was also confirmed by Yamasaki *et al*. (1993). The antiviral action of *P. vulgaris* was also reported against the herpes simplex virus type 1 and type 2 (Zheng 1990). The aqueous extract of this herb inhibits anaphylactic shock and immediate-type allergic reactions (Shin *et al*. 2001). It protects rat RBC against haemolysis and kidney and brain homogenates against lipid peroxidation (Liu and Ng 2000). An immune modulator effect of *P. vulgaris* was carried on monocytes (Xuya *et al*. 2005). It contains a high content of rosmarinic acid which makes plant more usable as far as its therapeutic applications are concerned

(Markova *et al*. 1997). The aqueous extract of this herb is recently used in clinical treatment of herpetic keratitis (Xu *et al*. 1999).

The wild sources of *P. vulgaris* will decrease dramatically due to the exhaustive collection for use in pharmaceutical preparations. To conserve the natural sources of *P. vulgaris*, tissue culture is being developed, which might be used as a potential substitute for wild *P. vulgaris* in the pharmaceutical industry. In the present study, therefore the antioxidant and antimicrobial activities of the product of tissue culture of *P. vulgaris* were investigated.

MATERIALS AND METHODS

Antibiotics (Himedia, Mumbai, India), ascorbic acid (SRL, Mumbai, India), calf thymus DNA (SRL), disodium hydrogen phosphate (Loba Chemie, Mumbai, India), TRIS-buffer (SISCO, Mumbai, India), DPPH (HIMEDIA), ferric nitrate (CDH, New Delhi, India), thiobarbituric acid (CDH), ammonium thiocyanate (Qualigens, Mumbai, India), NBT (BDH, Poole, UK) and EDTA (SISCO), sodium dihydrogen monophosphate (Loba Chemie), riboflavin (SISCO), trichloroacetic acid (Suvidnath Lab, Baroda, India), dimethyl sulfoxide (SISCO), linoleic acid (SRL), nutrient agar (CDH) and Mueller-Hinton agar (Micromaster, Mahrashtra, India). All other chemicals used in this study were either of analytical grade or of the highest purity grade available commercially.

Wild plant material

The wild plants of *P. vulgaris* used in this study were identified in the herbarium of department of Botany, University of Kashmir and

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collected from Naranag area of district Ganderbal, Kashmir. The plant material (prior to flowering stage) was shade dried and ground. The ground material (20 g) was extracted using different solvent systems (MeOH, EtOH, CHCl₃ and aqueous (Aq.)) using a Soxhlet extractor. The extract was collected and the solvent was evaporated. The filtrate was concentrated on a hot water bath at 35°C, then dried and weighed.

Tissue culture material

Explants (shoot tips, nodal buds) obtained from wild plants of *P. vulgaris* were cultured on both full and half-strength MS medium (Murashige and Skoog 1962) on different phytohormonal regimes i.e., BAP (5 to 20 μM) and NAA (2.5 to 15 μM). Cultures were kept for incubation under cool fluorescent tubes in a 16-hr photoperiod with light intensity of 21-42 μmol/m²/s¹ at a constant temperature of 25 ± 3°C. Relative humidity between 60 and 70% was maintained. The *in vitro* raised plant material was shade dried and ground. The ground material was extracted using different solvent systems (MeOH, EtOH, CHCl₃ and Aq.). The extract was collected and the solvent was evaporated. The filtrate was concentrated on a hot water bath at 35°C and dried. Then the dried extract was weighed and stored at 4°C in airtight bottles for further studies.

Both types of extracts (four *in vitro* and four wild) were redissolved in 30% DMSO with a concentration of 50 mg of extract per 50 ml of 30% DMSO. Different dilutions were also prepared from it.

Anti-oxidant studies

1. General free radical scavenging - DPPH assay

This is the primary method, in which the stable free radical i.e., DPPH (1, 1-diphenyl-2-picryl hydrazyl) which is purple in color is reduced to di-phenyl-picryl hydrazine (yellow color) based on the efficacy of the antioxidant. The method was done as described by (Kring and Berger 2001). 2 ml reaction mixture was prepared by adding 1 ml DPPH (500 μM) to different volumes (200, 300 and 400 μl) of crude extracts followed by TRIS buffer (100 mM, pH 7.4). The mixture was incubated at room temperature for 30 min. Absorbance of yellow colored complex was read at (517 nm) spectrophotometrically. Ascorbic acid was taken as positive control and reaction mixture without extract as negative control. The percentage inhibition was calculated from the following equation:

$$\%_{inhibition} = \left\{ \frac{control_{absorbance} - testsample_{absorbance}}{control_{absorbance}} \right\} \times 100$$

2. Superoxide anion scavenging – Riboflavin photo-oxidation method

In this method, the photo-oxidation of riboflavin leads to the generation of riboflavin radical which then auto oxidizes and generates superoxide radical. NBT i.e. nitro blue tetrazolium is a dye which is reduced by superoxide radical to diformazan and is detected by change in color of NBT in presence of extract. The method was taken from (Tevfik and Kadir 2008). 1.7 ml reaction mixture was made by adding 300 μl EDTA (0.1 M), 500 μl NBT (1.5 mM), phosphate buffer (0.067 M, pH 8) and 200 μl of wild and *in vitro* plant extract. The tubes were incubated at 37°C for 5-8 min. Finally 200 μl of riboflavin (0.12 mM) was added and then the tubes were kept in sunlight for 10-12 min until color change was observed (purple). The absorbance was then read at 560 nm. The percentage inhibition of superoxide anion generation was calculated using the following equation:

$$\%_{inhibition} = \left\{ \frac{control_{absorbance} - testsample_{absorbance}}{control_{absorbance}} \right\} \times 100$$

3. Hydroxyl radical scavenging – deoxyribose assay

The method used was that of Halliwell and Gutteridge (1981). The highly reactive radical i.e. hydroxyl radical was generated by using ferric nitrate (Fe³⁺), ascorbic acid, 30 mM H₂O₂. Reaction

mixture was made by adding 500 μl of DNA (1 mg/1 ml), 100 μl of different extracts, 100 μl of ascorbic acid (500 mM), 100 μl ferric nitrate (20 mM), 30 μl of H₂O₂ and final volume was made to 1 ml by Tris-HCL buffer (0.001 M, pH 7.5). The mixture was incubated at 37°C for 20 hrs. The reaction was terminated by adding 1 ml TCA (25%) and in case of any precipitation, tubes were centrifuged at 3000 × *g*. To the supernatant 1 ml of TBA (1.68%) was added. Tubes were kept in boiling water bath for 10 min and then cooled in an ice bath followed by centrifugation at 10000 rpm. TBARS (thiobarbituric acid reactive species) formation was estimated at 535 nm by spectrophotometer. The percentage of hydroxyl radical scavenging was estimated using the following equation:

$$\%_{inhibition} = \left\{ \frac{control_{absorbance} - testsample_{absorbance}}{control_{absorbance}} \right\} \times 100$$

4. Ferric thiocyanate (FTC) method

The method was described previously by Kikuzaki and Nakatani (1993). 2 ml of extract (1 mg/1 ml) was mixed with 2.88 ml of linoleic acid (2.51%, v/v in 4 ml of 99.5% (w/v) EtOH), 0.05 M phosphate buffer pH 7.0 (8 ml), and distilled water (3.9 ml) and incubated at 40°C for 96 hrs. To 100, 200 and 300 μl of this solution, 9.7, 9.6, 9.5 ml of 75% (v/v) EtOH was added, respectively followed by 0.1 ml of 30% (w/v) ammonium thiocyanate. Precisely after 3 min, 0.1 ml of 20 mM ferrous chloride in 3.5% (v/v) hydrochloric acid was added to the reaction mixture, the absorbance at 500 nm of the resulting red solution was measured, and it was recorded again every 24 hrs until the day when the absorbance of the control reached the maximum value. Vitamin C was used as positive control. The percentage inhibition of linoleic acid peroxidation was calculated by using the following formula:

$$\%_{inhibition} = \left\{ \frac{control_{absorbance} - testsample_{absorbance}}{control_{absorbance}} \right\} \times 100$$

5. Thiobarbituric acid assay

Thiobarbituric acid was added to the reaction mixture which interacts with malionaldehyde (MDA) (end product of LPO) and TBARS produced was measured spectrophotometrically according to Kishida *et al.* (1993). To 2 ml of the reaction mixture of ferric thiocyanate assay, 2 ml of trichloroacetic acid (20%) and 2 ml thiobarbituric acid (0.67%) was added and kept in boiling water for 10 min. It was cooled under tap water, centrifuged at 3000 rpm for 20 min and the supernatant was read at 500 nm. Reaction mixture without extract was taken as negative control and ascorbic acid as positive control. The percentage inhibition was calculated by using the following formula:

$$\%_{inhibition} = \left\{ \frac{control_{absorbance} - testsample_{absorbance}}{control_{absorbance}} \right\} \times 100$$

6. PMS preparation and lipid peroxidation assay

a) Liver from the freshly sacrificed sheep was perfused in icecold 0.9% (w/v) NaCl followed by removal of extraneous materials. After this it was weighed and minced, the pieces of liver were homogenized with 4 volumes of ice-cold 0.1 M potassium phosphate buffer (pH 7.4) containing 1.15% (w/v) KCl. The homogenate was centrifuged at 6000 rpm for 10 min. The supernatant was collected and further centrifuged at 15,000 rpm for 20 min at 4°C. The supernatant obtained was PMS (post mitochondrial supernatant).

b) Lipid peroxidation was measured as described by Halliwell (1990). Peroxidation was induced by 5 mM FeSO₄ and 500 mM ascorbate. In this assay to 1 ml of supernatant obtained above (PMS), 0.2 ml of ferric nitrate, 0.2 ml of ascorbic acid was added to 100, 150 and 250 μl of plant extracts and total volume was made to 2 ml with phosphate buffer. Then the solutions were incubated at 37°C for 1 hr. The reaction was then stopped by adding 1 ml of TCA (20%) followed by addition of 1 ml of 1.67% TBA. The mixture was then heated at 100°C for 10 to 20 min. After the addition of TCA, precipitation of proteins was removed by centri-

Table 1 Zones of inhibition (mm) of wild and *in-vitro* 10% (w/v) plant extracts of *Prunella vulgaris* L.

Type of strain	Methanol extract			Ethanol extract			Chloroform extract			Aqueous extract			Ant**
	Wild	*In-vitro*	Cn*	Wild	*In-vitro*	Cn*	Wild	*In-vitro*	Cn*	Wild	*In-vitro*	Cn*	
Echerchia coli	20	18	0	25	20	0	13	12	0	0	0	0	CD30=45 NA30=0 C30=10
Proteus vulgaris	0	0	0	0	0	0	10	10	0	0	0	0	C30=10 NA=0
Staphylococcus aureus	30	26	0	23	20	0	30	27	0	0	0	0	Az=35 C=40 NA=18 CD=45
Salmonella typhimurium	10	13	0	0	0	0	15	25	0	0	0	0	CD=15
Kliebsella Pneumonae	18	14	0	17	12	0	14	12	0	0	0	0	CD=0 Az=14

Data represents mean of 3 replicates/culture; 70 µl used in one well
*Control samples (MeOH, EtOH, CHCl₃, and water).
Antibiotics (Ant) used cefadroxyl 30 (CD 30); naldixic acid 30 (NA 30); chloramphenicol 30 (C 30); azithromycin 5 (Az 15)

fugation. Then the absorbance of the MDA-TBA complex in the supernatant was detected at 535 nm. The percentage inhibition of lipid peroxidation was calculated by using the following formula:

$$\%_{inhibition} = \left\{ \frac{control_{absorbance} - testsample_{absorbance}}{control_{absorbance}} \right\} \times 100$$

Antimicrobial studies

To assess the antimicrobial activity the concentration of extracts was 10% (w/v) prepared in the same solvent in which extractions were made. The studies were done according to method of (Lansing *et al*. 2006; Debnath 2008). Five pathogenic bacterial strains were used to find the antibacterial activity of *P. vulgaris* extracts. Certified strains of bacteria i.e. *Escherichia coli*, *Staphylococcus aureus*, *Kleibsella pneumonae*, *Salmonella typhimurium* and *Proteus vulgaris* were obtained from the Microbiology Lab, Sheri Kashmir Institute of Medical Sciences. Various media viz. nutrient agar (*E. coli*, *S. aureus* and *S. typhimurium*), Mueller-Hinton agar (*K. Pneumonae* and *P. vulgaris*) were used for culture maintenance. Stock cultures were maintained on respective agar slants. Subculturing was done once a month to maintain purity and viability. Experiments carried were done when the microbes were in the log phase. Overnight cultures were prepared by transferring a loop-full of stock cultures to tube having media and incubating at 37°C for 24 hrs. These cultures were then used as inocula for culturing pathogenic strains on Petri dishes for the antimicrobial activity using the agar diffusion method. The micro-organisms were used to inoculate different media agar plates; one strain per plate, wells were made on the plates with a sterile cork borer of 4 mm diameter for the different extracts and the plates were incubated at 37°C for 24 hrs. The same procedure was repeated with all extracts and strains as well with commercial antibiotics. Antimicrobial activity of both wild and *in vitro* grown plant extracts was determined by measuring the diameter of the zone of inhibition and the mean values (presented in **Table 1**) were compared with standard antibiotics like ampicillin, cefadroxyl, naldixic acid and chloramphenicol and azithromycin (Himedia). The effect of the extraction solvents alone (without extract) on the growth of micro-organisms was also measured (**Table 1**).

RESULTS AND DISCUSSION

In vitro raised plantlets

Out of number of trials the most successful concentration was BAP (15 µM) that yielded the highest number (30 ± 0.6) of shoots per shoot tip (Rasool *et al*. 2009). MS(x) + NAA (2.5µM) + BAP (15 µM) showed best proliferation potential in nodal bud culture (Rasool *et al*. 2008). By repeated sub culturing a better frequency multiplication rate was created for production of elite plants of *P. vulgaris*. The shoots obtained rooted well on half-strength MS basal medium with a mean of 8 roots per shoot. Plantlets transferred to open lab conditions showed 70% survival.

Antioxidant activity

Deoxy ribose assay: The scavenging of the hydroxyl radicals generated by Fenton's reaction by *P. vulgaris* extract is shown in (**Fig. 1**). It shows that MeOH and CHCl₃ extracts exhibit maximum activity. The activity of both *in-vitro* and wild grown plants are almost in same range. The amount of the extracts used per ml was 100, 50 and 25 µg.

Riboflavin photo-oxidation method: The scavenging of superoxide radicals by the different extracts are shown in **Fig. 2**. MeOH extract exhibits maximum activity in both the wild and *in vitro* grown plants. The amount of the extracts used per ml was 117.6, 58.8 and 29.41 µg. Reference antioxidant used were ascorbic acid and thiourea.

General free radical scavenging - DPPH assay: The general free radical scavenging assay shown in **Fig. 3** illustrates the anti-oxidant activity of EtOH, MeOH and Aq. extracts of both wild and *in vitro* plants. The amount of the extracts used per ml of reaction mixture was 100, 150 and 200 µg. Reference antioxidant used were ascorbic acid and thiourea.

Post mitochondrial supernatant assay: Here PMS and positive control ascorbic acid were used as a model to study peroxidation and inhibition of peroxidation by plant extracts. The amount of extract used was 50, 75 and 125 µg. Results were simply expressed by following the formation of MDA. The effect of *in-vitro* and wild extract of *P. vulgaris* on Fe^{3+}-Ascorbic acid/H_2O_2-mediated PMS lipid peroxidation is shown in **Fig. 4**. In both cases CHCl₃ and MeOH extracts showed maximum activity; *in vitro* CHCl₃ extract had more activity than the wild extract.

Ferric thiocyanate assay: This method evaluates the effect of extracts and reference antioxidant on preventing peroxidation of linoleic acid (**Fig. 5**). MeOH followed by EtOH extract is having high antioxidant activity in both *in-vitro* grown and wild collected plants. The amount of extract used is 30 µg/ml of reaction mixture. Reference antioxidants used were ascorbic acid and thiourea.

Thiobarbituric acid assay: In TBA method, formation of MDA is the basis for evaluating the extent of lipid peroxidation. At low pH and high temperature (100°C), MDA binds TBA to form a red complex. The amount of extract used is 10, 20 and 30 µg. MeOH extract (wild and *in-vitro*) had showed highest antioxidant activity (**Fig. 6**).

The FTC method was used to measure amount of peroxide at the beginning of lipid peroxidation and the TBA method measures free radicals present after peroxide oxidation. The anti-oxidant activity detected with TBA method was higher than that detected with the FTC method. This might suggest that the amount of peroxide in initial stage of lipid peroxidation was less than the amount of peroxide in
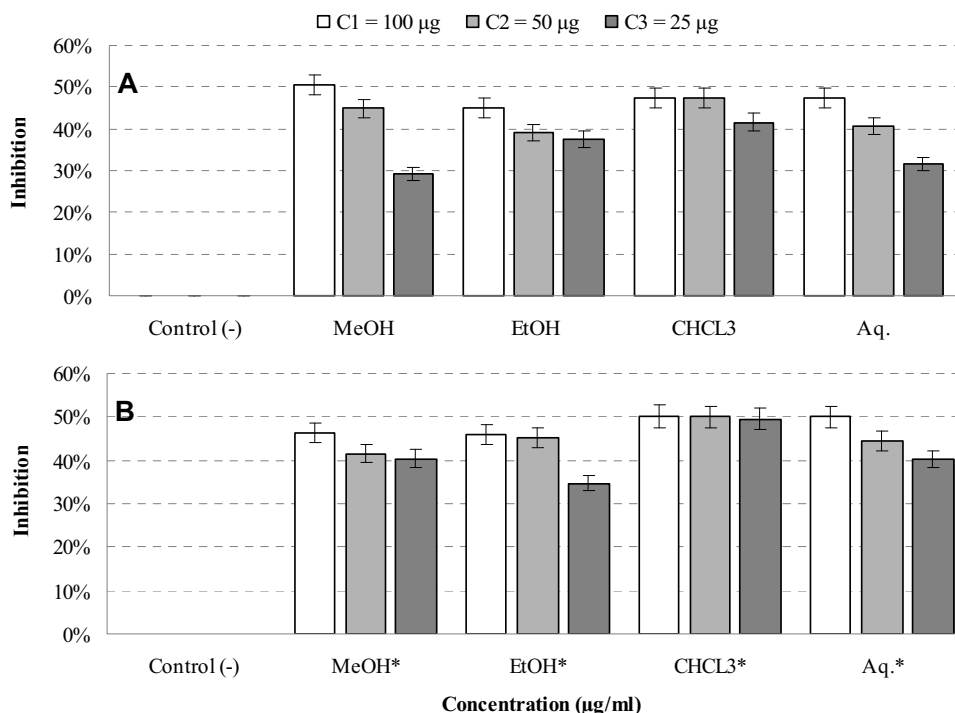
Fig. 1 Deoxyribose assay of wild (A) and *in-vitro* (B) plant extracts of *Prunella vulgaris* L. Values represents the mean ± standard deviation of three independent experiments (*n* = 3).
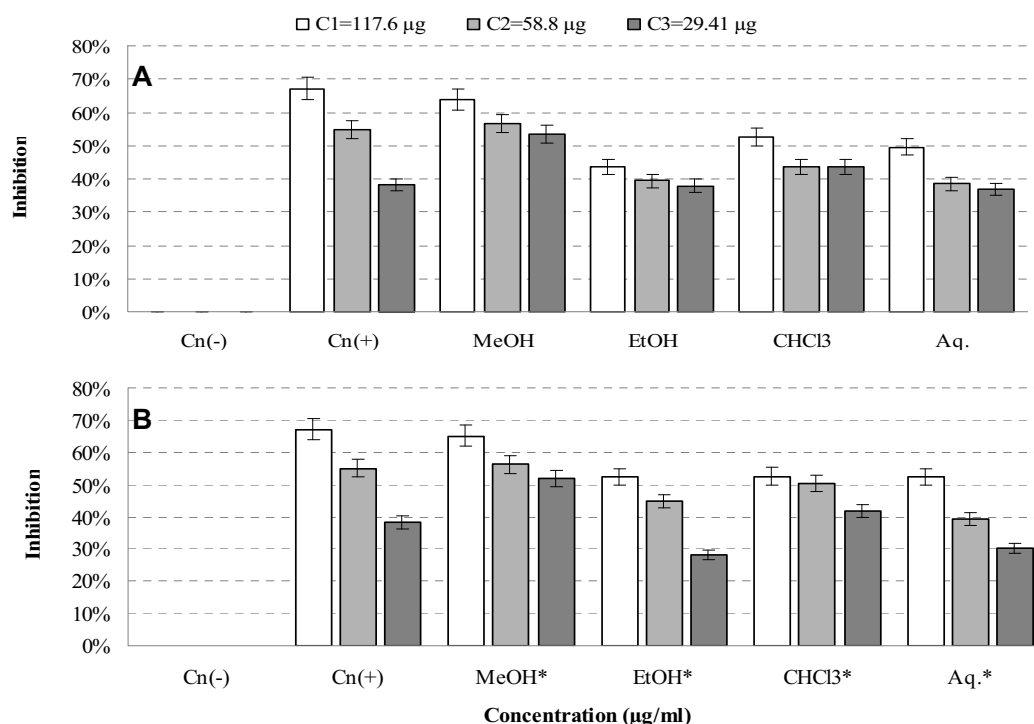
Fig. 2 Riboflavin photo-oxidation method of wild (A) and *in-vitro* (B) plant extracts of *Prunella vulgaris* L. Values represents the mean ± standard deviation of three independent experiments (n = 3).

the second stage. Furthermore secondary product was much more stable for a period of time.

Antibacterial activity

The results depicted in **Table 1** show that secondary metabolites present in *in-vitro* grown plants are in same range and of same type as found in wild plant extracts. The antimicrobial properties of the *in vitro* regenerated plantlets establish a fact, that these can be a source of elite plantlets. In some cases the activity of the *in vitro* extract was even more potent and effective than wild grown plant extract. Aq.

extract does not show any zone of inhibition against the five types of strains. CHCl₃ extract is very effective against all types of strains, particularly *S. aureus* (**Fig. 7**). It seems to be a good solvent for secondary metabolites. Besides CHCl₃, MeOH extract had also proven to be effective antimicrobial agent. Cefadroxyl 30 (CD 30) has proven ineffective against *K. pneumonae* and naldixic acid 30 (NA 30) is ineffective against *P. vulgaris*.

Plants are a tremendous source for the discovery of new products of medicinal value for drug development. Today several distinct chemicals derived from plants are important drugs currently used in one or more countries in the world.
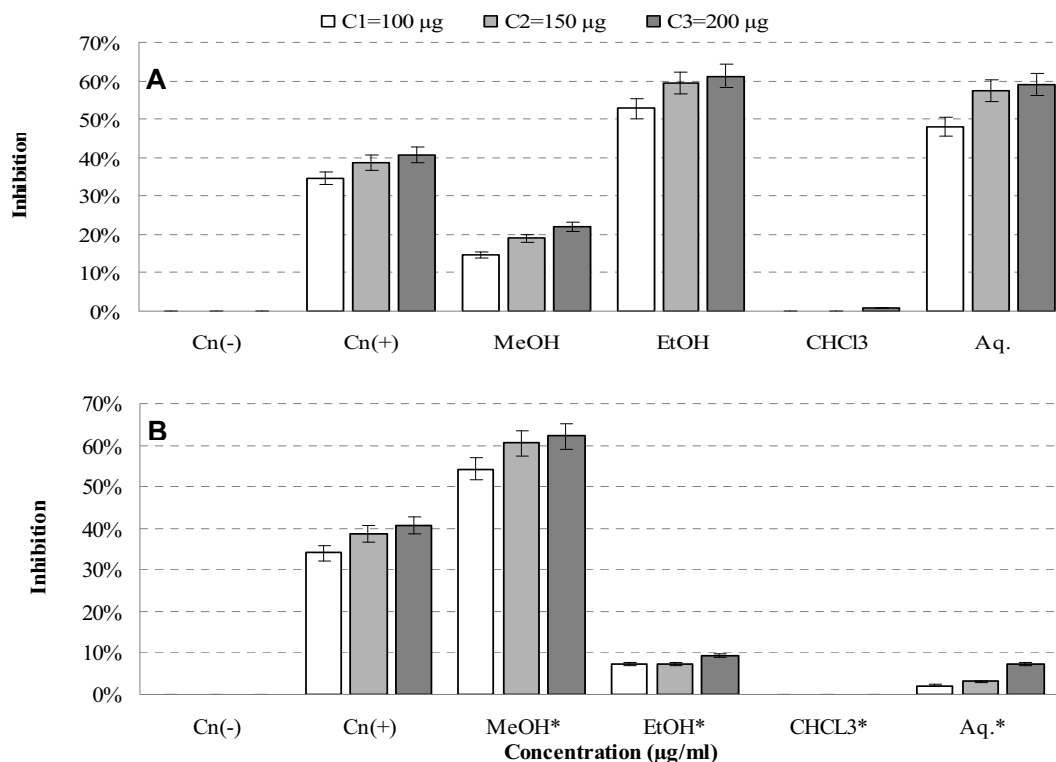
Fig. 3 DPPH assay of wild (A) and *in-vitro* (B) plant extracts of *Prunella vulgaris* L. Values represents the mean ± standard deviation of three independent experiments (n=3).
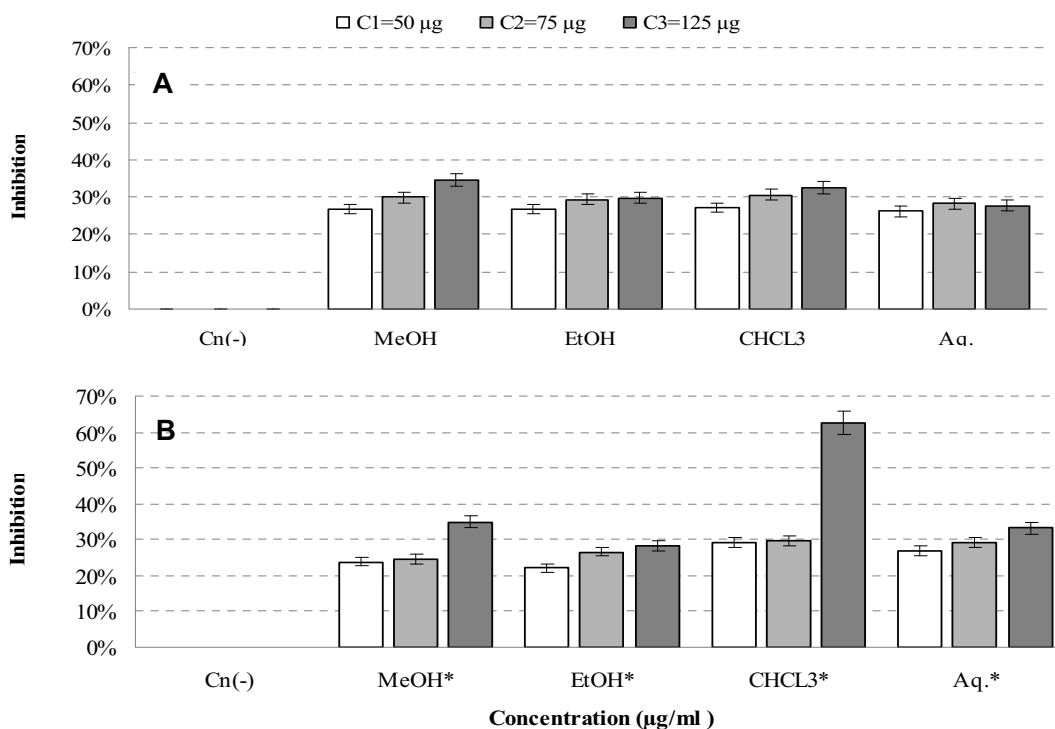
Fig. 4 PMS assay of wild (A) and *in-vitro* (B) plant extracts of *Prunella vulgaris* L. Values represents the mean ± standard deviation of three independent experiments (n = 3).

Many of the drugs sold today are simple synthetic modifications or copies of the naturally obtained substances. The evolving commercial importance of secondary metabolites in recent years has resulted in a great interest in secondary metabolism, particularly in the possibility of altering the production of bioactive plant metabolites by means of tissue culture technology. Plant cell culture technologies were introduced at the end of the 1960's as a possible tool for both studying and producing plant secondary metabolites. Different strategies, using an *in vitro* system, have been extensively studied to improve the production of plant chemicals (Vanisree *et al.* 2004). The medicinal properties are attributed to the primary and secondary metabolites synthesized by the plants (Faizi *et al.* 2003). In our studies we have compared the secondary metabolite production of wild and *in vitro* grown plants of *P. vulgaris* by exploiting their two medicinal attributes, antioxidant nature and antibacterial activity. Results suggest that the plant grown using tissue
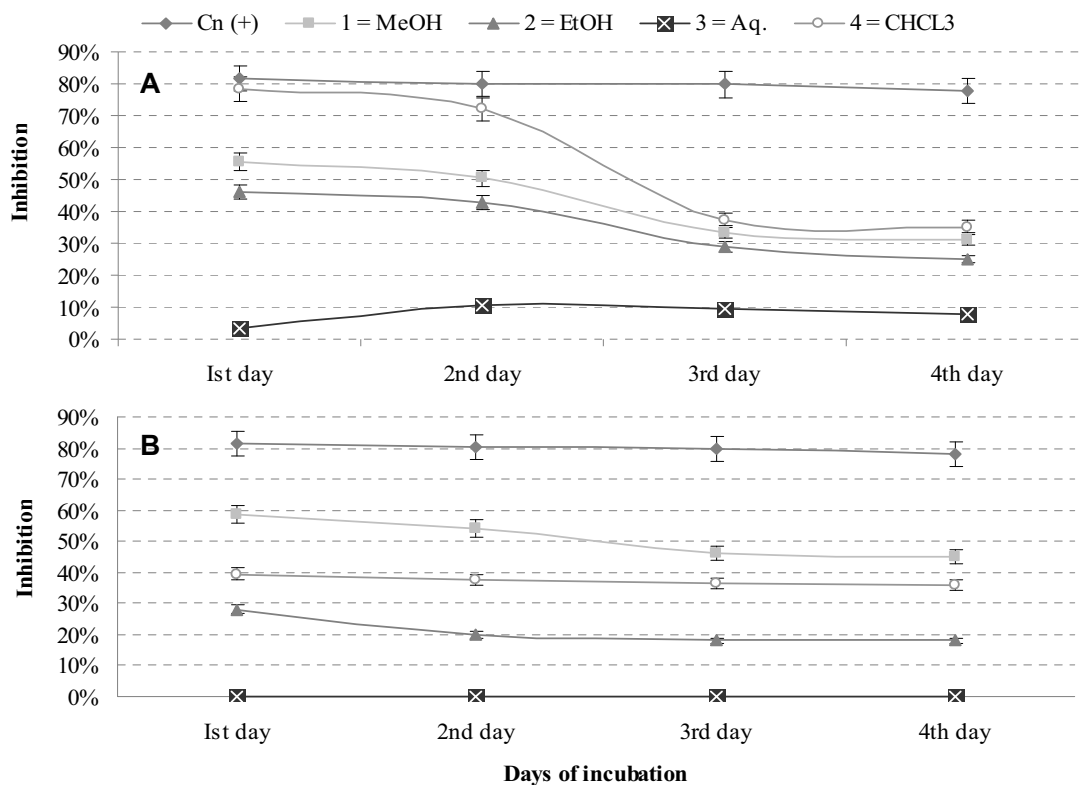
Fig. 5 Ferric thiocyanate assay of wild (A) and *in-vitro* (B) plant extracts of *Prunella vulgaris* L. Values represent the mean ± standard deviation of three independent experiments (n = 3).
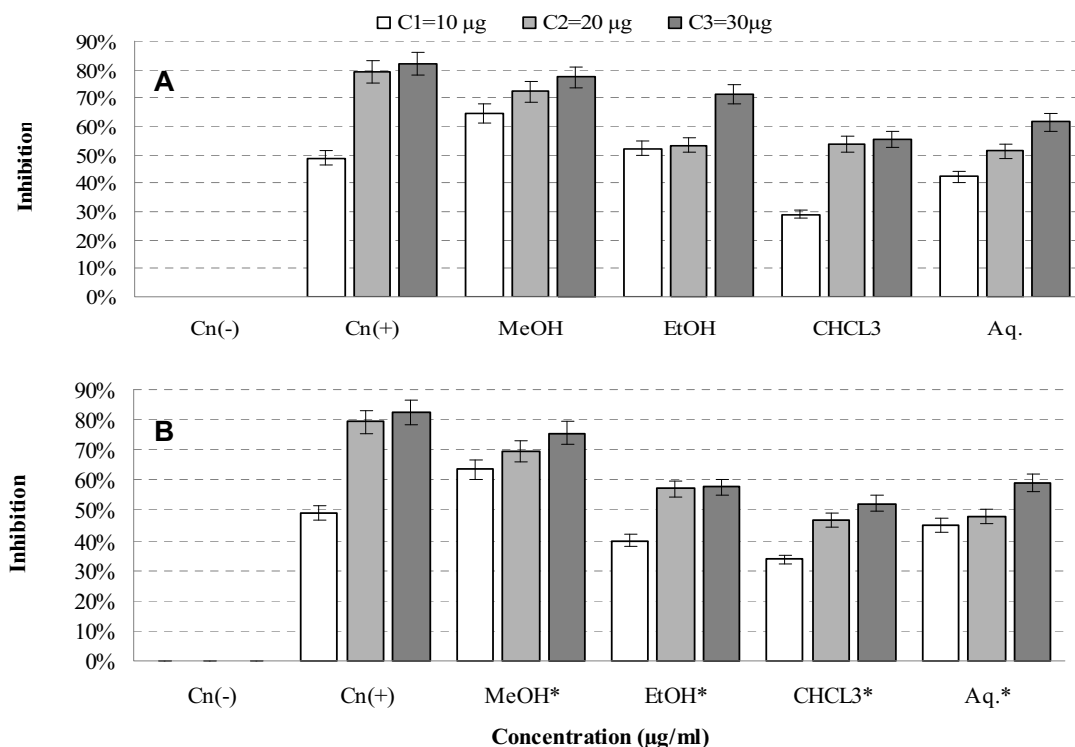

Fig. 6 TBA assay of wild (A) and *in-vitro* (B) plant extracts of *Prunella vulgaris* L. Values represents the mean ± standard deviation of three independent experiments (n = 3).

culture technology do contain the secondary metabolites in almost same range as in wild. Percentage inhibition against free radicals and zone of inhibition values against different bacteria are in same range, also in some cases higher than wild plant confessing that the *in vitro* grown plants are the elite clones of the parental stock and can be substituted against the wild plant so that further exploitation of medicinal plants can be curbed. Such findings are also reported by some authors (Jia *et al*. 2005; Landa *et al*. 2006; Deb-

nath 2008) otherwise overall reports on such type of work is rare. Crude methanol extracts from callus cultures of *Nigella* species were investigated for their antimicrobial activity. Results showed that the extracts of all calli tested exhibited significant antimicrobial activity, especially against *Bacillus cereus*, *S. aureus* and *Staphylococcus epidermidis* (Landa *et al*. 2006). Our results also confirm the antibacterial activity of tissue culture grown plant especially in *S. aureus*. Similar procedure has been outlined for plant regeneration and anti-
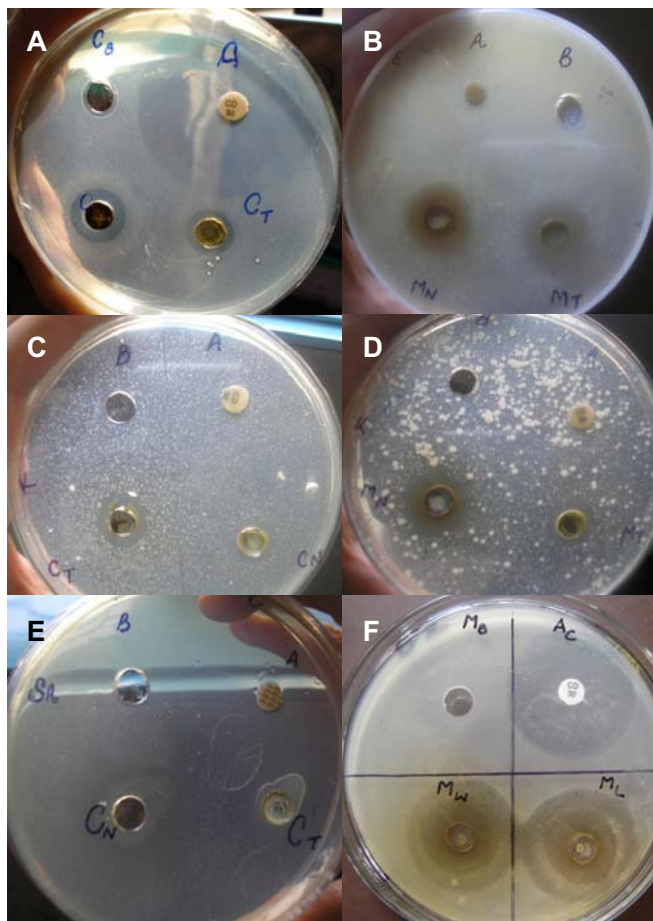
Fig. 7 Zones of inhibitions of *in-vitro* and wild plant extracts of *Prunella vulgaris* L. (A) Effect of CHCl₃ extract against *E. coli*; **(B)** Effect of MeOH extract against *E. coli*; **(C)** Effect of CHCl₃ extract against *K. peumonae*; **(D)** Effect of MeOH extract against *K. peumonae*; **(E)** Effect of CHCl₃ extract against *S. aureus*; **(F)** Effect of MeOH extract against *S. aureus*.

microbial screening of a medicinal herb, *Stevia rebaudiana* Bertoni, through *in vitro* culture of nodal segments with axillary buds on MS medium. *In vitro* and wild grown leaf extracts in different solvent system showed that the chloroform and methanol extract exhibited a concentration dependent antibacterial and antifungal inhibition. Both *in vitro* and wild dried leaf extract showed similar antimicrobial activity, which are in concordance with our results. Therefore, commercial manufacture of active constituents from these improved elite lines would be useful and profitable (Debnath 2008). The tissue culture of *Saussurea involucrata* was studied to determine its anti inflammatory and analgesic activities in experimental animals and study provided evidence that tissue culture raised plant has anti inflammatory and analgesic activities, suggesting the potential of the tissue culture technique to substitute for wild *S. involucrata* in the pharmaceutical industry (Jia *et al*. 2005). Same observations are recorded in this study which indicates the antioxidant and antibacterial activities of *in vitro*-cultured *P. vulgaris*. The sodium dodecyl sulphate polyacrylamide gel electrophoresis protein profile of *in vitro* grown and wild plants of *Clitoria ternatea* was same between regenerated and naturally growing shoots. Total soluble protein in aerial part as well as in seeds of *in vitro* regenerated and wild grown plants was almost the same (Shahzad *et al*. 2007). Methanolic and acetone extracts from *Salvia officinalis*, as well as from shoots and roots of *in vitro* regenerated plants were evaluated for their antioxidant properties. The methanolic hairy root and roots of regenerated plant extracts possessed the strongest effects on reducing molybdenum. DPPH radical scavenging and protective effect against linoleic acid oxidation was observed

for acetone extracts of shoots obtained from *in vitro* culture followed by the extracts of shoots of intact plants grown in the field (Grzegorczyk *et al*. 2007). In contrast, our results showed methanol and chloroform extracts to be good antioxidants except in the DPPH assay where results showed variations possibly due to different reaction mechanisms.

CONCLUSIONS

Reports suggest good similarities in medicinal properties between micropropagated plants and wild grown plants of *P. vulgaris* L. Therefore, commercial manufacture of active constituents from these improved elite lines would be useful and profitable without any loss of biodiversity.

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