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Full Length Research Paper

DPPH-scavenging antioxidant potential in regenerated tissues of *Stevia rebaudiana*, *Citrus sinensis* and *Saccharum officinarum*

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DPPH-scavenging antioxidant potential of *in vitro* regenerated tissues of *Stevia rebaudiana*, *Citrus sinensis* and *Saccharum officinarum* was carried out to evaluate and explore new potential sources for natural antioxidants. These species are used in everyday life and produced valuable secondary metabolites that scavenge toxic free radicals. Toxic free radicals can cause different diseases in human body. The ethanol extracts of callus, regenerated shoots and plantlets along with DPPH solution was checked after 10, 20, 30 and 40 min time intervals. The present study revealed that significantly higher activity (87.7%) was observed in callus of *Stevia rebaudiana* followed by regenerated shoots (86.3%) and regenerated plantlets (83.5%), respectively. The antioxidant potential in callus of *C. sinensis* was recorded 51.8% while regenerated shoots and plantlets exhibit 56.4 and 49% activity. Minimum activity was recorded in callus (48.8%) of *S. officinarum* in overall experiment but regenerated shoots exhibit 77.01% activity seconded by plantlets (54.94%). The present study revealed that *in vitro* regenerated tissues of *S. rebaudiana* scavenge and detoxify more DPPH free radicals than *C. sinensis* and *S. officinarum* (*S. rebaudiana* > *C. sinensis* ≥ ≤ *S. officinarum*).

Key words: *Stevia rebaudiana*, *Citrus sinensis*, *Saccharum officinarum*, antioxidant.

INTRODUCTION

The active constituents of plant tissues are mainly its secondary metabolites. These metabolites are naturally produced during different plant growth phases. Natural antioxidants of plants origin are known to exhibit a wide range of biological effects, including antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, antithrombotic and vasodilatory activity (Liyana-pathirana and Shahidi, 2006). It has been recorded that free radicals are involved in causing many diseases (Ames et al., 1993). In living bodies unsaturated fatty acids in the biomembranes are attacked by free radicals causing in membrane lipid peroxidation, a decrease in membrane fluidity, loss of enzymes and receptor activity and damage membrane proteins leading to cell inactivation (Dean and Davies, 1993). Free radicals also attack DNA and cause mutation leading to cancer. For these reasons natural antioxidants are of interest for the treatment of many kinds of cellular degeneration (Tutour, 1990). Restriction on the use of synthetic antioxidants is being imposed, because of their carcinogenicity (Bronen, 1975). As resources of natural antioxidants much attention has been paid to plants (Couladis et al., 2003). Especially, the antioxidants present in edible plants have been considered as food additives (Fukuda et al., 1990). Several methods have been developed to evaluate the

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total antioxidant activity of fruits and medicinal plant tissues. One of the methods is the DPPH assay, which can accommodate a large number of samples in a short period of time and is sensitive enough to detect natural compounds at very low concentrations so it was used in the present study for the primary screening of antioxidants (Evelson et al., 2001). The objective of the current study is to evaluate the DPPH activity of regenerated tissues of three economical and important species to find new potential sources of natural antioxidants.

MATERIALS AND METHODS

Procurement of regenerated plant materials

Callus, *in vitro* regenerated shoots and plantlets of *Stevia rebaudiana*, *Citrus sinensis* and *Saccharum officinarum* were procured from Plant Tissue Culture Laboratory, Biotechnology and Plant Breeding Division, Nuclear Institute for Food and Agriculture, Peshawar Pakistan.

Extracts preparation

Dried callus shoots and plantlets were grinded to get fine powder for extracts preparation. Ethanol extracts was prepared by taking 5.0 g of powdered material in a container along with 50 ml of ethanol and kept for 1 week with periodic shaking. The solution was filtered and the filtrate was collected and the filtrates were pooled. The final extracts were passed through Whatman filter paper No.1. The pooled ethanol extracts were concentrated by rotary vacuum evaporator at 40ºC and the collected extracts was stored at 4ºC in an air tight bottle (Ahmad et al., 2010). The extracts obtained from each part were dissolved in ethanol independently to get stock solutions. The stock solution was prepared by dissolving pure extract of 5 mg in 20 ml of methanol independently.

DPPH free radical scavenging activity

The free radical scavenging activity of ethanol extracts of regenerated tissues was measured in terms of hydrogen donating or radical scavenging ability using the stable radical (1, 1-diphenyl-2-picrylhydrazyl (DPPH) according to the methods of Ahmad et al. (2010). The test extracts were prepared in ethanol therefore the DPPH was also prepared in ethanol. 3.96 mg of DPPH was dissolved in 20 ml of methanol to get stock solution. 1 ml of sample solution was added to 2 ml of DPPH solution separately. These solution mixtures were kept in the dark for 30 min (incubation period) at room temperature. 30 min later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. All tests were carried out in triplicate. Finally the radical scavenging activity was calculated as percentage of DPPH discoloration using the equation:

$$\text{Scavenging DPPH free radical (\%)} = 100 \times (1\text{-AE/AD})$$

Where AE is absorbance of the solution, when extract has been added at a particular level and AD is the absorbance of the DPPH solution with nothing added (blank, without extract).

RESULTS AND DISCUSSION

Antioxidant constituents of plant origin are vital substances that possess the ability to protect the body from damage caused by free radical induced oxidative stress (Ahmad et al., 2010). Regenerated plantlets can accumulate secondary metabolites similar to those found in mother plant. Antioxidant potential in regenerated tissues of *S. rebaudiana*, *C. sinensis* and *S. officinarum* was determined by using DPPHº-free radical (Figure 1). Significantly higher antioxidant potential (89.4%) was observed in callus of *S. rebaudiana* than shoots and plantlets. The antioxidant activity was determined in time dependent manner, the results were taken with consecutive 10 min gap to evaluate and compare maximum activity. Maximum activity recorded for regenerated shoots was 86.3%, while the regenerated plantlets of *S. rebaudiana* exhibit 87.0% activity. Abbasi et al. (2010), observed the same results in *Silybum marianum*. It means that callus of *S. rebaudiana* had significantly higher capacity to detoxify DPPH free radicals than other plant tissues (Figure 2). Ahmad et al. (2010) also observed higher antioxidant activity in regenerated tissues of *Piper nigrum* L. The World Health Organization (WHO) performed a thorough evaluation of recent experimental studies of stevioside and steviols conducted on animals and humans, and concluded that "stevioside and rebaudioside are not genotoxic *in vitro* or *in vivo* and that the genotoxicity of steviol and some of its oxidative derivatives *in vitro* is not expressed as an *in vivo*" (Benford et al., 2006). It has also been reported that ethanol and methanol leaf extracts of *S. rebaudiana* possess maximum antioxidant activity (Shukla, 2009). In the present investigation *Citrus sinensis* and *S. officinarum* exhibit lower activity than *S. rebaudiana* (Figure 4). After different time intervals maximum activity recorded for callus of *C. sinensis* was 51.8%. However in different *in vitro* regenerated tissues, regenerated shoots represent best activity of 56.4%, while 49% activity was observed in regenerated plantlets (Figure 3). Citrus species produce secondary metabolites like ascorbic acid, flavonoids, carotenoids (Johnston and Bowling, 2002), antocianin and acid cinamic derivatives which are responsible for antioxidant activity. Ghasemi et al. (2009) reported the antioxidant activity of 13 commercially available citrus species through DPPH. Similar work on antioxidant activity through DPPH on four citrus species was represented by Ghafar et al. (2010). The antioxidant activity of juices of two citrus species was also reported (Hoyle and Santos, 2010).

Sugarcane extract has displayed a wide range of biological effects including immunostimulation, anti-thrombosis activity, anti-inflammatory activity, vaccine adjuvant, modulation of acetylcholine release (Barocci et al., 1999) and anti-stress effects. In the present investigation of sugarcane tissues extract the best activity of 77.12% was observed in regenerated shoots than callus (48.8%) and plantlets (54.94%). The present data

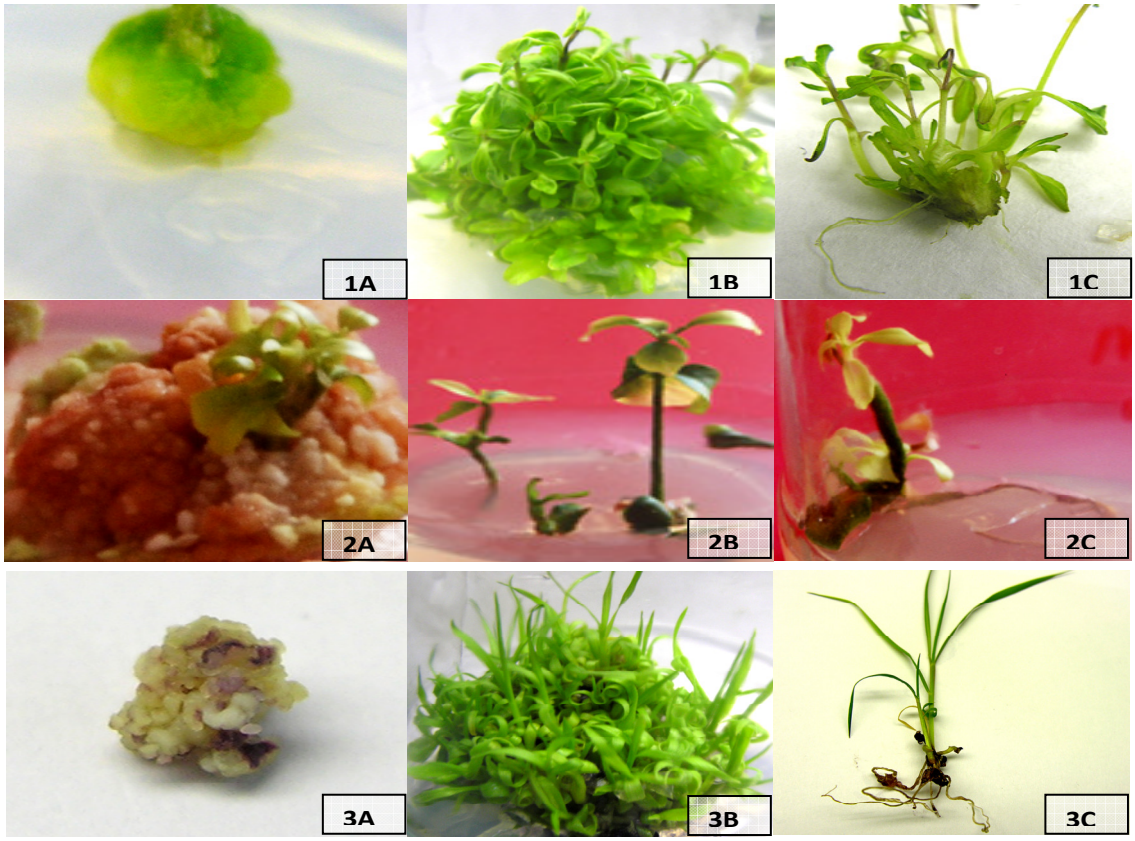

Figure 1. *In vitro* regenerated tissues of *Stevia rebaudiana*, *Citrus sinensis* and *Saccharum officinarum*. Callus, multiple shoots and *in vitro* regenerated plantlets *S. rebaudiana* (1A to C). Callus with shoots, singlet shoot and plantlet of *C. sinensis* (2A to C). Embryogenic callus, shoot multiplication and plantlet of *S. officinarum* (3A to C).

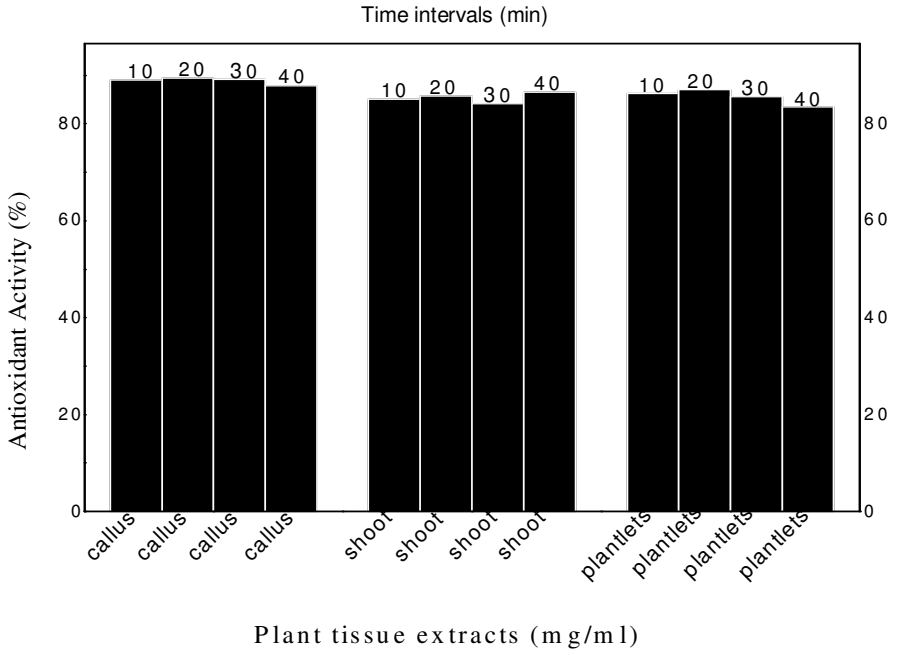

Figure 2. Time dependent antioxidant (free radical scavenging) activity of *in vitro* regenerated tissues of *Stevia rebaudiana*. Values are means of 3 replicates. Each values are not significantly different at P<0.05

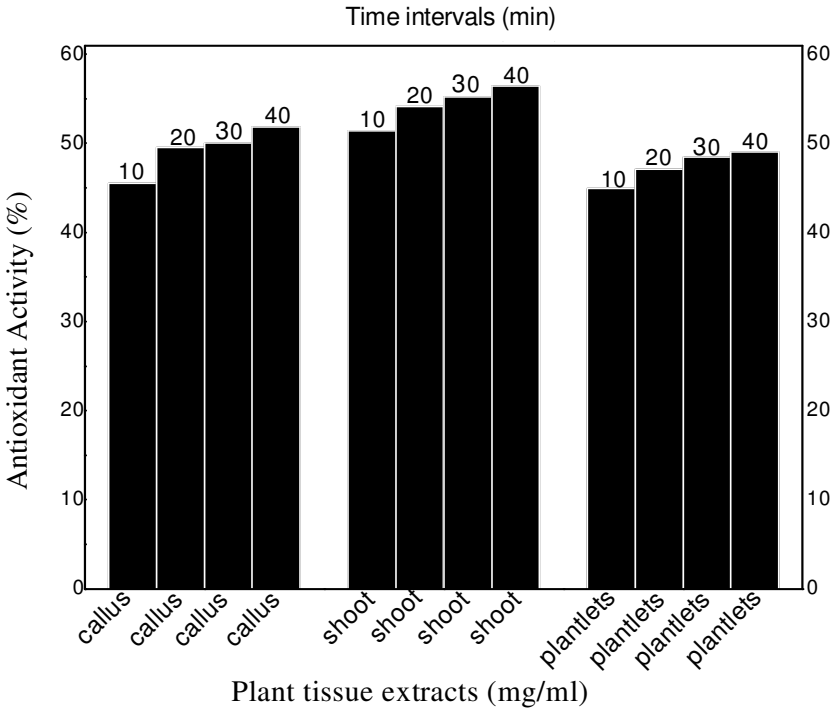

Figure 3. Time dependent antioxidant (free radical scavenging) activity of *in vitro* regenerated tissues of *Citrus sinensis*. Values are means of 3 replicates. Each values are not significantly different at P<0.05.

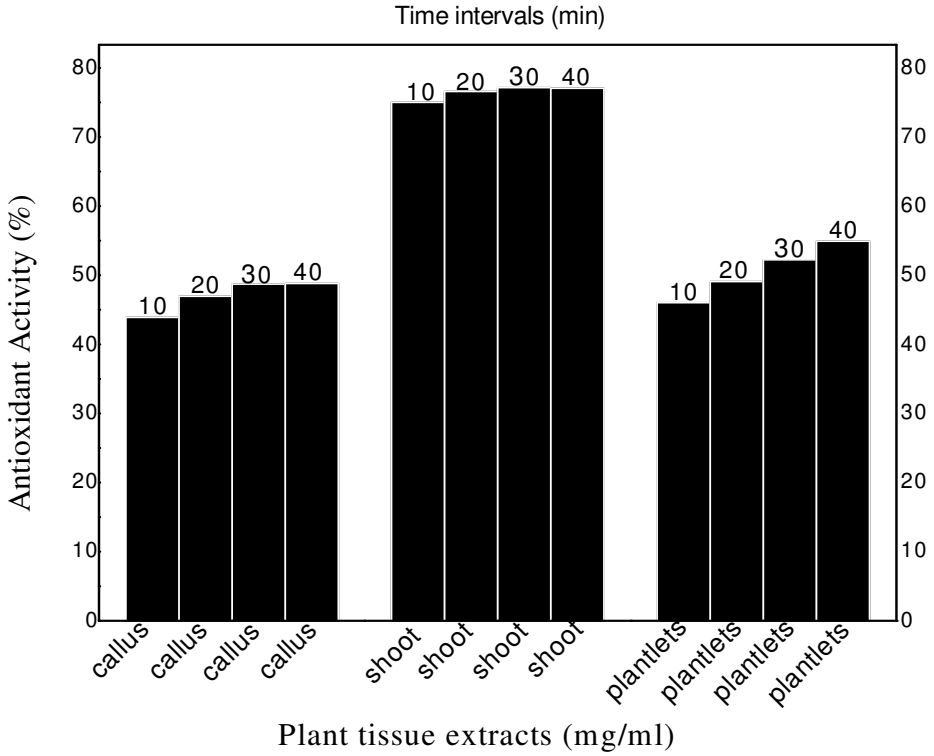

Figure 4. Time dependent antioxidant (free radical scavenging) activity of *in vitro* regenerated tissues of *Saccharum officinarum*. Values are means of 3 replicates. Each values are not significantly different at P<0.05.

are consistent with the findings of many research groups who reported such positive correlation between total phenolics content and antioxidant activity of sugarcane extracts (Zheng and Wang, 2001). The overall objective of the current study was to investigate the antioxidant activity through DPPH of *in vitro* regenerated tissues of *S. rebaudiana*, *C. sinensis* and *S. officinarum*. Comparatively maximum activity was exhibited by all the tissues of *S. rebaudiana*. However due to lower scavenging of DPPH radical's, lower activity was recorded in callus of *C. sinensis* and lower activity was also recorded in callus and plantlets of *S. officinarum*.

REFERENCES

Abbasi BH, Khan MA, Mahmood T, Ahmad M, Chaudhary MF, Khan MA (2010). Shoot regeneration and free-radical scavenging activity in *Silybum marianum* L. Plant Cell Tiss. Org. Cult., 101: 371-376.

Ahmad N, Fazal H, Abbasi BH (2011b). *In vitro* Larvicidal potential and Antioxidative enzymes activities in Ginkgo biloba, *Stevia rebaudiana* and *Parthenium hysterophorous*. Asian Pacific J. Trop. Med., 169-175.

Ahmad N, Fazal H, Abbasi BH, Farooq S (2011a). Efficient free radical scavenging activity of Ginkgo biloba, *Stevia rabaudiana* and *Parthenium hysterophorous* leaves through DPPH. Int. J. Phytomed.,. 2: 231-239.

Ahmad N, Fazal H, Abbasi BH, Rashid M, Mahmood T, Fatima N (2010). Efficient regeneration and antioxidant potential in regenerated-tissues of *Piper nigrum* L. Plant Cell Tiss. Org. Cult., 102: 129-134.

Ahmad N, Fazal H, Ahmad I, Abbasi BH (2011c). Free Radical Scavenging (DPPH) Potential in Nine *Mentha* Species. Toxicol. Ind. Health. Doi., 10.1177/0748233711407238.

Ames BN, Shigenaga MK, Hagen TM (1993). Oxidants, antioxidants and the generative disease of aging. Proc. Nat. Acad. Sci. USA., 90: 7915-7922.

Barocci S, Re L, Capotani C, Vivani C, Ricci M, Rinaldi L (1999). Effects if some extracts on the acetyl-choline release at the mouse neuromuscular joint. Pharmacol. Res., 39: 239-245.

Benford DJ, DiNovi M, Schlatter J (2006). Safety Evaluation of Certain Food Additives: Stevio Glycosides. WHO Food Addit. Ser., 5: 117-144.

Bronen AL (1975). Toxicology and biochemistry of butylated hydroxy anizole and butylated hydroxy toluene. J. Am. Oil Chem. Soc., 52: 59-63.

Couladis M, Tzakou O, Verykokidou E (2003). Screening of some Greek aromatic plants for antioxidant activity. J. Phytother. Res., 17: 194-196.

Dean RT, Davies MJ (1993). Reactive species and their accumulation on radical damaged proteins. Trends Biochem. Sci., 18: 437-441.

Evelson P, Travacio M, Repetto M (2001). Evaluation of total reactive antioxidant potential of tissue homogenates and their cytosols. Arch. Biochem. Biophys., 388: 261-266.

Fukuda Y, Osawa T, Namiki M (1990). Studies on antioxidant substance in sesame seeds. Agric. Biol. Chem., 49: 301-306.

Ghafar MFA, Prasad KN, Weng KK, Ismail A (2010). Flavonoid, hesperidine, total phenolic content and antioxidant activities from *Citrus* species. Afr. J. Biotechnol., 9: 326-330.

Ghasemi K, Ghasemi Y, Ebrahimzadeh MA (2009). Antioxidant activity, phenol and flavonoid contents of 13 *Citrus* species peels and tissues. Pak. J. Pharm. Sci., 22: 277-281.

Hoyle CHV, Santos JH (2010). Cyclic voltammetric analysis of antioxidant activity in citrus fruits from Southeast Asia. Int. Food Res. J., 17: 937-946.

Johnston CS, Bowling DL (2002). Stability of ascorbic acid in commercially available orange juices. J. Am. Diet. Assoc., 102: 525-529.

Liyana PCM, Shahidi F (2006). Antioxidant properties of commercial soft and hard winter wheats (*Triticum aestivum* L.) and their milling fractions. J. Sci. Food Agric., 86: 477-485.

Shukla (2009). *In vitro* antioxidant activity and total phenolics content of ethanolic leaf extract of *Stevia rebaudiana* Bert. Food Chem. Toxicol., 47: 2338-2343.

Tutour BL (1990). Antioxidative activities of algal extracts. Synergistic effect with vitamin E. Phytochemistry, 29: 3759-3765.

Zheng W, Wang SY (2001). Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem., 49: 5165-5170.