Comparative antioxidant activity in sugarcane leave's samples against polyethylene glycol

Syed Rizwan Abbas[1], Syed Dilnawaz Ahmad[3], Syed Mubasher Sabir[2], Sardar Ali Khan[1],Wajid Aziz[5], Asad Hussain Shah[1], Aourang Zeb Rao[1], Muhammad Rehan Abbas[5*], Rizwan Taj Khan[4], Attiya Batool[4].

[1]Department of Plant Breeding and Molecular Genetics, Faculty of Agriculture, University of Poonch, Rawalakot A.J.K. Pakistan
[2]Faculty of Agriculture, University of Poonch, Rawalakot, A.J.K. Pakistan
[3]Vice Chancellor of University of Azad Jammu and Kashmir
[4]Department of Botany, University of Azad Jammu and Kashmir
[5]Department of Computer Science and IT, University of Azad Jammu and Kashmir

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*Address for Author:
Muahmmad Rehan Abbas,
Department of Computer Science and IT, University of Azad Jammu and Kashmir, Muzaffarabad.
E-mail:
drmuhammadrehanabbas@gmail.com

Abstract

Sugarcane is an important crop of Pakistan. There are lacks of water in sugarcane growing area of Pakistan. Therefore, to evaluate the best producing and high potential genotypes against drought and good antioxidant genotype, lunched an experiment of thirteen genotypes of sugarcane. In that experiment DPPH have been used against five treatment of PEG. HSF-240, CP-43-33 and HSF-242 performed highest antioxidant activity. CP-77-400, NSG-45 and CSSG-668 performed lowest antioxidant activity. NSG-555 is highly drought resistant and recommended for those areas where shorting of water exist. However, CP-43-33 is drought susceptible.

Introduction

Sugarcane (*Saccharum officinarum* L.) is a resource not only for sugar, but it has other important uses as animal feed, as the raw material for the production of alcohol, and other derivatives (FNP Consultoria & Com´ercio., 2005). Sugarcane extract often have strong polyphenolic antioxidants such as anthocyanin and tannins (Wang *et al*., 1997). Sugarcane is one of the world's most significant sugar crops', providing over 76% of sugar for human conservation. Recent reports have shed light into several organic properties of sugar cane and its resulting products. Antioxidants are known for their capability to scavenge the free radicals and defend living beings from oxidative harm (Sies, 1996). Plants in addition to animals are constantly exposed to free radicals. Free radicals are extremely unbalanced and reactive molecules and present a terrible challenge to all living systems (Halliwell & Gutteridge, 1997). If left unchecked, they can cause oxidative damage by initiating chain reactions that disorder membranes, denature proteins, splinter DNA and finally participate in cell death, ageing and cancer (Ames, Gold, & Willet, 1995).

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 decline in membrane fluidity, loss of enzymes and receptor activity and harm membrane proteins leading to cell inactivation (Dean and Davies, 1993). Free radicals also hit DNA and cause alteration leading to cancer. For these reasons natural antioxidants are useful for the treatment of many kinds of cellular deterioration (Tutour, 1990). Constraint on the use of synthetic antioxidants is being forced, because of their carcinogenicity (Bronen, 1975). As resources of natural antioxidants much attention has been paid to plants (Couladis et al., 2003). Particularly, the antioxidants present in suitable for eating plants have been considered as food additives (Fukuda et al., 1990). Antioxidants can expire or retard the oxidation procedure by scavenging free radicals. These antioxidants are considered as possible defense agents for decreasing oxidative damage of human body from ROS and retard the development of many chronic diseases as well as lipid peroxidation (Peryor, 1991; Kinsella et al., 1993; Lai et al., 2001). However, more recently the polyphenols have found to be advantageous as strong antioxidants (Vinson et al., 2002; Wang et al., 1997). Flavonoids, flavon-3-ols, flavones, flavanones and flavanonols are supplementary classes of flavonoids that vary in their oxidation state from the anthocyanin. (Ronald E.wrolstad). Current studies have revealed that many flavonoids and associated Polyphenols contribute significantly to the total antioxidant activity of many fruits and vegetable (Luo, Basile & Kenelly 2002; Vinson et al., 1999). Only very newly physiological effects of four types of extracts were described by Japanese researchers (Nagai et al., 2011; Koge et al., 2002) viz., endorsement of resistance against viral and bacteria infections, stimulation of immune response, defense against liver injuries, free radical scavenging activity and development in chickens. It is anticipated that human eat between a few 100mg to 1g flavonoids every day (Halloman and Katan 1999; Pieta 2000). Human studies have bring into being the flavonoids become visible in blood plasma, at pharmacologically active levels, after eating certain foods but do not accumulate in the plasma (Cao, Booth, Sadowski & Prior 1998; Holloman & Katan 1999). Sugarcane juice has been used in the Ayurveda and Unani systems of medicine in India, since time immemorial. Sugarcane extract has displayed a large range of biological special effects including immunostimulation (El-Abasy et al., 2002), anti-thrombosis activity, anti-inflammatory

activity, vaccine adjuvant, modulation of acetylcholine release (Barocci et al., 1999) and anti-stress effects. The current study reports that sugarcane juice has strong antioxidant activity under different experimental conditions. Some investigators (Noa M et al., 2002, Molina V et al., 2005) have consistently proved on in vivo models the antioxidant properties of high molecular weight alcohols enclosed in the wax. Sugar cane also contains phenolic acids, flavonoids and other phenolic compounds (Paton NH and DuongM 1992, McGhie TK 1993), which could account for definite antioxidant activity. Certainly, a direct association between antioxidant activity and phenolics content in vegetable extracts has been convincingly established (Rice-Evans C et al., 1996). In addition, Payet et al. 2005, bring into being antioxidant activity in different samples of cane brown sugars and projected various phenolic acids and flavonoids compounds to be at least to some extent responsible for the observed activity. However, the statistical relationship between phenolics content and antioxidant activity was low. Hence, the authors recommended on the option of other metabolites to be also involved, possibly, those produced during the sugar production.

The objectives of this study are to detect and isolate the best antioxidant genotypes of sugarcane. To classify the drought tolerant genotypes of sugarcane, which could perform best for those areas of Pakistan where drought problems exist.

Materials and Methods

Plant material

Sugarcane (*Saccharum officinarum* L.) has collected from different research station of sugarcane growing areas of Pakistan. The experiment were conducted on thirteen genotypes which are HSF-240, SPF-213, CP-77-400, CP-43-33, HSF-242, NSG-60, NSG-45, CPF-198, Lho 83-153, NSG-555, CSSG-668 and S-2003-US-718 and S-2002-US-133. All genotypes were cultivated in CRBD design in the glasshouse of Faculty of agriculture Rawlakot Pakistan.

Polyethylene glycol treatment

T1	T2	T3	T4	T5
CONTROL	PEG-5%	PEG-7.5%	PEG-10%	PEG-12.5%

Equal concentrations of fertilizer were applied to the plants. Newly growing leaves were collected from the top five leaves of the plant for those experiments.

Preparations of leaves sample concentrations

Treatments	Concentration	DPPH	Ethanol	Samples
T1	25ul/ml	500ul	962.5ul	37.5ul
T2	50ul/ml	500ul	925ul	75ul
T3	100ul/ml	500ul	850ul	150ul
T4	150ul/ml	500ul	775ul	225ul
T4	300ul/ml	500ul	550ul	450ul

Treatment concentrations with samples

Treatments	Leaves sample's concentrations				
T1(CONTROL)	25ul	50ul	100ul	150ul	300ul
T2 (PEG-5%)	25ul	50ul	100ul	150ul	300ul
T3 (PEG-7.5%)	25ul	50ul	100ul	150ul	300ul
T4 (PEG-10%)	25ul	50ul	100ul	150ul	300ul
T5 (PEG-12.5%)	25ul	50ul	100ul	150ul	300ul

DPPH radical-scavenging

Scavenging of the stable radical, DPPH_, was assayed in vitro (Hatano, Kagawa, Yasuhara, & Okuda, 1988). The extract (10– 100 µg) was added to a 0.5 ml solution of DPPH (0.25 mM in 95% ethanol). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm in a spectrophotometer. Percent inhibition was calculated from the control. Vitamin C was used as a standard compound in the DPPH. assay.

Result and Discussions

Sugarcane juice considered a strong and nourishing drink in the tropics and subtropics. Patients suffering from jaundice and liver-related disorders are optimistic to drink sugarcane juice in the conventional system of medicine. DPPH is usually used as a

substracte to evaluate anti-oxidative activity of antioxidant. The method is based on the reduction of the maternal DPPH solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction. The sugarcane juice positively regulates host natural resistance against viral, bacterial and protozoan infections (El-Abasy et al., 2003; El-Abasy et al., 2002), special effects on the levels of macrophages, neutrophils and natural killer cells (Lo et al., 2005). By products of sugar manufacture from sugarcane have shown a wide range of biological behavior (Tanaki, Man, Ohta, Katsuyama, & Chinen, 2003), particularly antioxidative activities, prophylactic behavior and other physiological functions (Takara, Matsui, Wada, Ichiba, & Nakasone, 2002).

Fig 14&15 showed average total antioxidant potential of sugarcane leaves extracts against different PEG concentrations of thirteen genotypes. Among these HSF-240, CP-43-33 and HSF-242 had highest average of five-sample antioxidant activity, which are 70.04%, 69.06% and 68.26%. However, CP-77-400, NSG-45 and CSSG-668 showed average of five-sample lowest antioxidant activities, which are 51.55%, 56.24% and 56.37 all thirteen genotypes performed huge variations in antioxidant activities. In Fig-1 HSF-240, five leaves samples concentrations have been used (25, 50, 100, 150 and 300ul/ml) against five treatments (T1, T2, T3, T4 and T5). HSF-240 showed highest antioxidant activity and increased when sample concentrations was increased e.g. at 25ul/ml, 50ul/ml, 100ul/ml, 150ul/ml and 300ul/ml showed 66.63%, 67.68%, 69.46%, 70.93% and 75.49% respectively. Therefore, this showed that sugarcane is good antioxidant because; as sample concentration increased than oxidant, percentage is increased. When, PEG concentration or osmotic pressure increased on that same samples showed different results, as PEG concentrations increased than antioxidant percentage decreased i.e. at control, PEG-5%, PEG-7.5%, PEG-10% and PEG-12.5% of 25ul/ml sample concentration showed decreasing results which are 66.63%, 60.86%, 59.91%, 52.15% and 36.41%. These results showed that when stress increased than antioxidant activities is gradually decreased. CP-77-400 showed same results at lowest antioxidant activity level (Fig-3) at 25ul/ml sample concentration CP-77-400 showed lowest results at PEG-12.5% concentration, at this stress CP-77-400 didn't survived the cell membrane of this genotype completely ruptured and cell reached to death. In (Fig-4) CP-43-33 showed different results as compared to other genotypes, it showed good antioxidant activity at control or at normal condition but as stress started immediately it lose the survival and showed very bad results. NSG-555 showed excellent results of antioxidant activity at all concentrations (Fig-10). NSG-555 showed resistant against PEG concentrations, the antioxidant percentage decreased in minor readings as compare to other genotypes, so this genotype is highly favorable for those areas of Pakistan where agriculture land having drought problems.

The assays used for checking antioxidant activity of sugarcane leaves extracts at different PEG concentrations showed different levels of antioxidant action. When, Antioxidants are present in low concentration in substances that prevent and/or delay the oxidation of substrates. Non-enzymatic antioxidants react with pro-oxidants and inactivate them. In this redox reaction antioxidants act as reductants. In this context, antioxidant power can be referred to as 'reducing ability'. In the FRAP assay, an easily reducible oxidant, Fe (III) is used in excess. Thus on reduction of the Fe(III)–TPTZ complex by antioxidant, blue colored Fe(II)–TPTZ is formed, which can be measured spectrophotometrically at 595 nm (Pulido, Bravo, & Saura-Calixto, 2000).

Conclusions

In Pakistan, sugarcane occupied huge area of agriculture land in all provinces (Punjab, Sindh, KPK and Baluchistan). CP-43-33 is not suitable for drought stress in lower Punjab and interior Sindh but NSG-555 having good results against drought resistant and recommended for these areas. Overall HSF-240 is the best genotype for all areas of Pakistan, which can contribute a well and up growing production for economy of Pakistan.

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Figure.1. Antioxidant activity of aqueous extract of (HSF-240) leaves for five concentrations. DPPH radical scavenging activates against PEG with different treatments. Values are mean±SD (n=3).

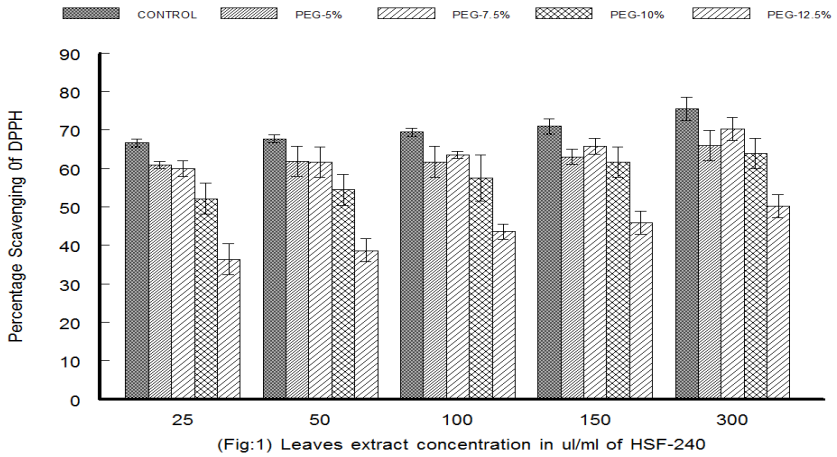

Figure.2. Antioxidant activity of aqueous extract of (SPF-213) leaves for five concentrations. DPPH radical scavenging activates against PEG with different treatments. Values are mean±SD (n=3).

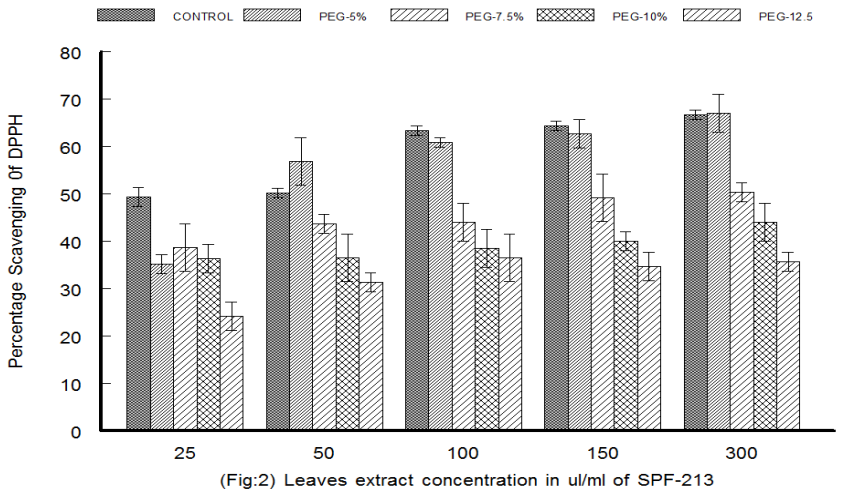

Figure.3. Antioxidant activity of aqueous extract of (CP-77-400) leaves for five concentrations. DPPH radical scavenging activates against PEG with different treatments. Values are mean±SD (n=3).

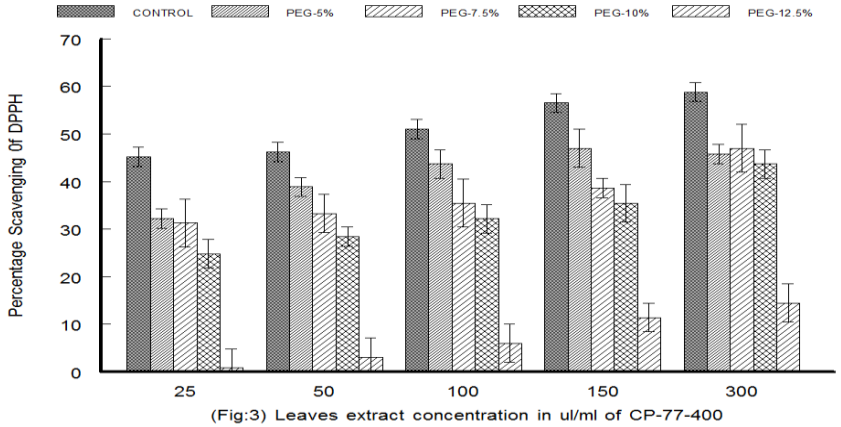

Figure.4. Antioxidant activity of aqueous extract of (CP-43-33) leaves for five concentrations. DPPH radical scavenging activates against PEG with different treatments. Values are mean±SD (n=3).

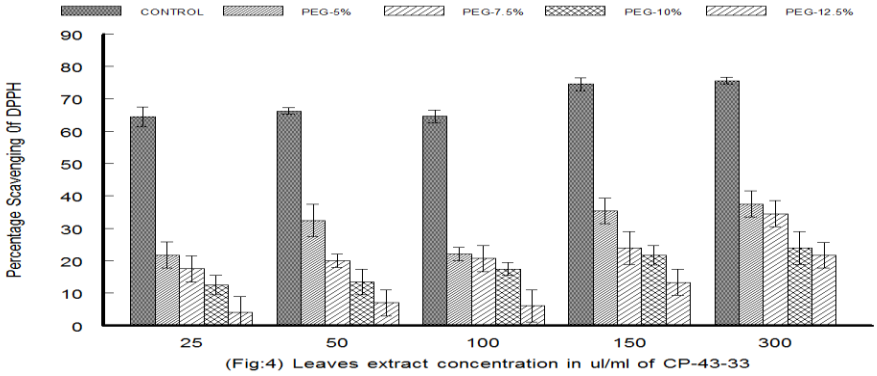

Figure.5. Antioxidant activity of aqueous extract of (HSF-242) leaves for five concentrations. DPPH radical scavenging activates against PEG with different treatments. Values are mean±SD (n=3).

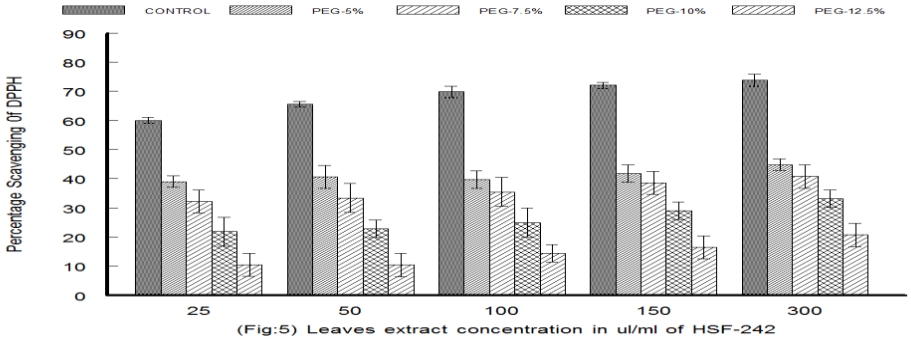

Figure.6. Antioxidant activity of aqueous extract of (NSG-60) leaves for five concentrations. DPPH radical scavenging activates against PEG with different treatments. Values are mean±SD (n=3).

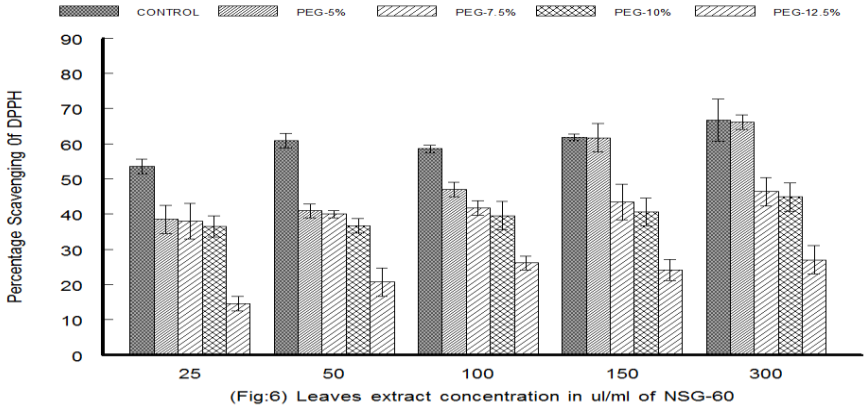

Figure.7. Antioxidant activity of aqueous extract of (NSG-45) leaves for five concentrations. DPPH radical scavenging activates against PEG with different treatments. Values are mean±SD (n=3).

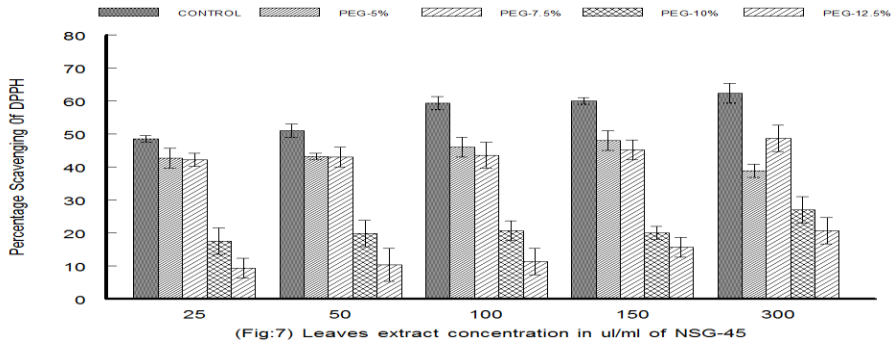

Figure.8. Antioxidant activity of aqueous extract of (CPF-198) leaves for five concentrations. DPPH radical scavenging activates against PEG with different treatments. Values are mean±SD (n=3).

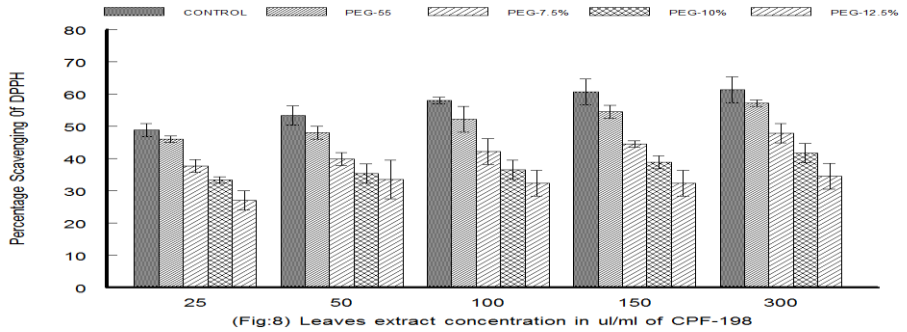

Figure.9. Antioxidant activity of aqueous extract of (Lho 83-153) leaves for five concentrations. DPPH radical scavenging activates against PEG with different treatments. Values are mean±SD (n=3).

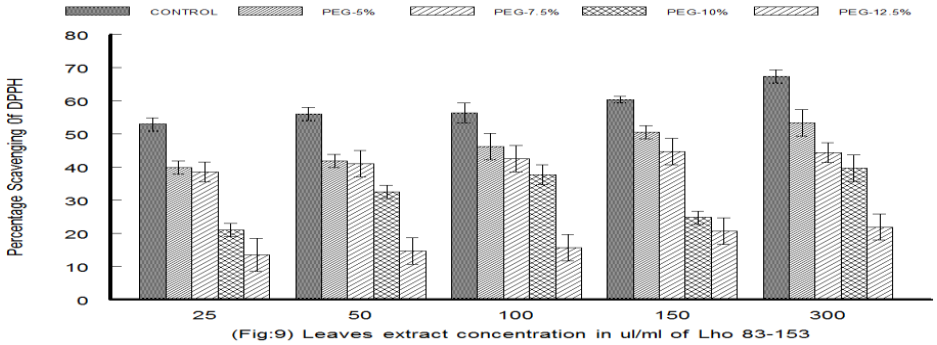

Figure.10. Antioxidant activity of aqueous extract of (NSG-555) leaves for five concentrations. DPPH radical scavenging activates against PEG with different treatments. Values are mean±SD (n=3).

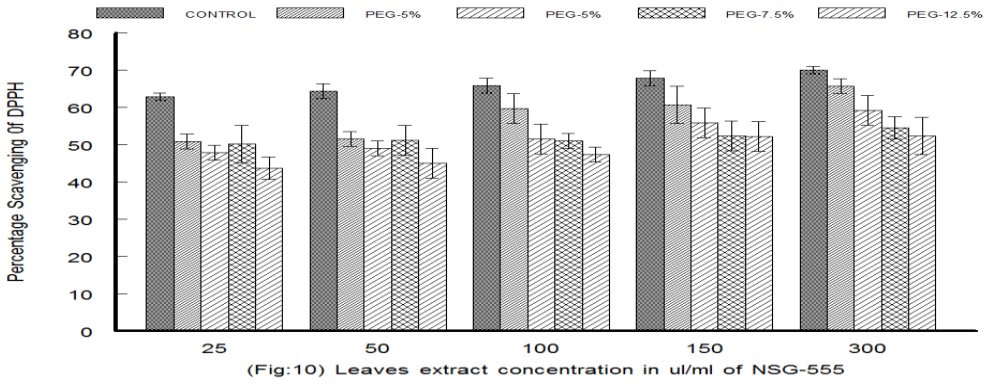

Figure.11. Antioxidant activity of aqueous extract of (CSSG-668) leaves for five concentrations. DPPH radical scavenging activates against PEG with different treatments. Values are mean±SD (n=3).

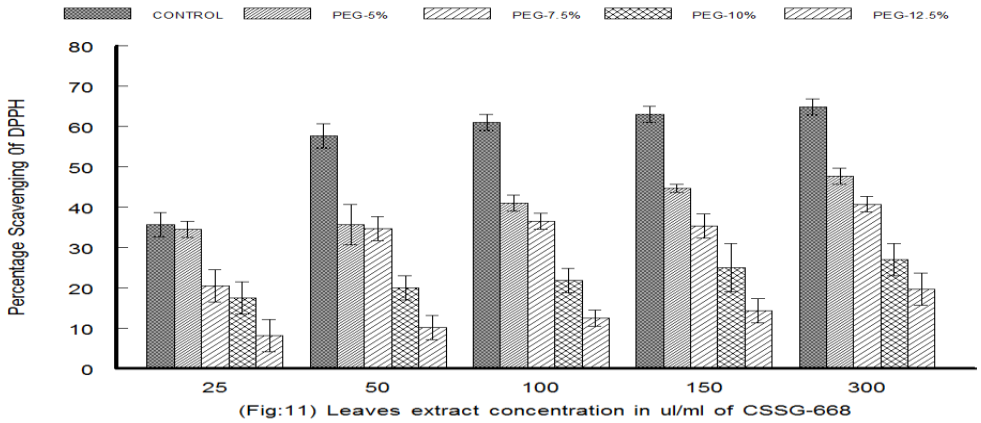

Figure.12. Antioxidant activity of aqueous extract of (S-2003-US-718) leaves for five concentrations. DPPH radical scavenging activates against PEG with different treatments. Values are mean±SD (n=3).

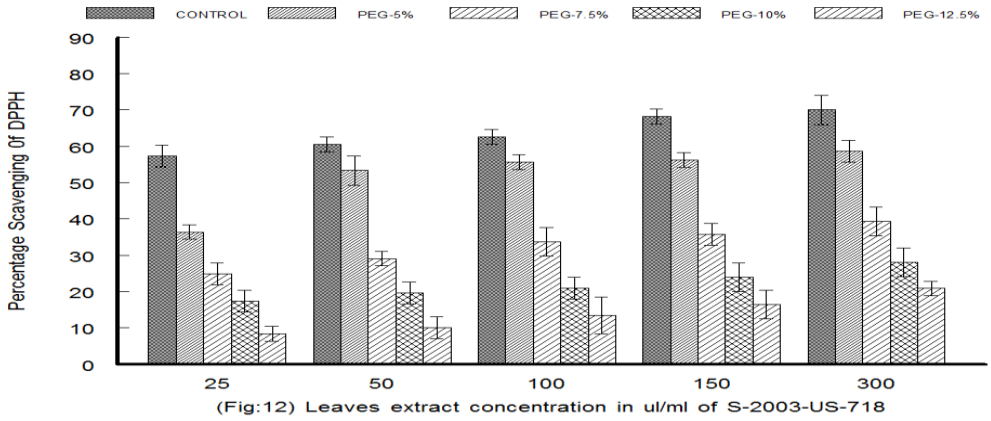

Figure.13. Antioxidant activity of aqueous extract of (S-2002-US-133) leaves for five concentrations. DPPH radical scavenging activates against PEG with different treatments. Values are mean±SD (n=3).

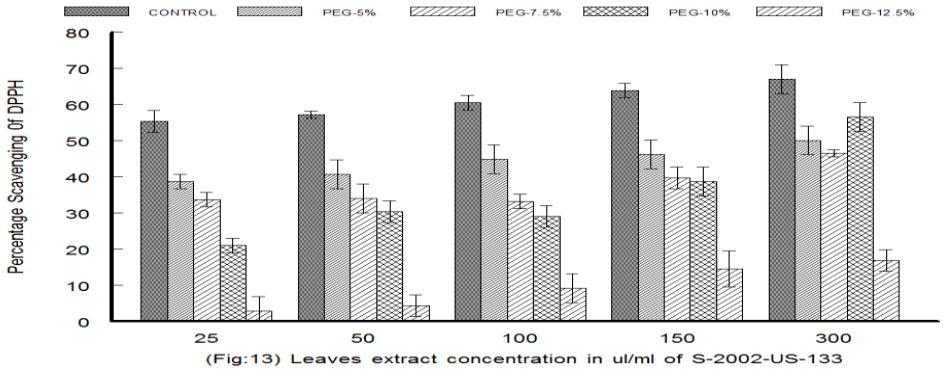

Figure.14. Average antioxidant activity of aqueous extract of sugarcane leaves for five concentrations. DPPH radical scavenging activates against PEG with different treatments. Values are mean±SD (n=3).

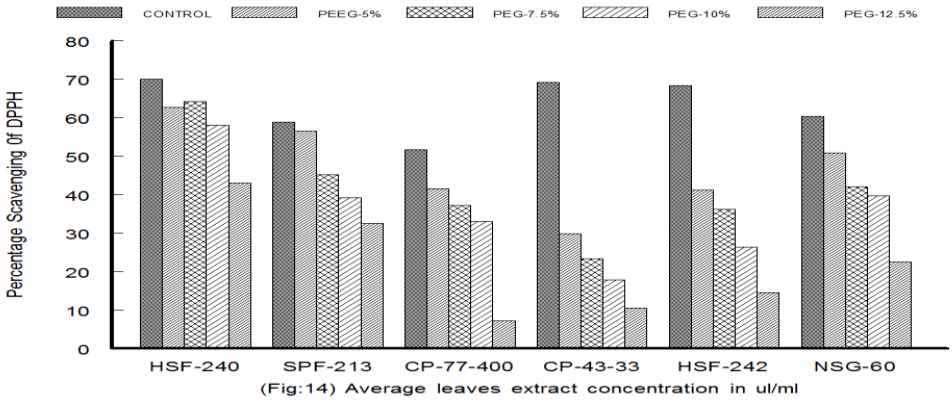

Figure.15. Average antioxidant activity of aqueous extract of sugarcane leaves for five concentrations. DPPH radical scavenging activates against PEG with different treatments. Values are mean±SD (n=3).