RESULT AND DISCUSSION

4. RESULT AND DISCUSSION

Beetroot is a vegetable having a beautiful colour and plenty of nutrients. Many like to eat this especially for its colour. Since the carbohydrates in the beetroot are in the form of sugar particles, this quickly digests and mixes in our blood. Beetroot contains water, protein, fat, minerals, fiber and carbohydrate. Minerals and vitamins like calcium, phosphorus, iron, vitamin C, vitamin A together with vitamin B_1, B_2, B_3, B_6, sodium, potassium, sulfur, chlorine, iodine, copper etc. are found in beetroot. Beetroot is also a good tonic for liver problems. If beetroot leaves are cooked like other greens and consumed, diseases like ulcer and jaundice will be cured.

Beetroot is a good source of inorganic nitrate. Nitrate is believed to be metabolised to nitric oxide and other bioactive nitrogen species and there is strong evidence that regular consumption has a major effect on cardiovascular system including lowering blood pressure, reducing hypertension, inhibition of platelet aggregation and other vasoprotective properties. A protective role against ischemic stroke has also been observed in animal models.

Most research on beetroot and betalains has focused on antioxidant activity. Various studies ranked beetroot among the 10 most potent vegetables in terms of antioxidant capacity (Stintzing & Carle, 2004). A study investigating the radical scavenging capacity of different betalains found that structural features were related to antioxidant potential (Cai *et al.*, 2005).

Hence a study was conducted to compare the antioxidant level of two beetroot varieties like detroit dark red and Ooty. Plate 1 and 2 shows respectively the detroit dark red and Ooty beetroot varieties.

Plate 1: Detroit Dark Red Beetroot

Plate 2: Ooty Beetroot

The results of the study are discussed under the following headings.

4.1 Enzymic antioxidants

4.1.1 Catalase, Peroxidase and Superoxide dismutase

4.1.2 Glutathione-S-transferase and Glutathione reductase

4.1.3 Glutathione peroxidase and Polyphenol oxidase

4.2 Non enzymic antioxidants

4.2.1 Ascorbic acid, α-Tocopherol and Carotenoids

4.2.2 Lycopene, Glutathione and Polyphenol

4.1 Enzymic antioxidants

Reactive oxygen species (ROS) are generated from leakage of electrons onto oxygen from mitochondrial electron transport chain, microsomal cytochrome P_{450} and their electron donating enzymes and other systems (Hansford *et al.*, 1997).

For useful purposes, ROS(eg. O_2, HOCL and H_2O_2) are produced from activated phagocytes (Prakash *et al.*, 1998). Inactivation and removal of ROS depend on reactions involving the antioxidative defense system. The endogenous antioxidant defense includes enzymatic (eg. Superoxide dismutase, catalase, peroxidase etc,) and non enzymatic (eg. Ascorbic acid, α-tocopherol, glutathione etc,) systems (Chatterjee, 1998).

4.1.1 Catalase, Peroxidase and Superoxide dismutase

The levels of Catalase, Peroxidase and Superoxide dismutase of different beetroot varieties are represented in Table III and Figure 1.

Table III: Activities of Catalase, Peroxidase and Superoxide Dismutase in Beetroot Varieties

S.No	Beetroot Varieties	Catalase (U^*/g)	Peroxidase ($U^•$/g)	Superoxide dismutase (U^+/g)
1.	Detroit dark red	0.588	0.445	0.524
2.	Ooty	0.470	0.667	0.474
	SED	0.052	0.140	0.035
	CD	0.154	0.201	0.005

*Amount of enzyme required to decrease the optical density by 0.05 units

• 1µmole of pyrogallol oxidized /min

+ Amount that cause 50% reduction in the extent of NBT oxidation

Catalase

Catalase is produced naturally in all living organisms. It helps the body to breakdown hydrogen peroxide into oxygen and water. It prevents the accumulation of carbon dioxide in blood. Among the two varieties of beetroot, the highest activity of catalase was observed in Detroit dark red variety (0.588U/g). It indicates that the hydrogen peroxide formed by SOD is efficiently removed by catalase. Plant catalases are reported to be very sensitive to environmental conditions (Hartwig *et al.*, 1992). A highest activity of catalase was also reported by Kartikeyan and Rani (2003) in *Piper longum.*

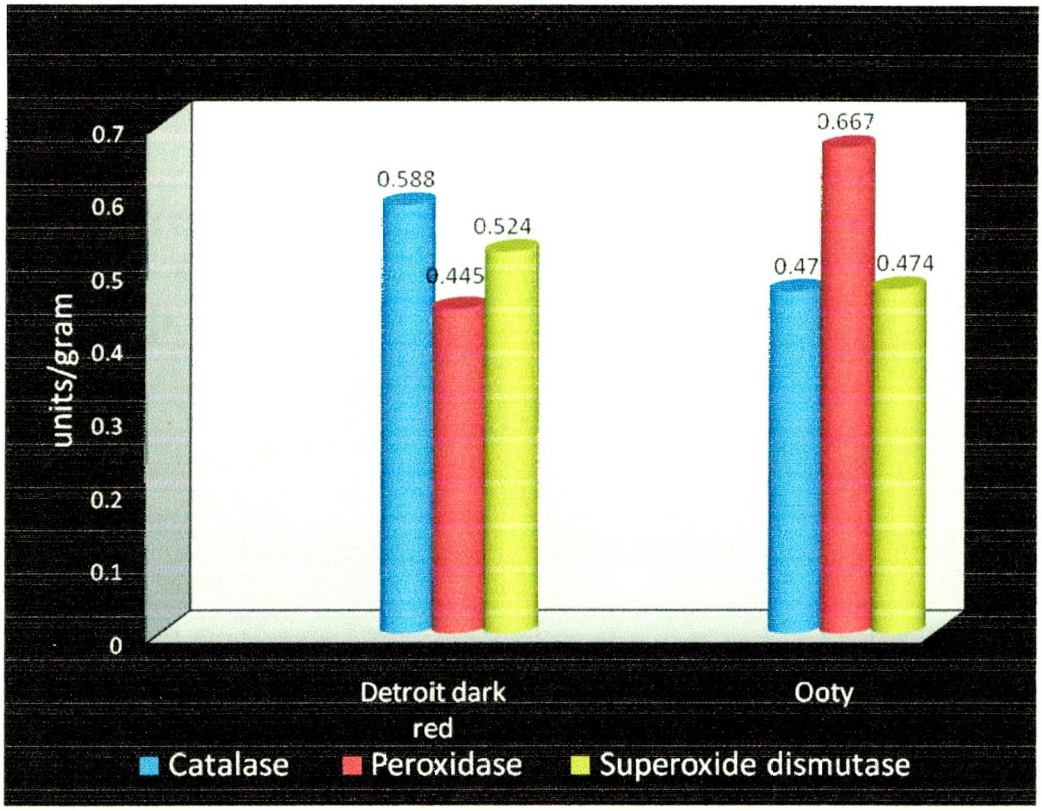

Figure 1: Activities of Catalase, Peroxidase and Superoxide Dismutase in Beetroot varieties

Peroxidase

In plants, peroxidases are involved in many physiological processes, involving responses to biotic and abiotic stress and the biosynthesis of lignin. The peroxidase activity was found to be higher in Ooty variety (0.667U/g) when compared to the detroit dark red variety. Chen *et al*., (1993) showed the relationship between total peroxidase activity and changes in cell wall and membrane integrity under salt stress. Hydrogen peroxide was involved in peroxidase mediated oxidative polymerization, which inturn results in cell wall strengthening, the activation of peroxidase may have a protective role (Velikova *et al.*, 2000).

Gomaa *et al.*, (2008) has reported that the application of Nitrogen, phosphorus and potassium (NPK) fertilizer had enhanced the peroxidase activity in wheat. The activity of peroxidase was increased under potassium chloride stress condition in *Chenopodium album* (L.) (Yao *et al.*, 2010).

Superoxide dismutase

Superoxide dismutase isozymes play a major role in combating oxygen radical mediated toxicity. The highest superoxide dismutase activity was found in Detroit dark red (0.524U/g) and the lowest superoxide dismutase activity (0.474 U/g) was observed in Ooty variety.

Superoxide dismutases are reported widely in plant sources and have free radical scavenging effect of fresh juice. The free radical scavenging activities of vegetables decreased after cooking. Poontariga *et al.*, (2003) had reported that chilling stress induced a significant increase of superoxide dismutase activity. Gaballah and Gomaa (2005) also reported that the SOD activity was found to be increased in fababean varieties by the effect of *Rhizobium* and sodium.

4.1.2 Glutathione-S-transferase and Glutathione reductase

The activity of Glutathione-S-transferase and Glutathione reductase in beetroot varieties were predicted in Table IV and Figure 2 and 3 respectively.

Table IV: Activities of Glutathione-S-transferase and Glutathione reductase

S.No	Beetroot Varieties	Glutathione –S-transferase (U*/g)	Glutathione reductase (U*/g)
1.	Detroit dark red	2.57	0.02
2.	Ooty	1.95	0.015
	SED	0.025	0.005
	CD	0.151	0.011

- μ moles of CDNB –GSH conjugated /min/g sample
- μ moles of GSH consumed/min/g sample

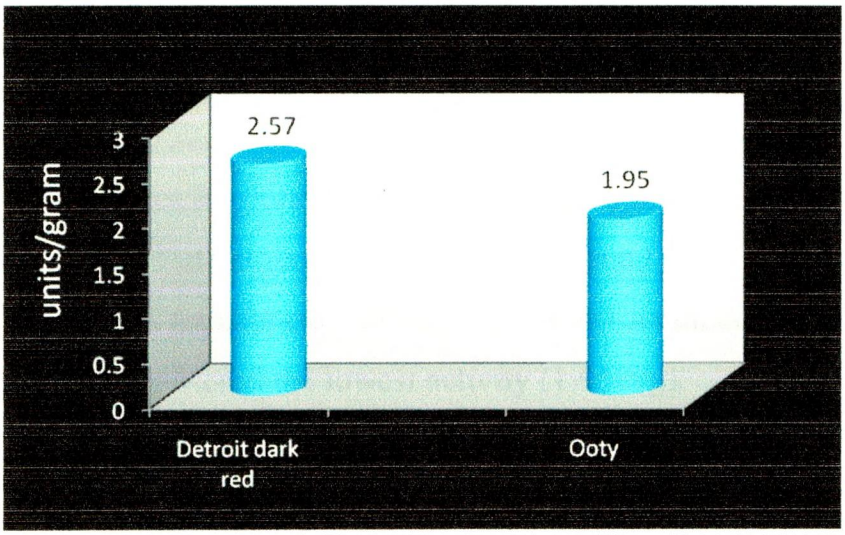

Figure 2: Activity of Glutathione-S-transferase

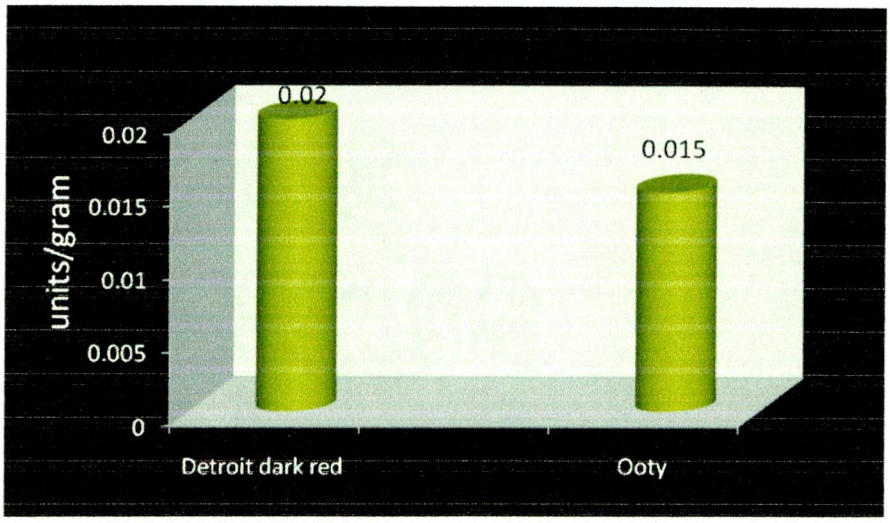

Figure 3: Activity of Glutathione reductase

Glutathione-S-transferase

The role of glutathione-S-transferase is to catalyse the conjugation of electrophilic substrates to glutathione, these enzymes also carry out a range of other functions. The Detroit dark red variety was found to be superior with the higher glutathione-S-transferase activity (2.57U/g) compared to the Ooty variety, which had registered the lowest activity (1.95U/g).

Ali *et al.*, (2005) also reported an increased level of enzymatic antioxidants like glutathione peroxidase and glutathione-S-transferase in the roots and leaves of *Phalaenopsis*, which led to the breakdown of oxidants such as H_2O_2, organic hydroperoxides and lipid hydroperoxide resulting in greater protection against oxidative damage.

Glutathione reductase

The Ooty variety exhibited a very poor glutathione reductase activity. Again the Detroit dark red variety was found to be superior registering highest glutathione reductase activity. It is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds as well as maintaining exogenous antioxidants such as vitamin C and E in their reduced forms (Amit khatria *et al.*, 2009).

4.1.3 Glutathione peroxidase and Polyphenol oxidase

Table V gives the activities of glutathione peroxidase and polyphenol oxidase in the selected beetroot varieties. Figure 4 and 5 represents the same.

Table V: Activities of glutathione peroxidase and Polyphenol oxidase in the selected beetroot varieties

S.No	Beetroot Varieties	Glutathione peroxidase (U*/g)	Polyphenol oxidase (U•/g)
1.	Detroit dark red	0.88	0.003
2.	Ooty	0.65	0.004
	SED	0.012	0.001
	CD	0.022	0.000

* μ moles of GSH consumed /min/g of sample

• Amount of enzymes that transform 1μ mole of dihydrophenol /μ mole of quinone/ min

Glutathione peroxidase

Glutathione peroxidase protects the organism from oxidative damage. It reduces the lipid hydroperoxides into corresponding alcohols and to reduce free hydrogen peroxide to water. Glutathione peroxidase activity was found to be significantly ($P<0.05$) higher in the Detroit dark red (0.88U/g) variety when compared to the Ooty variety.

Glutathione peroxidase is involved in the reduction of H_2O_2 and organic peroxide, a continuous flow of reducing equivalents through glutathione system necessarily has to be balanced by continuous formation of NADPH maintaining the steady state. A study by Karthikeyan and Rani (2003) had shown the highest activity of glutathione peroxidase in *Piper nigrum.*

Polyphenol oxidase

The Polyphenol oxidase activity in the beetroot varieties was to be very low. Both the beetroot varieties were shown comparable activities. Polyphenol oxidase or monophenol mono oxygenase is a tetramer that contains four atoms of copper per molecule and binding sites for 2 aromatic compounds and oxygen.

The enzyme catalyzes the hydroxylation of monophenols to diphenols. They can also further catalyse the oxidation of diphenols to produce quinones (Mayer, 2006). *Solanum berthaultii* has shown high polyphenol oxidase activity (45% of soluble protein) in glandular trichomes (Kowalski *et al.*, 1992).

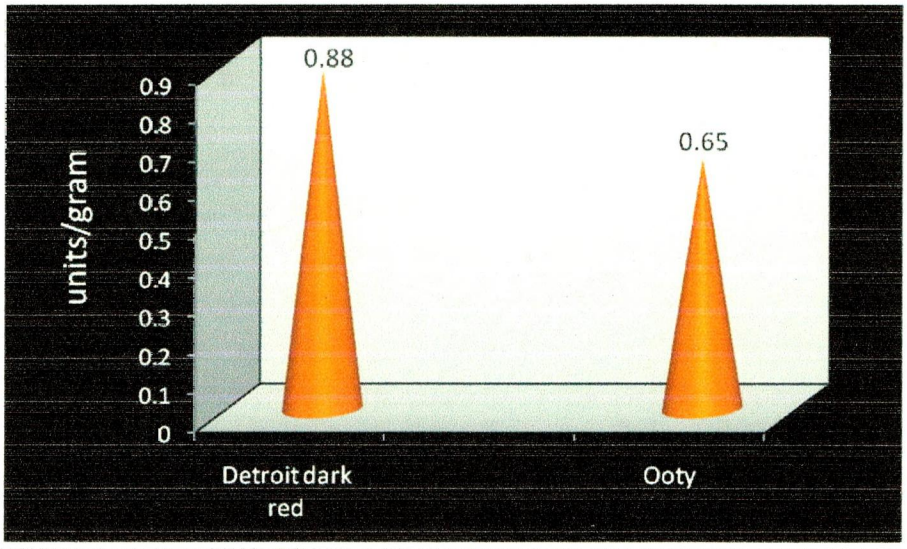

Figure 4: Activity of glutathione peroxidase

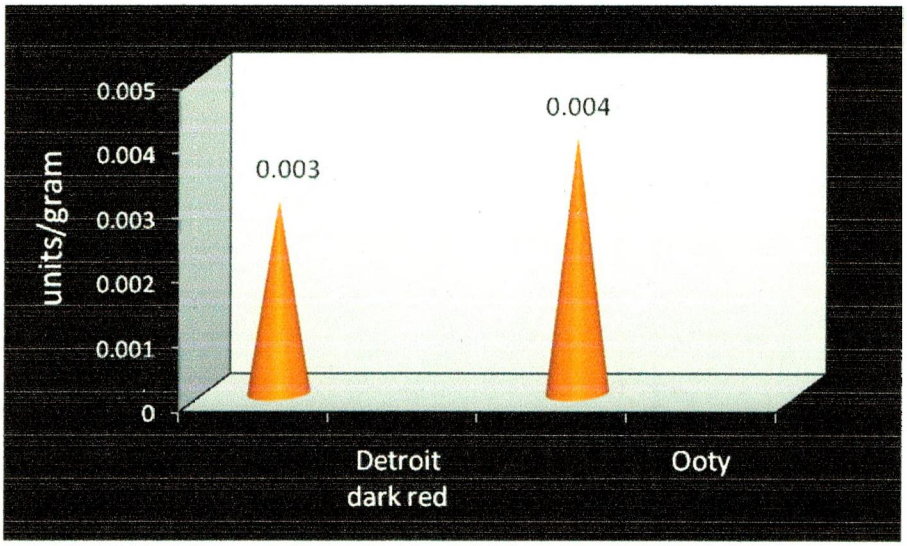

Figure 5: Activity of polyphenol oxidase

4.2 Non enzymic antioxidants

Apart from enzymic antioxidants, spectrum of non-enzymatic antioxidants namely vitamin C, vitamin E, carotenoid and glutathione are important in cellular system in curtailing reactive oxygen species.

4.2.1 Ascorbic acid, α-tocopherol and carotenoid

The levels of ascorbic acid, α-tocopherol and carotenoid were assessed and the results are represented in Table VI and Figure 6 respectively.

Table VI: Activities of Ascorbic Acid, α-Tocopherol and Carotenoid in Beetroot varieties

S.No	Beetroot Varieties	Ascorbic acid (mg/g)	α -Tocopherol (mg/g)	Carotenoid (mg/g)
1.	Detroit dark red	0.35	0.12	0.27
2.	Ooty	0.12	0.13	0.22
	SED	0.015	0.004	0.005
	CD	0.069	0.012	0.040

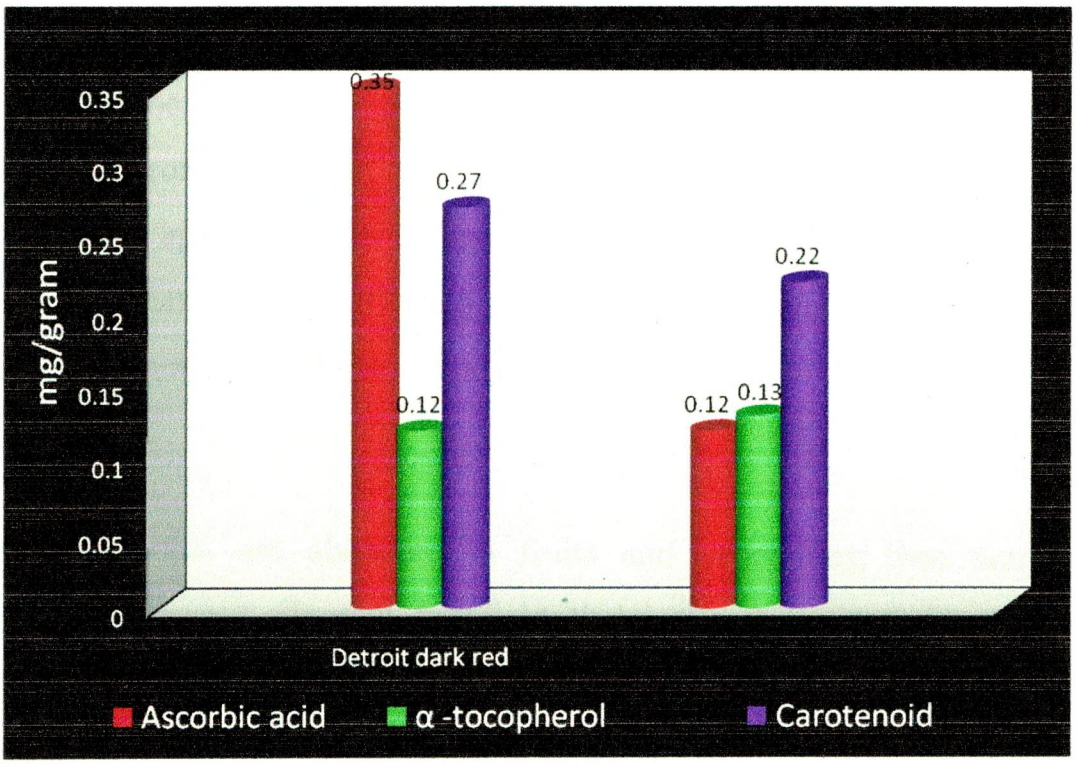

Figure 6: Activities of Ascorbic acid, α-Tocopherol and Carotenoid

Ascorbic acid

Ascorbic acid is reported to be associated with better free radical scavenging activities *in vivo* than the antioxidant enzymes because they are present both intracellular as well as in the extracellular fluid (Chatterjee and Nandi, 1991). It is evident from the table VI and figure 6 that the highest value of ascorbic acid (0.35mg/g) was found in the detroit dark red variety. Bheroo and Yadav (2003) and Chander *et al.*, (2003) were also reported a high content of ascorbic acid in the fruit of beer and plum.

α-Tocopherol

α-Tocopherol is located in chloroplast and thylakoid membranes. It protects the plant from stress tolerance. The α-tocopherol levels in the two beetroot varieties were found to be similar with 0.13mg/g for the Ooty variety and 0.12mg/g for the detroit dark red beetroot variety. The antioxidant properties of tocopherol are the result of its ability to quench both singlet oxygen and peroxides (Fryer, 1992). Within the membrane tocopherol is the only protective agent that can act against the toxic effects of oxygen radicals (Suntress and Shek, 1995). Gunadi (2009) had reported that the potassium fertilizers had increased the α-tocopherol content in potato.

Carotenoid

Carotenoids are abundant in fruits and vegetables. The numerous studies on carotenoid pigment highlight their attractive properties (Britton, 1997). The carotenoid content of the detroit dark red beetroot variety was higher (0.27mg/g) when compared to the Ooty variety (0.22mg/g). Carotenoids have antioxidant activity against singlet oxygen and oxygen free radical. Carotene is rich in yellow and green leafy vegetables, when ingested with minimal fat, enhance serum carotenoids and the total-body vitamin A pool size and can

restore low liver vitamin A concentrations to normal concentrations (Judy *et al.*, 2007).

4.2.2 Lycopene, Glutathione and Polyphenol

The levels of lycopene, glutathione and polyphenol contents of the two beetroot varieties were represented in Table VII. Figure 7 depicts the levels of lycopene and glutathione and figure 8 represents the level of Polyphenol contents of the selected beetroot varieties.

Table VII: Levels of Lycopene, Glutathione and Polyphenol in the selected Beetroot varieties

S.No	Beetroot Varieties	Lycopene (mg/g)	Glutathione (mg/g)	Polyphenol (mg/g)
1.	Detroit dark red	1.30	0.0047	1.06
2.	Ooty	1.06	0.0051	0.99
	SED	0.060	0.002	0.015
	CD	0.178	0.000	0.088

Lycopene

In plants, their function is to absorb light in photosynthesis, protecting plants against photosensitization. The lycopene content of the detroit dark red variety was found to be higher than the Ooty variety. A study by Saini and Singh (1994) had also reported a high content of lycopene in tomato fruits. Lycopene is a member of the carotenoid family and is responsible for the red colour.

Glutathione

The highest glutathione was found in Ooty variety which was followed by Detroit dark red variety. The glutathione is a non enzymic mode of defence against free radicals (Govindrajan *et al.*, 2003). Glutathione reduced the formation of toxic lipid peroxide and hydrogen peroxide in biological systems by acting as a substrate for glutathione peroxidase (Thurnham, 1994). Glutathione react chemically with singlet oxygen, super oxide and hydroxyl radicals and therefore function directly as a free radical scavenger. Younis *et al.* (2009) had reported that the application of phosphate solubilising bacteria and *Azospirillum* had increased the glutathione content in *Lactuca sativa.*

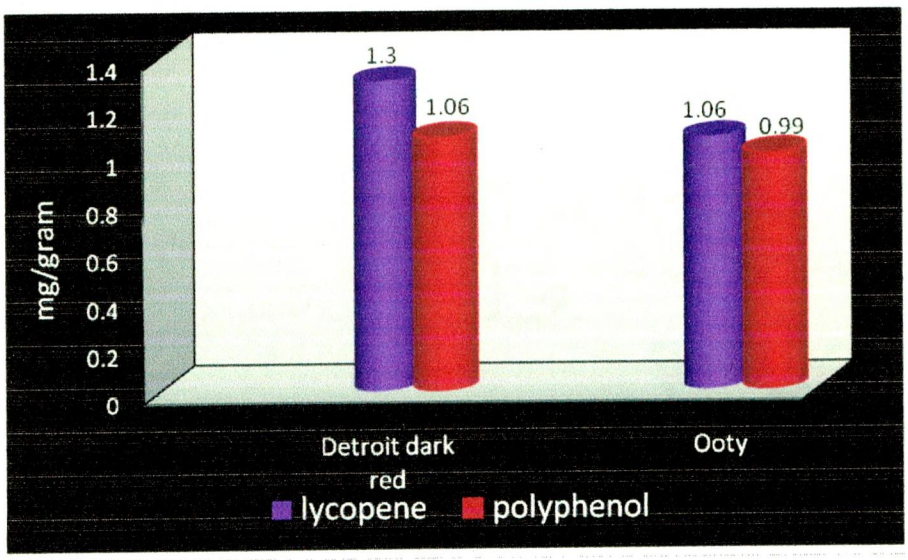

Figure 7: Activities of lycopene and Polyphenol

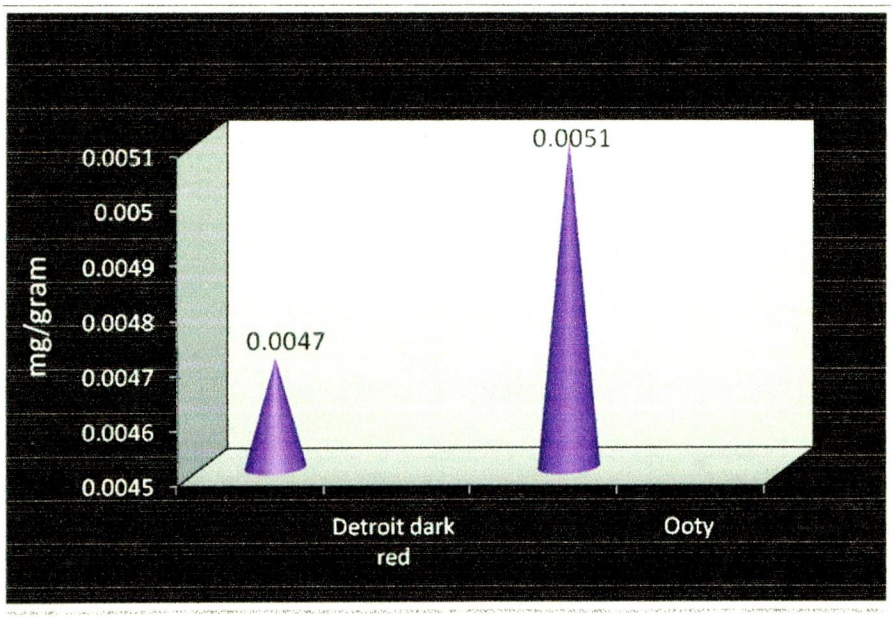

Figure 8: Activity of reduced glutathione

Polyphenol

The highest polyphenol content was shown in the detroit dark red variety which was followed the Ooty variety. The study is in accordance with the results of Yadav and Vijayakumari (2004), that the application of vermicompost had enhanced the polyphenol content of the beetroot. Polyphenols include flavanoids, flavanols, isoflavones, proanthocyanins and tannins. They are widely distributed in fruits like grapes, tea and berries and were used as dietary supplements because of their role in preventing heart disease, cancer and inflammation conditions.

The results of the present study revealed that the level of enzymic and non-enzymic antioxidants were found to be higher in the detroit dark red variety and low level in the Ooty variety. The antioxidants present in beetroot may wipe out the free radical bodies and lower cholesterol level. Beetroot can also act as detoxifier as they remove waste products from our body and increases nutrient supply to the tissues.

Beetroot helps to normalize the pH balance of the body and build the blood and also increases the uptake of oxygen. Beetroot has been used as a supportive therapy in diseases of the liver and fatty liver. It supports and stimulates the liver, gallbladder, kidney and spleen and increases the flow of bile.

Beets have been used successfully in conditions of acne, anemia, cancer, diverticulitis, dysmenorrhea, hepatitis, jaundice, nausea, hypoglycemia, leukemia, poor circulation, diarrhoea, constipation, haemorrhoids and dysentry. Hence by including beetroot regularly in our diet one can prevent several disorders as well as improve the health conditions.