In vitro evaluation of enzymic antioxidants in the seed and leaf samples of *Syzygium cumini* and *Momordica charantia*

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***Abstract*-** Antioxidant properties of plants are well established. Hence this study aims to evaluate the various enzymic antioxidants such as superoxide dismutase(SOD), Catalase (CAT), Peroxidase(POD), Polyphenol oxidase(PPO), Glutathione-S transferase (GST), Glutathione peroxidase (GSH-Px) and Glutathione reductase (GR) in the methanolic extracts of *Syzygium cumini* and *Momordica charantia* seed, leaf, seed mix and leaves mix samples.. The seed extract of *Syzygium cumini* showed the maximum activity of catalase, superoxide dismutase, polyphenol oxidase, Glutathione-S-transferase, Glutathione reductase and registered lowest value in the peroxidase activity. The leaves mixture showed the maximum activity for polyphenol oxidase. Glutathione reductase activity was formed to be highest in the methanolic extract of *Momordica charantia* leaf.

***Index Terms*-** *Syzygium cumini*, *Momordica charantia*, enzymic antioxidants

I. INTRODUCTION

Medicinal plants represent rich source of antimicrobial agents. Based on WHO reports, more than 80% of the world population relies on traditional medicine for their primary healthcare needs (Varahalarao Vadlapudi and Kaladhar, 2012). Medicines from natural products are the growing world-wide interest and act as complementary or alternative medicine to ameliorate many diseases (Thirupathi *et al*., 2008). The inadequate supplies, high cost, side effects with the modern medicines have led to a reawakening of interest in the plants utilization and their products in recent years (Magaji *et al*., 2008).

India stands as the rich source of medicinal plants and their plant products for the development of therapeutic materials (Prabhu *et al*., 2008). Chopde *et al*., (2008) have viewed medicinal herbs as an indispensable part of the traditional medicine practised all over the world for its low costs, easy access and ancestral experience. It is very important to undertake studies relating to screening of the folklore medicinal plants for their proclaimed biological efficacy (Mali *et al*., 2008).

Antioxidants are naturally present in the spices and herbs and thus play an important role in the chemoprevention of diseases and aging (Khalaf *et al*., 2007). Many ailments like inflammation, hyperlipemia, artherosclerosis, osteoporosis, bone resorption, cardiovascular diseases, immune deficiency, central nervous system disorders and cancer have also been effectively treated with the medicinal plants (Talhouk *et al*., 2008). Severe ill-fated diseases like diabetes have been shown to have significant relief with the plant extracts and their products (Sathishsekar and Subramanian, 2005).

Syzygium cumini is being widely used to treat diabetes by the traditional practitioners over many centuries. Ithas been shown to decrease the oxidative stress in diabetes which was greatly increased due to prolonged exposure to hyperglycemia and impairment of oxidant/antioxidant equilibrium(De Bona *et al*., 2010).

Padmaja Chaturvedi (2012) stated that *Momordica charantia* has also used for management of diabetes in the Ayurvedic and Chinese systems of medicine from the time immemorial. Many reports are there on its effects on glucose and lipid levels in diabetic animals and some in clinical trials. The extract of *Momordica charantia* has shown significant decrease in ulcer index, total acidity, free acidity and pepsin content (Samsul Alam *et al*., 2009).

II. MATERIALS AND METHODS

1. Preparation of Plant Extracts:

The air-dried leaves and seeds of *S. cumini* and *M. charantia* were pulverized into powdered form. The dried powders (0.5 g) of the samples were extracted by soaking with methanol (Me-OH) using orbital shaker for 48 hrs at room temperature. Using Whatman No.1 filter paper the extracts were filtered. Residues were re-extracted twice with fresh aliquots of the same solvents. Solvents from the combined extracts were evaporated using a vacuum rotary evaporator and the resulting residues were used for the analyses.

2. Procedure:

Estimation of Catalase

The estimation of catalase was done according to the method described by Luck, 1947. Each sample was homogenized in a blender with M/150 phosphate buffer (assay buffer diluted 10 times) at 4˚C and centrifuged. The sediment was stirred with cold phosphate buffer, allowed to stand in the cold with occasional shaking and then the extraction was repeated. The combined supernatants were used for the assay. Read against a control cuvette containing the enzyme solution as in the experimental cuvette, but containing H_2O_2 free phosphate buffer (M/150). 3 ml of H_2O_2 phosphate buffer was pipetted out into the experimental cuvette and 0.01-0.04 of the sample was added

and mixed with a glass or plastic rod flattened at one end. Note the time (Δt) required for a decrease in absorption from 0.45 to 0.40. The value was used for the calculations. If 't' is more than 60 sec. The measurements have to be repeated with a more concentrated solution of the samples.

Estimation of Peroxidase

The estimation of peroxidase was carried with the method described by Reddy *et al*., 1995. One part of each sample was macerated with five parts (W/V) of 0.1 M phosphate buffer (pH 6.5) in a homogenizer. Centrifuge the homogenate at 500 rpm for 15 min and the supernatants were used as the enzyme source. All procedures were carried out at 5˚C. 3 ml of 0.05 M pyrogallol solution and 0.02 ml of enzyme extract were pipetted out in a test tube and the spectrophotometer was adjusted to read '0' at 430 nm. Then 0.5 ml of 1% H_2O_2 was added in the cuvette and change in absorbance for every 30 seconds up to 3 minutes was recorded.

Determination of Superoxide dismutase (Misra and Fridovich, 1972)

The incubation medium contained a final volume of 3ml, 50mM potassium phosphate buffer (pH 7.8), 45µM methionine, 5.3µM riboflavin, 84µM Nitro Blue Tetrozolium (NBT) and 20mM potassium cyanide. The tubes were placed in an aluminum Foil-lined box maintained at 25^{o}C and equipped with 15W fluorescent lamps. Reduced NBT was measured spectrophotometrically at 600nm after exposure to light for 10 minutes. The maximum reduction was evaluated in the absence of the amount of enzyme giving 50% inhibition of the reduction of NBT.

Determination of Polyphenol oxidase (Esterbauer *et al.*, 1977)

5 g of the plant sample was ground and made up to 20 ml with the medium containing Tris-HCl, sorbitol and NaCl. Then, the homogenate was centrifuged at 1500 rpm for 15 minutes at 4°C. The supernatant was used for the assay. Catechol solution (0.3 ml) and phosphate buffer (2.5 ml) were added into a cuvette and the spectrophotometer was set at 495 nm. The enzyme extracts (0.2 ml) were added and the change in absorbance was recorded for every 30 seconds up to 5 minutes. One unit of catechol oxidase or laccase is defined as the amount of enzyme that transform one µmole of dihydrophenol to one µmole of quinine/minute.

Estimation of Glutathione-S-transferase (Habig *et al.*, 1974)

The enzyme activity was determined by monitoring the change in absorbance at 340nm in a spectrophotometer. 0.1 ml of both substrates (GSH and CDNB were taken in 0.1M phosphate buffer (pH 6.5) at room temperature to make a volume of 2.9 ml. The reaction was started by the addition of 0.1 ml of sample to this mixture; the readings were recorded against distilled water blank for a minimum of three minutes. The complete assay mixture without the sample served as the control to monitor non-specific binding of the substrate. Care was taken to ensure that final concentration of ethanol in the mixture was always less than 4%.

Estimation of Glutathione Peroxidase (Rotruck *et al.*, 1973)

To 2ml of Tris buffer, 0.1ml of sodium azide, 0.2ml of EDTA and 0.5ml of plant extracts were added. 0.2ml of glutathione followed by 0.1ml of hydrogen peroxide were added to the mixture, mixed well and incubated at 37°C for 10 minutes along with a tube containing all the reagents except sample. The reaction was arrested after 10 minutes by the addition of 0.5 ml of 10% TCA. The samples were centrifuged and the supernatant was assayed for glutathione.

The activities are expressed as µg GSH consumed /minute/mg protein.

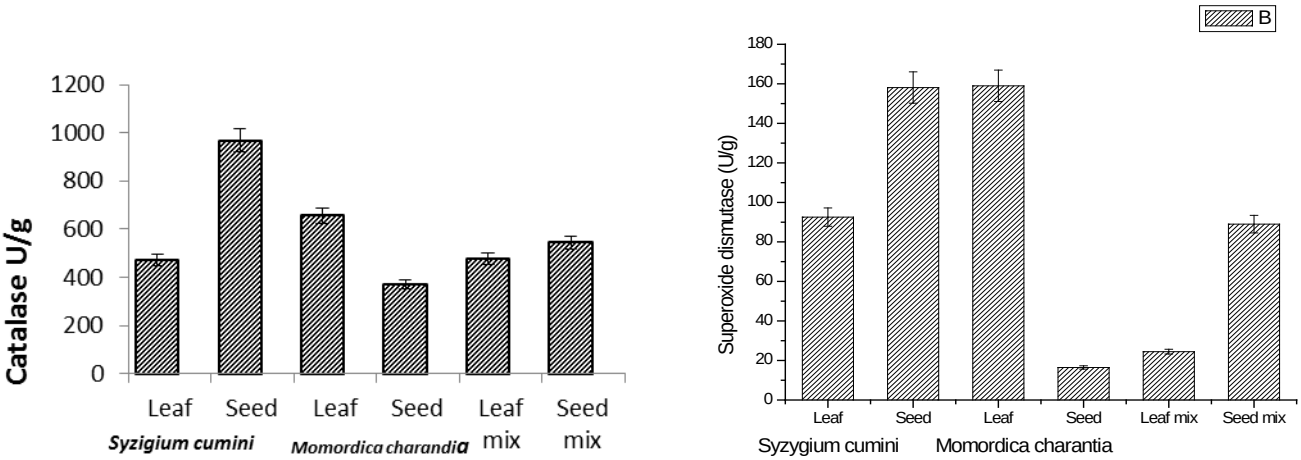
Estimation of Glutathione Reductase

Glutathione Reductase was estimated by following the method proposed by David and Richard, 1983. According to them 20% aqueous extract was prepared in 0.12 M phosphate buffer of pH 7.2, used as the source of enzyme. The assay system contained 1 ml of 0.12M potassium phosphate buffer, 0.1ml of 10mM sodium azide, 0.1 ml of 15mM EDTA, 0.1 ml 0f 6.3mM oxidized glutathione and 0.1ml of enzyme source and water in the final volume of 2 ml. Kept for 3 minutes. The 0.1 ml of NADPH was added. At 340nm the absorbance was recorded at an interval of 15 seconds for 2 to 3 minutes. For each series of measurement controls were done that contained water instead of oxidized glutathione. The enzyme activity was expressed as milli moles of NADPH oxidized/minutes/ g sample.

III. RESULTS AND DISCUSSION

The levels of various antioxidative enzymes were determined and presented in Figures I and II.

Figure 1- Levels of enzymic antioxidants in the seed and leaves samples of *S. cumini* and *M. charantia*



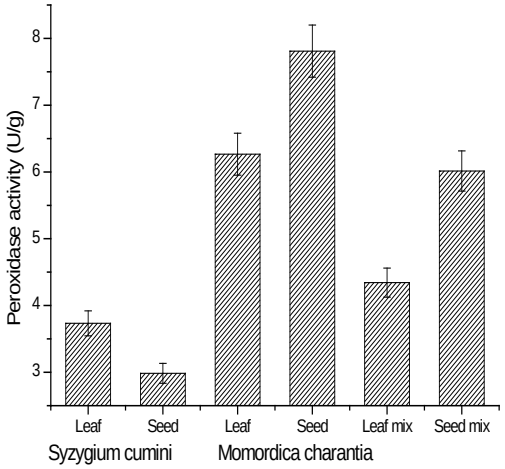
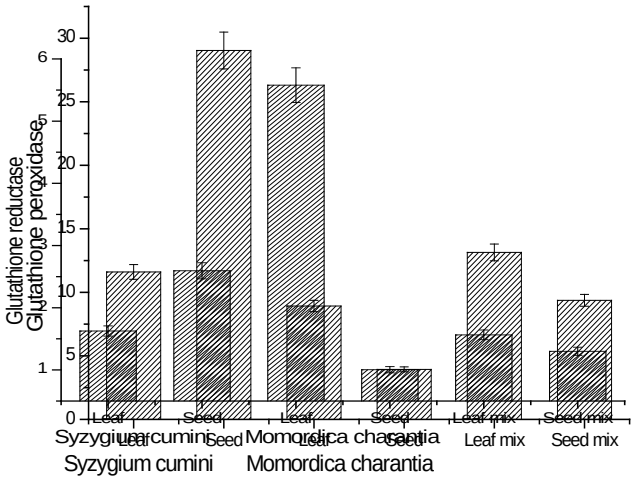
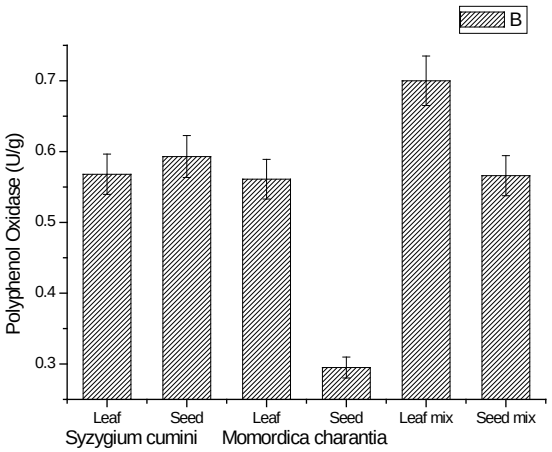
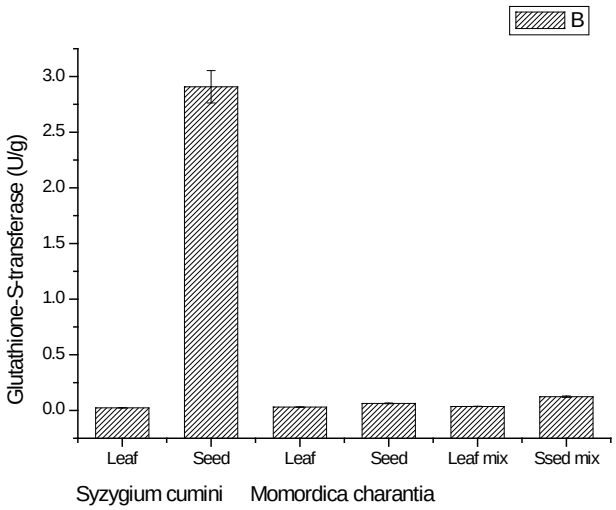
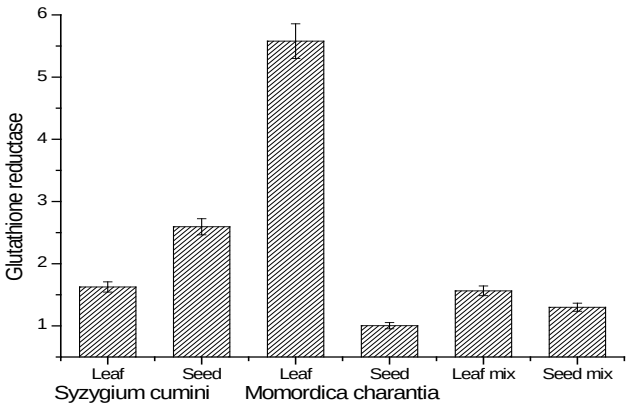


Figure 2- Levels of enzymic antioxidants in the seed and leaves samples of *S. cumini* and *M. charantia*









From figure 1 it is evident that the maximum activity of Catalase and Superoxide dismutase was observed in the seed extract of *Syzygium cumini*, 158.122 U/g) whereas the seed extract of *Momordica charantia* was found to be the poorest source of the above mentioned enzymes(368.958 U/g, 16.471 U/g). But in the case of peroxidase the seed extract of *Syzygium cumini* registered the lowest value (2.985U/g) though it exhibited maximum activity for Catalase and Superoxide dismutase.

The crude methanollc extracts and essential oils of leaves of *S. cumini* exhibited higher antioxidant activity (Mohamed *et al*. 2013). The extract of leaf mixture recorded the maximum activity of polyphenol oxidase (0.700U/g) and the minimum activity was shown by the seed extract of *Momordica charantia* (0.295U/g).

Some isoenzymes of peroxidases are reported to exhibit polyphenol oxidase (PPO) activity which is involved in active scavenging of oxygen radicals (Okpuzor and Omidyii, 1998).
From figure 2 it is observed that the activity of Glutathione-S-transferase (2.908U/g) and Glutathione peroxidase (29.036U/g) was found to be the maximum in the seed extract of *Syzygium cumini* and the leaf extract of *Momordica charantia* showed the highest activity of Glutathione reductase (5.578 U/g). Aqueous and ethanolic crude extract of *S. cumini* skeels have the highest total phenolic content and antioxidant activity (Lanchakon Chanudom *et al*. 2015). Least activity of Glutathione peroxidase and Glutathione reductase was noticed in the seed extract of *Momordica charantia* and Glutathione-S-transferase in the leaf extract of *Syzygium cumini*.

Ali *et al*. (2005) have reported that the increased level of enzymic antioxidants glutathione peroxidase and glutathione-S-transferase in root and leaves segments of Phalaenopsis led to the breakdown of oxidants such as H_2O_2, organic hydroperoxides and lipid hydroperoxide resulting in greater protection against oxidative damage.

IV. CONCLUSION

The various enzymic antioxidants have been evaluated in the methanolic extracts of leaves and seed samples of *S.cumini* and *M.charantia*. Hence the *S.cumini* and *M.charantia* have proved to be potent source of antioxidants in eradicating the free radicals. Further purification can be done and drug designing can be focussed from these samples for the future benefit of the society.

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