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Evaluation of Non-Enzymatic and Enzymatic Antioxidant Activity in Leaves Extracted from Medicinal Plants.

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ABSTRACT

Five medicinal plants were taken to investigate and evaluate levels of non-enzymatic and enzymatic antioxidants in leaves of *Calotropis procera*, *Datura stramonium*, *Argemone mexicana*, *Withania somnifera*, *Solanum nigrum*. The results indicate that among these medicinal plant leaves; the enzymatic activities of enzymatic antioxidant viz. catalase, glutathione reductase activity were highest in leaves of *Withania somnifera* but superoxide dismutase activity was high in *Calotropis procera*. The level of non-enzymatic antioxidant viz. total phenols, flavonoids and carotenoid were highest in medicinal plant leaves of *Argemone Mexicana* but ascorbic acid in *Datura stramonium*.

Keywords- Antioxidants, *Argemone mexicana*, *Withania somnifera*, *Solanum nigrum*, *Calotropis procera*, *Datura stramonium*

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INTRODUCTION

Medicinal plants possess antioxidant defence system comprised of enzymatic and non-enzymatic components, which normally maintain reactive oxygen species balance within the cell. For instance, they use diverse array of enzyme like superoxide dismutase, catalase and peroxidases as well as low molecular mass antioxidants to scavenge different types of reactive oxygen species Dong *et al.*, (2006). Antioxidants play a significant role in the prevention of diseases and do have a capacity to reduce oxidative stress by chelating trace elements or scavenging free radicals and protecting antioxidant defenses (Banerjee and Dasgupta, 2005). Consumers should increase their intake of foods rich in antioxidant compounds that lower the risk of chronic health problems associated with the above diseases (Klipstein-Grobusch *et al.*, 2000).

Medicinal plants contain large amounts of antioxidants, such as polyphenols, vitamin C, vitamin E, selenium, β -carotene, lycopene, lutein, and other carotenoids, which play important roles in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Free radicals or ROS (reactive oxygen species) are highly reactive oxygen metabolites, which can attract electrons from surrounding molecule to induce accumulation of cellular damage and this result in skin aging (Ma *et al.*, 2001; Cook and Samman, 1996), As the free radicals play major role in causing the diseases, the supply of antioxidants, in the diet is of great importance for a healthy life (Scalbert and Williamson, 2000).

Medicinal plant based antimicrobials represent a vast untapped source of pharmaceuticals and further exploration of plant antimicrobials need to occur for treatment of infectious diseases both in plants and humans while simultaneously for mitigating many of the side effects that are often associated with synthetic antimicrobials. Out of the several hundred thousand medicinal plant species around the globe, only a small portion has been investigated both phytochemically and pharmacologically (Hostettmann and frost, 1999).

MATERIALS AND METHODS

Collection of sample

The following fresh medicinal plants: (i) *Argemone Mexicana* (Mexican poppy) (ii) *Withania somnifera* (Ashwagandha) (iii) *Solanum nigrum* (Makoy) (iv) *Calotropis procera* (Madar) (v) *Datura stramonium* (Datura) were collected from local area of Allahabad, U.P., India.

Removal of moisture from leaves extract

The leaves were washed thoroughly with tap water and rinsed with distilled water. The leaves were dried using filter paper and in hot air oven maintained at $55^{\circ}\text{C} \pm 20^{\circ}\text{C}$. The dried leaves of medicinal plants were weighed till constant weight was obtained and homogenized to a fine powder and stored in air tight polypropylene bottles.

Extraction method

Extraction of powdered leaves was done by the method given by Kalpna *et al.*, (2010). 5g of dried powder was taken in conical flask containing methanol (50mL) respectively, plugged with cotton and kept on a rotary shaker (120rpm) for 72hrs at RT. After 72h, the extract was filtered with moist muslin cloth. Supernatant was collected and solvent was evaporated.

Preparation of enzyme extracts

The leaves of medicinal plant were washed thoroughly with tap water and rinsed with distilled water. The leaves were patted dry using filter paper. A 10% homogenate (w/v) was prepared in the pre-chilled extraction buffer prescribed for each enzyme (usually the buffer used for assay of the enzyme) using mortar and pestle. The homogenate was filtered over three layers of moist muslin cloth. The residue was discarded and supernatant used immediately for enzyme assay. The entire process was carried out at a temperature close to 4 °C.

Determination of Antioxidant Activity

Estimation of Ascorbic acid

Ascorbic acid was estimated according to the method given by Harris and Ray (1935).

Estimation of carotenoids content

Carotenoids were estimated according to the method given by Mahadevan and Sridhar (1986).

Estimation of flavonoids content

Flavonoids content was estimated according to the method given by Chang *et al.*, (2002).

Estimation of total phenols content

Total phenols content was estimated according to the method given by McDonald *et al.*, (2001).

Enzyme assay

Catalase

Catalase activity was assayed by estimating the residual H₂O₂ in the mixture, which was then determined by oxidation with KMnO₄ titrimetrically using modified Hosetti *et al.*, (1994) method.

Glutathione reductase

Glutathione reductase activity was done according to the method given by cakmark *et al.*, (1992).

Superoxide dismutase

The activity of superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) according to the method given by Bauchamp & Fridovich (1971).

RESULTS AND DISCUSSIONS

These medicinal plant species; the level of non-enzymatic antioxidants *viz*, total phenol, flavonoids and carotenoid content were high in *Argemone Mexicana* leaves 28.91±0.04 mg/g; 32.67±0.21 mg/ml and 29.5±0.32 µg/ml respectively in table 1 however, but Veeru *et al.*, (2009) who reported ascorbic acid content 8.43 mg/100gm and carotenoids content was 17.66 mg/100gm in *Solanumnigrum* leaves; Kumar *et al.*, (2010) reported 17.32±0.099 mg/gm quercetin equivalent fresh tissue of total flavonoid content in healthy leaves of *Withania somnifera*. Jaleel (2009) reported that the ascorbic acid content 7.5 mg/gm in *Withania somnifera* leaves but we have got high 21.33± 1.49 mg/100g fresh wt. tissue) in *Datura stramonium*.

The activity of enzymatic antioxidant *viz*. catalase and glutathione reductase were high in leaves of *Withania somnifera* 1192±0.21 and 0.084±0.76 U/mg whereas, Jaleel (2009), reported catalase activity 3.43 U/mg and glutathione reductase contents in leaves 6.5 µg/gm of *Withania somnifera* leaves. Nevertheless, Chinathana and Ananthi (2012) reported the activity of catalase in experimental group contains albino mice treated with aqueous leaves extract of *Solanum nigrum* as 35.06±0.09 U/mg.

Superoxide dismutase activity was high in *Calotropis procera* leaves with 1.908±0.62 U/mg in table-2. Whereas, Chinathana and Ananthi (2012) reported the activity of superoxide dismutase in experimental group contains albino rats treated with aqueous leaves extract of *Solanum nigrum* as 2.73±0.14 U/mg and Jaleel (2009) reported superoxide dismutase activity 9.43 U/mg of *Withania somnifera* leaves.

Table 1: Non-enzymatic antioxidant contents in medicinal plant leaves

Medicinal plant	Common name	Ascorbic acid contents ^a	Total Phenol contents ^b	Flavonoid contents ^c	Carotenoid Contents ^d
<i>Solanum nigrum</i>	Makoy	8.33± 0.2	5.6±0.14	9.6±0.04	1.66±0.21
<i>Withania somnifera</i>	Ashwaganda	6.5±0.23	13.51±0.61	7.23±0.21	21.98±0.19
<i>Argemone mexicana</i>	Mexican poppy	15.21±0.61	28.91±0.04	32.67±0.21	29.5±0.32
<i>Calotropis procera</i>	Madar	12.89±0.34	15.98±0.53	12.7±0.43	28.05±0.32
<i>Datura stramnim</i>	Datura	21.33± 1.49	21.23±0.69	11.23±0.15	12.76±0.72

a (mg/100g fresh wt. tissue); b (mg gallic acid equivalent/gm of extract powder); c (mg quercetin equivalent/ml of extract powder); d (µg/ml); values are expressed as mean ± S.D. (n = 3)

Table 2: Activity of enzymes in medicinal plant leaves

Medicinal plant	Catalase	Glutathione reductase	Superoxide dismutase
<i>Solanum nigrum</i>	1137±0.43	0.034±0.54	1.374±0.23
<i>Withania somnifera</i>	1192±0.21	0.084±0.76	1.128±0.41
<i>Argemone mexicana</i>	765±0.18	0.056±0.23	0.852±0.11
<i>Calotropis procera</i>	1090±0.61	0.045±0.20	1.908±0.62
<i>Datura stramonium</i>	876±0.23	0.023±0.13	0.552±0.43

Activity (U/mg); values are expressed as mean ± S.D. (n = 3)

CONCLUSION

The was to determine the non-enzymatic antioxidants viz. ascorbic acid, carotenoid, flavonoids and total phenolic content in two medicinal plant species along with assay of enzymatic antioxidants, catalase and glutathione reductase. The level of non-enzymatic antioxidant contents were high in *Argemone Mexicana* compare to other four medicinal plants but enzymatic antioxidant contents were in *Withania somnifera*.

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