



EVALUATION OF ANTI-DENATURATION PROPERTY AND ANTI-OXIDANT ACTIVITY OF TRADITIONALLY USED MEDICINAL PLANTS

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ABSTRACT

The antigenic property of the denatured proteins leads to some of the diseases like serum sickness, glomerulonephritis, rheumatoid arthritis and systemic lupus erythematosus. Metabolism of xenobiotics leads to the production of reactive oxygen species (ROS), which leads to oxidative stress. Many literatures stated that, chronic oxidative stress is one of the important causes for the production of auto antibodies which leads to autoimmune diseases. Different parts of *Ventilago maderaspatana* Geartn, *Acacia nilotica* linn, and *Polyalthia longifolia* were powdered and extracted by cold percolation using ethanol. The extracts were subjected for *in-vitro* protein anti-denaturation using Bovine serum albumin and reducing antioxidant activity. All the extracts have shown mild to moderate anti-denaturation and antioxidant activity. Among them *Acacia nilotica* showed anti-denaturation percentage inhibition of 97% at 1µg/ml. 1whereas the standard drug (Diclofenac sodium) showed a percentage inhibition of 66% at The result also shows that *Acacia nilotica* Linn has good reducing property than other plant extracts.

KEY WORDS

Anti-Denaturation, Inflammation, *Acacia nilotica*, *Ventilago Maderaspatana*

INTRODUCTION

The age old herbal remedies and herbal supplements often give miraculous results in treating many dreadful illnesses. The demand on plant based therapeutics is increasing in both developing and developed countries due to their recognition as non-narcotic, easily biodegradable, producing minimum environmental hazards, no adverse effects and easily available at affordable prizes. Moreover synthetic drugs are very expensive to develop since, for the

successful introduction of a new product approximately 3000-4000 compounds are to be synthesized, screened and tested, whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs¹.



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Drugs presently used for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. NSAIDS which includes both selective and non-selective cyclooxygenase (COX) inhibitors are most widely used for the treatments of pain. All of these drugs are reported for their side effects and toxic effects². The activity of non-steroidal anti-inflammatory drugs (NSAIDs) in rheumatoid arthritis and other rheumatic diseases does not seem to be only due to the inhibition of the production of endogenous prostaglandins (which could be affected at much lower doses than those required in these conditions), it also prevents the denaturation of proteins (which acts as antigens and leads to autoimmune diseases)³. When BSA is heated it undergoes denaturation, it expresses antigens associated to Type III hyper-sensitive reaction and which are related to diseases such as serum sickness, glomerulonephritis, rheumatoid arthritis and systemic lupus erythematosus¹.

Cytochrome P450 (CYP) enzymes participate in the detoxification of xenobiotics. Paradoxically, they can produce reactive oxygen species (ROS) that can damage DNA, as well as lipids and proteins⁴. Metabolism of xenobiotics leads to the production of reactive oxygen species (ROS), which leads to oxidative stress. Many literatures have reported that, chronic oxidative stress is one of the important causes for the production of auto antibodies which leads to autoimmune diseases⁵. An increased risk of hematological malignancies, compared with the general population was found among patients with rheumatoid arthritis and systemic lupus erythematosus⁶. The products of free radical reactions have been detected in the blood and joints of patients with rheumatoid arthritis and in

other inflammatory diseases such as glomerulonephritis. Thus it can be correlated that autoimmune phenomena and rheumatic diseases may be a result of auto antibodies generated against various auto antigens.

Antioxidants are free radical quenching agents and used for the prevention of many diseases. Polyphenolic compounds are commonly found in both edible and non-edible plants and reported to have multiple biological effects due to their antioxidant activity. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents⁷. Many reports claim that all the NSAIDs and antioxidants possess anti-denaturation activity¹. The plants selected in the present study are used by the traditional physicians as analgesics to treat chronic joint pains and for inflammatory conditions. Thus the present study was framed to evaluate reduced antioxidant activity and BSA anti-denaturation property to assess its anti-inflammatory activity.

MATERIALS AND METHODS

(i) Collection and Authentication

The plant selected for the study are *Ventilago maderapatana* Geartn (stem) (fam. Rhamnaceae), *Acacia nilotica* Linn (leaves) (fam. fabaceae), and *Polyalthia longifolia* (leaves) (fam. Annonaceae). The plants were collected from the various parts of Andhra Pradesh, India and authenticated by the botanist, S.V. University, Tirupati and by the taxonomist, Nagurjuna government degree college, Nalgonda. The plant materials were air dried and powdered.



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(ii) Preparation Of Extract

200gms of powdered plant materials were extracted by cold percolation using ethanol as solvent for seven days. The extract was concentrated under reduced pressure and air dried.

(iii) Anti-denaturation Activity

A solution of 0.2% W/V of BSA was prepared in Tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid. Stock solutions of 10,000µg/ml of all extracts were prepared by using methanol as a solvent. From these stock solutions 3 different concentrations of 100, 200 and 500 µg/ml were prepared by using methanol as a solvent. 50µl of

each extract was transferred to Eppendorf tubes using 1ml micro pipette. 5ml of 0.2% W/V BSA was added to all the above Eppendorf tubes. The control consists of 5ml 0.2% W/V BSA solution with 50 µl methanol. The standard consist 100 µg/ml of Diclofenac Sodium in methanol with 5ml 0.2% W/V BSA solution. The test tubes were heated at 72° C for 5 minutes and then cooled for 10 minutes. The absorbance of these solutions was determined by using UV/Vis Double beam spectrophotometer (Elico SL-196) at a wavelength of 660nm. The % inhibition of precipitation (denaturation of the protein) was determined on a % basis relative to the control using the following formula⁸.

$$\frac{(\text{Abs of control} - \text{Abs of extract})}{\text{Abs of control}} \times 100$$

% inhibition of denaturation = ----- X 100

Abs of control

(iv) Reducing Antioxidant Activity

Different concentrations of plant extracts (100 – 500mg) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferri-cyanide [K₃Fe(CN)₆] (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1%). The absorbance was measured at 700 nm against a blank using UV-Vis spectrophotometer (Elico –SL 196). Increased absorbance of the reaction mixture indicates increase in reducing power⁹.

RESULTS

The extracts percentage yield was calculated and the colour of the extracts was observed. The % yield and colour of the extracts are mentioned in table no.1

Table 1.

% yield and colour of the extracts

S.No	Name of the plant	Part used	Solvent	Colour	% yield
1	<i>Ventilago Maderaspatana</i> Geartn	Stem	Ethanol	Light green	9.25
2	<i>Acacia Nilotica</i> Linn	Leaves	Ethanol	Dark green	12.1



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3	<i>Polyalthia Longifolia</i>	Leaves	Ethanol	Dark green	10.87
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The % yield w/w of *Ventilago Maderaspatana Geartn* (Stem), *Acacia Nilotica Linn* (Leaves) and *Polyalthia Longifolia* (Leaves) extracts were 9.25, 12.1, and 10.87 % w/ w respectively.

1. Anti-Denaturation activity

This study establishes the anti-denaturation activity of plant extracts and represented in Table no 2.

Table 2. Percentage anti-denaturation of Bovine serum Albumin against heat in presence of various plant extracts.

S. No	Plant extract	Concentration $\mu\text{g/ml}$	% Inhibition
1	<i>Acacia nilotica</i> (leaves)	1	97.4 \pm 0.08
		100	62.7 \pm 0.02
		200	43.7 \pm 0.09
		500	22.2 \pm 0.06
2	<i>Polyalthia longifolia</i> (leaves)	1	86 \pm 0.03
		100	32.2 \pm 0.03
		200	21 \pm 0.07
		500	16 \pm 0.04
3	<i>Ventilago maderaspatana</i> (stem)	1	76.6 \pm 0.05
		100	44.4 \pm 0.07
		200	33 \pm 0.02
		500	5 \pm 0.04
4	Diclofenac sodium	100	88.8 \pm 0.07

The results are expressed as mean \pm SD. $p < 0.05$.

All the plant extracts has protected Bovine Serum Albumin (BSA) against heat denaturation. *Acacia nilotica* plant extract has shown 22.2 % and 43% anti-denaturation activity on BSA at 500 $\mu\text{g/ml}$ and 200 $\mu\text{g/ml}$ respectively. *Acacia nilotica* and *Polyalthia longifolia* showed 97% and 86% of anti-denaturation effect at 1 $\mu\text{g/ml}$ respectively. All the extracts were found to have good % of anti-denaturation at lowest concentrations. This result was coinciding with the statement of Williams et al., that the anti-denaturation action of extract is more when the concentration is less. The control Diclofenac sodium showed 88% anti-denaturation effect at 100 $\mu\text{g/ml}$.



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2. Reducing Antioxidant Activity

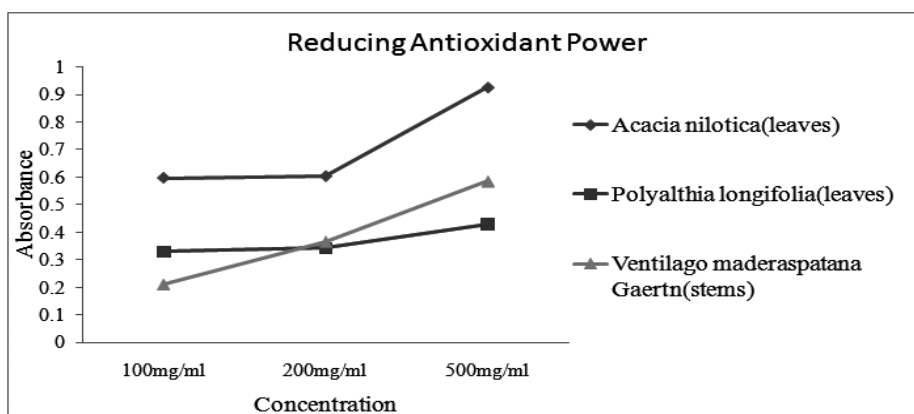


Fig 1.Reducing antioxidant Power of Plant extracts.

The reducing anti-oxidant activity shows the reducing property of the plant extracts on potassium ferri-cyanide. The absorbance is directly proportional to the reduction of ferric ions to ferrous ions, thus an increase in the absorbance denotes the reducing property of the plant extracts. The result shows that all the plant extract has mild antioxidant activity, among them *Acacia nilotica* and *Ventilago maderaspatana Gaertn* showed a constant increase in absorbance with increase in the concentration. The graphical representation of reducing antioxidant property of the plant extracts has been shown in Fig. No.1.

DISCUSSION

The study was designed to explore the therapeutic values of traditionally used medicinal plants. The plants selected for the study is being used by traditional physicians to cure severe chronic joint

pains and inflammation. Many literatures have correlated the protein denaturation activity and autoimmune diseases. Many studies on antioxidants have proved that oxidative stress has great importance in generation of autoimmune bodies responsible for autoimmune diseases. Since most of plants have polyphenolic compounds which has a good reducing, singlet oxygen quenching effect on free radicals. Thus in this study *in-vitro* anti-denaturation of Bovine Serum Albumin and reducing antioxidant activity was evaluated. The results have clearly demonstrated that all the plant species have mild to moderate antioxidant and anti-denaturation activity. Literatures suggest that, the anti-denaturation property of BSA was due to the presence of two interesting binding sites in the aromatic tyrosine rich and aliphatic threonine and lysine residue regions of the BSA¹⁰. They have also reported that therapeutic molecules could be activating the tyrosine motif rich receptor dually with



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threonine that regulate signal transduction biological pathways for their overall biological action^{11,12}. Compounds interacting with the aliphatic regions around the lysine residue on the BSA could be interesting as anti-oxidants with anticancer activity such as the polyphenols, phenyl propanoids and the disulphides (eg alpha-lipoic acid)¹¹⁻¹⁴. Therefore, this may be these phenolic compounds present in the plant extracts may be the reason for the possible anti-denaturation and reducing antioxidant activity.

CONCLUSION

The present study clearly states that the plants used in this study have both antioxidant activity and anti-denaturation activity. Among them *Acacia nilotica* showed good anti-denaturation and antioxidant activity. Further *in-vivo* investigations have to be performed to authenticate the biological activities. Phytochemical investigation is also proposed in order to isolate the active fraction and eventually the pure compound.

REFERENCES

1. Fayyaz Ahmad, rafeeq A. Khan, Shahid Rasheed, Study of analgesic and anti inflammatory activity from plant extracts of lactuca scariola and artemisia absinthium. J. Islam. Acad. Sci, 5: 111-114, (1992).
2. May Hamza and Raymond A. Dionne. Mechanisms of Non-Opioid Analgesics Beyond Cyclooxygenase Enzyme Inhibition: Curr. Mol. Pharmacol, 2: 1-14, (2009).
3. Insel, P. A. Analgesic-antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout. In: Hardman JG Limbird LE, Molinoff PB, Ruddon RW and Goodman Gilman A, *The pharmacological Basis of Therapeutics*, 9th Edn, McGraw Hill, New York, 1996, pp. 617-657.
4. Neber DW, Dalton PT, The role of cytochrome p-450 enzymes in endogenous signaling pathways and environmental carcinogenesis. Nature Rev Cancer. 6: 947-960. (2006).
5. Namazi MR. Neurogenic dysregulation, oxidative stress, autoimmunity, and melanocytorrhagy in vitiligo: can they be interconnected? Pigment Cell Res. 20: 360-363, (2007).
6. Abu-Shakra M, Buskila D, Ehrenfeld M, Conrad K, Shoenfeld Y. Cancer and autoimmunity: autoimmune and rheumatic features in patients with malignancies. *Ann Rheum Dis*. 60: 433-440, (2001).
7. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB, The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radical Res. 23: 375-383, (1995).
8. Williams LAD, Vasquez EA, Milan PP, Zebitz C, Kraus W. *In vitro* anti-inflammatory and anti-microbial



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- activities of phenylpropanoids from *Piper betle* (Piperaceae). APRauter, PB Palma, J. Justino, Araujo ME, Santos SP (Eds). *The Phytochemical Society of Europe: Natural products in the new millennium: Prospects and industrial application*. Kluwer Academic Publisher, Dordrecht. The Netherlands, 2002, pp. 221 – 227.
- Hajimahmoodi M, Sadeghi N, Jannat B, Oveisi M.R, Madani S, Kiayi M et al. Antioxidant activity, reducing power, total phenolic content of iranian olive cultivar. 2008. *J. Biol. Sci.* 8: 779 – 783 (2008).
 - Williams LAD, Rosner H, Conrad J, Moller W, Beifuss U, Chiba K, Selected secondary metabolites from *Phytolaccaceae* and their biological/pharmaceutical significance, Research Signpost. In: *Recent Res Devel In Phytochem*, 6: 13–68. (2002).
 - Williams LAD, Connar AO, Latore L, Dennis O, Ringer S, Whittaker JA, Conrad J, Vogler B, Rosner H, Kraus W, The in vitro anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. *West Indian Med J*, 57: 327 – 331, (2008).
 - Rosner H, Williams LAD, Jung A, Kraus W, Disassembly of microtubules and inhibition of neurite outgrowth, neuroblastoma cell proliferation, and MAP kinase tyrosine dephosphorylation by dibenzyl trisulphide. *Biochim Biophys Acta.* 1540: 166–77, (2001).
 - Kannan S, Free radical theory of autoimmunity. *Theor Biol Med Model*, 3: 22, (2006).
 - Kawabata T, Packer L, α -lipoate can protect against glycation of serum albumins but not low density lipoproteins. *Biochem Biophys Res Commun.* 203: 99–104. (1994).