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Research Article

# *IN VITRO* ANTIINFLAMMATORY AND ANTIOXIDANT ACTIVITY OF LEAF EXTRACTS OF *Datura metal*

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**ABSTRACT**

Objective: Roots of *Datura metel* has been proved by many scientists as potential anti-inflammatory and antioxidant agent, however much work has not been done to evaluate leaves of the plant for these activities. This study was undertaken to evaluate the in vitro anti-inflammatory and antioxidant potential of leaves of *Datura metel*.

Methods: Aqueous and methanolic extracts of the leaves of *D.metel* were subjected to *in vitro* screening models such as DPPH free radical scavenging activity, total phenolic content, total antioxidant capacity and total anti-inflammatory activity by HRBC membrane stabilization. Ascorbic acid was used as the standard.

Results & Conclusion: The results thus obtained suggested that leaves of *D.metel* possess considerable activity and therefore further in vivo studies can establish it as a potential anti-inflammatory and antioxidant remedy.

**Keywords:** Antiinflammatory, Antioxidant, Plant extract, Phenolic compound, HRBC membrane lysis, DPPH radical.

## INTRODUCTION

Inflammation is a local response of living mammalian tissues to injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agent. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury [1, 2].

Reactive oxygen species (ROS) and reactive nitrogen species (RNS), generated in the human body, can cause oxidative damages associated with many diseases such as atherosclerosis, coronary heart diseases, Parkinson's diseases, diabetes, neurodegenerative (Alzheimer's disease), aging and cancer. Antioxidants, such as phenolic compounds including flavonoids, chalcones, lignoids, stylbenoids, tannins, and diarylheptanoids, are distributed in the plant kingdom and may prevent oxidative damage by scavenging ROS. Therefore, the phenolic constituents of plants are of interest as potential chemo preventive agents, and plants may be an attractive alternative to currently available commercial antioxidants, because they are biodegradable to non-toxic products [3, 4]. Antioxidants may act by decreasing oxygen concentration, intercepting singlet oxygen, preventing first chain initiation by scavenging initial radicals, binding metal ion catalysts, decomposing primary products to non-radical compounds and chain breaking to prevent continued hydrogen abstraction from substrates. The balance of free radical production and level of antioxidant defenses is essential for health. Too high level of free radicals and low amount of antioxidants can lead to a condition of oxidative stress and chronic injury. This effect is observed due to the conversion of oxygen molecule intracellularly into the reactive oxygen species or free radicals (reduction mechanism) which is toxic to the cells and tissues. These reactive oxygen species trigger the production of pro- inflammatory cytokinin and chemokinin mediators [5].

The mechanism of inflammation is attributed, to release of ROS from activated neutrophils and macrophages. ROS over production results tissue injury by damaging macromolecules and lipid peroxidation of membranes. In addition, it propagate inflammation by stimulating release of cytokines such as IL-1, TNF-α and interferon-γ which are responsible for the recruitment of additional neutrophils and macrophages. Thus free radicals are important mediators that provoke or sustain inflammatory responses and their neutralization by antioxidants and radical scavengers can reduce inflammation [6].

A large number of Indian medicinal plants are attributed with various pharmacological activities because they contain a diversified class of photochemical. It is believed that current analgesia-inducing drugs such as opoids and non – steroidal anti-inflammatory drugs are not useful in all cases, because of their side effects and potency. As a result, a search for alternatives seems necessary and beneficial [7]. Medicinal plants having a wide variety of chemicals from which novel anti-inflammatory agents could be discovered. Scientific studies are required to judge their efficacy. Traditional and folklore medicines play an important role in health services around the globe. About three quarters of the world population relies on plants and plant extracts for health care. India has an extensive forest cover enriched with plant diversity [8]. Several plants have been used in folklore medicine. The rational design of novel drugs from traditional medicine offers new prospects in modern health care. Ayurveda the traditional medicinal system in India described certain plants which strengthen the host immune system. The present study was undertaken to evaluate in vitro anti-inflammatory and antioxidant activity of methanolic and aqueous extracts of *Datura metel* leaves.

## MATERIALS & METHODS

### *Plant material*

Plant leaves of *Datura metel* were collected from the Simhachalam area, Visakhapatnam, Andhra Pradesh, India.

### *Plant extraction*

Fresh and uninfected leaves of plants under study were shed dried and then grinded to fine powder. Later the plant powders were subjected to Soxhlet extraction using 90% methanol and distilled water as solvents. The extracts were subjected to solvent recovery under vacuum and the condensed extracts were collected in sterile plastic bottles. Dilutions were made as and when required.

### Determination of DPPH Radical Scavenging Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity was measured by the spectrophotometric method. A stock solution of 25mg of DPPH (150M) was prepared in 100ml of ethanol. 0.1ml of extract of different concentration (50, 100, 250, 500 and

1000µg/0.1ml) and 1.9ml of DPPH was added. Control without test compound was prepared in an identical manner. In case of blank, DPPH was replaced by ethanol. The reaction was allowed to be completed in the dark for about 20 minutes. Then the absorbance of test mixtures was read at 517nm.The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical. Ascorbic acid (50,100, 250, 500 and 1000g/0.1ml) was used as standard. The percentage DPPH inhibition was calculated from the following formula.

%DPPH inhibition = [(OD of control –OD of test) /(OD of control)]x100

**Determination of Total Phenolic Content**

The total phenolic content in the methanol leaf extract of *D. metel* was measured using Folin-Ciocalteu reagent based on procedures described by Singleton et al. [9], with some modifications. Briefly, 0.5 ml of plant extract (1mg/ml) was mixed with 1.5 ml (1:10 v/v diluted with distilled water) Folin- Ciocalteau's reagent and allowed to stand for 22°C for 5 min. Then 2 ml of sodium carbonate ($Na_2CO_3$, 7.5%, w/v) was added and the mixture were allowed stand for another 90 min and kept in the dark with intermittent shaking. Then the absorbance of the blue color that developed was measured at 725 nm using spectrophotometer. The experiment was carried out in triplicates.

Gallic acid was used for constructing the standard curve (50 to 500µg/ml; Y= 0.001X- 0.014; $R^2$= 0.997) and the total phenolic compounds concentration in the seeds extract was expressed as

milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g) of extract.

**Determination of Total Antioxidant Capacity**

The total anti oxidant activity was evaluated by Prieto et al. [10]. An aliquot of 0.1 ml of sample solution/ ascorbic acid equivalent to 500µg was combined with 1ml of the reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM sodium molybdate). In case of blank, 0.1ml of methanol was used in place of sample. The tubes were capped and incubated in a boiling water bath at 95$^0$C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695nm against blank. The anti-oxidant capacity was expressed as equivalents of ascorbic acid (mg/g) using an ascorbic acid standard curve (25 to 125µg/ml; Y= 0.014X- 0.063; $R^2$= 0.979).

**In vitro Anti-inflammatory Activity by HRBC Membrane Stabilization Method**

The principle involved here is stabilization of HRBC (human red blood cell) membrane by hypotonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH7.4, 0.15M], 2ml hypo saline [0.36%], 0.5ml HRBC suspension [10% v/v] with 0.5ml of plant extracts of various concentrations (50, 100, 250, 500 and 1000 µg/0.5ml), standard drug diclofenac sodium (50, 100, 250, 500 and 1000 µg/0.5ml) and control [distilled water instead of hypo saline to produce 100% hemolysis] were incubated at 37$^0$C for 30 min and centrifuged respectively. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm. The percentage hemolysis produced in the presence of HRBC membrane stabilization or protection was calculated using the formula:

Percentage Stabilization = 100 - [(Optical Density Of Drug ) / (Optical Density Of Control) X 100]

**Statistical Analysis**

The experiments were replicated thrice for each parameter and the standard deviation was calculated. Values were expressed in standard deviation from mean n=3.

**RESULTS & DISCUSSION**

The strength of antioxidant activity was determined by DPPH radical scavenging activity assay. This assay is being used widely as a preliminary test which provides information on the reactivity of test compound with a stable free radical since odd electron of DPPH gives strong absorption band at 517nm (violet color) and when it is bleached by the extract, there is a decrease in absorbance. This method is based on the reduction of alcoholic DPPH solution in the presence of hydrogen donating anti-oxidant(AH) due to the formation of non- radical form DPPH – H by the reaction DPPH+AH→ DPPH –H +A. The remaining DPPH measured after certain time, corresponds inversely to the radical scavenging activity of the anti-oxidant. The sensitivity of the method is determined by the strong absorption of DPPH.

**Table1: DPPH radical scavenging activity**

| Test sample | Concentration (µg/ml) | % inhibition of DPPH radical | IC50 (µg/ml) |
|---|---|---|---|
| Aqueous extract of *Datura metel* (leaves) | 5 | 16.26±0.23 | |
| | 10 | 22.03±0.31 | |
| | 25 | 41.32±0.03 | 74.0 |
| | 50 | 48.91±0.16 | |
| | 100 | 53.52±0.55 | |
| Methanolic extract of *Datura metel* (leaves) | 5 | 22.16±0.43 | |
| | 10 | 34.32±0.15 | 56.34 |
| | 25 | 45.54±0.64 | |
| | 50 | 59.21±0.33 | |
| | 100 | 62.67±0.75 | |
| Ascorbic acid | 5 | 23.55±0.56 | |
| | 10 | 45.37±0.73 | |
| | 25 | 60.12±0.11 | 36.28 |
| | 50 | 74.98±0.41 | |
| | 100 | 78.06±0.23 | |

The antioxidant activity of the extracts might be due to the presence of phenolic compounds [11, 12]. Many plant extracts have been reported to have multiple biological effects, including antioxidant properties due to their phytoconstituents including phenolics. The antioxidant activity of phenolics is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [13, 14]. Total phenolics were assayed by Folin- Ciocalteau's colorimetric method, which can estimate all flavonoids, anthocyanins and nonflavonoid phenolic compounds, that is, all the phenolic compounds present in the extracts. This method is based on an oxidation-reduction reaction in which phenolic compounds are oxidized with simultaneous reduction of a phosphotungsten-phosphomolybdate complex in an alkaline medium, reacting blue. Table 2 gives the results for total phenolics, expressed as gallic acid equivalent (mg GAE/g).

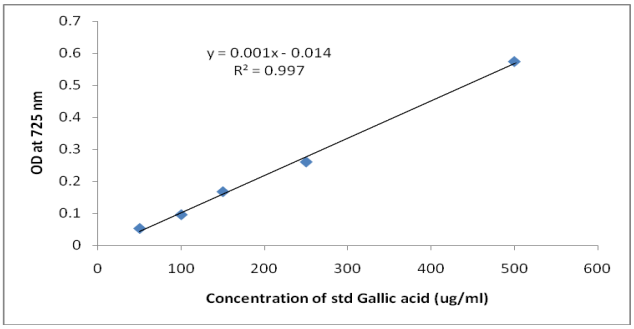


**Fig1: Calibration plot for total phenols**

**Table2: Total phenolic content of leaf extracts of *D.metel***

| Methanolic leaf extract | GAE mg/gm of plant | % phenolic content in plant |
|---|---|---|
| *Datura metel* (Methanolic) | 19.893±0.52 | 1.989±0.58 |
| *Datura metel* (Aqueous) | 12.352±0.73 | 1.235±0.46 |

Total antioxidant activity was estimated by phosphorous-molybdenum method. The total antioxidant capacity was expressed

as equivalent of ascorbic acid per gram of plant sample. The total antioxidant activity in methanolic leaf extract of *D. metel* was found to be high and considerable as compared with the aqueous extract.

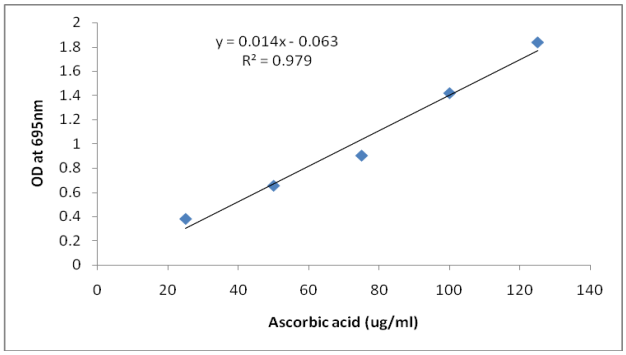


**Fig1: Calibration plot for total antioxidant capacity**

**Table3: Total antioxidant capacity of *D.metel* extracts**

| Total antioxidant capacity | Ascorbic acid equivalent mg/g of plant | |
|---|---|---|
| | Methanolic leaf extract | Aqueous leaf extract |
| *D.metel* | 22.360±0.67 | 13.334±0.64 |

Aqueous extract of *D.metel* was not much effective as an anti-inflammatory agent however the methanolic extract stood better. The percentage protection of RBC membrane lysis by methanolic extracts of *D. metel* was 79.77% at 1000µg/0.5ml (Table-4) which can be understood as a considerable value with IC50 value 431.53µg/0.5ml when compared with that of standard drug diclofenac sodium (84.43% at 1000µg/0.5ml with IC50 value 238.07µg/0.5ml). It possessed significant activity comparable with that of the standard Diclofenac sodium. *D. metel* showed significant anti-inflammatory activity which may be due to presence of chemical profile such as Flavones, Tri-Terpenoids, Flavonones and Phenols [15].

**Table4: % inhibition of HRBC membrane lyses by extracts of *D.metel***

| Test sample | Concentration (µg/0.5ml) | In vitro Antiinfammatory activity % inhibition of RBC membrane lysis | IC50 (µg/ml) |
|---|---|---|---|
| Aqueous extract of *D.metel* | 50 | 40.93±0.44 | 882.50 |
| | 100 | 49.23±0.12 | |
| | 250 | 56.07±0.34 | |
| | 500 | 62.13±0.37 | |
| | 1000 | 63.96±0.15 | |
| Methanolic extract of *D.metel* | 50 | 46.13±0.21 | 431.53 |
| | 100 | 59.92±0.17 | |
| | 250 | 70.84±0.34 | |
| | 500 | 77.91±0.35 | |
| | 1000 | 79.77±0.25 | |
| Diclofenac sodium | 50 | 55.21±0.18 | 238.07 |
| | 100 | 67.32±0.06 | |
| | 250 | 74.71±0.24 | |
| | 500 | 82.38±0.31 | |
| | 1000 | 84.43±0.15 | |

## CONCLUSION

Today, the search for natural compounds rich in antioxidant, anticancer and antimicrobial properties is escalating because of their importance in controlling many chronic disorders such as cancer and cardiovascular diseases. It has been estimated that approximately two-thirds of anticancer drugs approved worldwide are derived from plant sources. It is increasingly being realized that many of today's diseases are due to the "oxidative stress" that results from an imbalance between the formation and neutralization of pro-oxidants. These excess free radicals react with biological macromolecules such as proteins, lipids and DNA in healthy human cells and this results in the induction of carcinogenesis, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases. These harmful radicals have to be eliminated from biological systems by enzymes such as superoxide dismutase, catalase and peroxidase, or compounds such as ascorbic acid, polyphenpls, tocopherol and glutathione, which possess antioxidant properties.

Aqueous and methanolic extracts of plants *D.metel* were studied for their ability to stabilize human RBC membrane lyses in hypotonic saline. The results obtained were also compared with standard anti-inflammatory agent diclofenac sodium and standard antioxidant ascorbic acid respectively. It was found that the plant had considerable activity as compared to diclofenac sodium. The plants were also tested for other antioxidant features like DPPH radical scavenging, total antioxidant and total phenolic content as the oxidative radicals can also cause problems like inflammation by membrane destabilization. Although the plant extracts under study were found low in total phenolic content, the DPPH radical scavenging and total antioxidant capacity were found considerable specially in methanolic extract. The results therefore suggest that the plants under study can be used as potential cure for anti-inflammatory agents. Further *in vivo* studies are required to elucidate its exact mechanism of action.

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