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Evaluation of anti-oxidant, anti-inflammatory and toxicity potential of ethanolic extract of *C.diurnum* leaves

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ABSTRACT: Plants contain several different phytochemicals which can act on various metabolic pathways of the human system at a time thus effectively treating several conditions at once. Main objective of this research was to identify and assess the anti-oxidant, anti-inflammatory and toxicity potential of *Cestrum diurnum* leaves extract. Phytochemical screening of plant extract was performed, followed by DPPH radical scavenging potential assay, FRAP assay and protein denaturation inhibition assay with both the plant extract and the standard drugs. Hemolytic toxicity was also determined to calculate the safe dosage of plant extract for consumption as a possible drug candidate against oxidative and inflammatory stress conditions. This study confirmed the presence of high quantities of total phenols and total flavonoids which in turn showed notable anti-oxidant potential but slightly reduced reduction potential. Higher protein denaturation inhibition rates indicated the efficiency of plant extract as an effective anti-inflammatory agent and nominal hemolytic activity at 100μ g/ml of plant extract. Further studies are required to ascertain the specific phytocompounds responsible for such activities and their potential applications as pharmacologically active compounds.

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I. INTRODUCTION

Humans have had to deal with a variety of illnesses, discomforts, and attempts to combat them using a variety of strategies over the years. The use of medicinal plants to treat various illnesses is one of the many strategies used to fight illnesses[1]. The majority of these illnesses are associated with the generation of free radicals. A vital component of aerobic life and metabolism are free radicals. Antioxidants shield cells from the harm that free radicals can do. It has been demonstrated that antioxidants slow down or stop other molecules from oxidizing[2]. Another key player in disease pathogenesis is the process of inflammation. Inflammation's primary function is to shield organisms from microbial infections; in other cases, it serves as a defense mechanism against illnesses like cancer. But in other cases, inflammation can become dangerous and result in severe clinical diseases. By triggering many signaling pathways, the long-term overexpression of inflammatory factors can change a number of physiological processes[3].

Because of their various therapeutic uses, medicinal plants have been prized for ages in conventional medical systems around the world.Many of the bioactive substances found in these plants, such as polyphenols, flavonoids, carotenoids, vitamins, and terpenoids, have strong antioxidant properties as well as anti-inflammatory properties. The day-blooming jasmine, day-blooming cestrum, wild jasmine, Chinese inkberry, or *Cestrum diurnum* L. (Family: Solanaceae), commonly known as Din-ka Raja in Urdu, is an upright, evergreen, woody shrub with many leafy branches and simple leaves. The plant produces black fruits that resemble globular berries and short clusters of fragrant white blooms.Many Cestrum species are utilized in Ayurveda, traditional Chinese medicine, and South and North American folk medicines to treat burns and swelling because they contain similar bioactive phytoconstituents[4,5].

Main objective of this study was to evaluate and analyze the anti-oxidant and anti-inflammatory potential of locally grown *C.diurnum* leaves as a source of potentially therapeutic and pharmacologically active compounds.

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II. MATERIAL AND METHODS

A. Plant Collection and Extract Preparation

Fresh plants were collected and identified at the Pakistan Museum of Natural History, Islamabad, Pakistan. Leaves of C*estrum diurnum* were separated from stems, cleaned and shade dried. Dried leaves were then crushed and strained to form fine powder which was used for extract preparation using absolute ethanol through soxhelt apparatus. Resulting extract was then filtered and dried at room temperature, and stored in refrigerator at 4°C for further use[6].

B. Phytochemical screening of C.diurnum

Plant extract's phytochemical analysis was done using standard methods; Hager's test for alkaloids, ferric chloride for phenols, lead acetate for flavonoids, Braymer's test for tannins, Salkowski's test for steroids, froth test for saponins, Liebermann's test for glycosides, acetic acid for cardiac glycosides, Ninhydrin test for amino acids and acetic acid and sulphuric acid for deoxy sugars[7].

C. Total Phenolic Content

Total phenolic content of plant extract was calculated by slightly modified Folin-Ciocalteau method of Kang et. al. with gallic acid as standard[8]. Four different concentrations of gallic acid (25, 50, 75 and 100μ g/ml) were taken for the standard curve and single concentration of plant extract was taken. Test was done in triplicates. Linear regression analysis was performed to obtain the value of x from;

$$y = cx + m$$

where $y =$ dependant variable, $x =$ independent variable, $c =$ slope and $m =$ intercept at $R^2 < 1$ Putting the value of x in the formula $C = c(V/\mathrm{m})$, gave us the concentration of total phenols in the plant extract (gm) in mg as per gallic acid equivalent;

where $c = x, V = \mathrm{vol}(1mL), m =$ mass (convert in gram $= 0.001$ g)

D. Total Flavonoid Content

Colorimetric method using aluminium chloride was employed with few changes to evaluate the total flavonoid content of plant extract[9]. Rutin was taken in various concentrations ($25, 50, 75, 100\mu$ g/ml) to generate a standard curve. Plant extract (1mg/ml) was taken, and test was done in triplicates. Linear regression analysis was performed to obtain the value of x from;

$$y = cx + m$$

where $y =$ dependant variable, $x =$ independent variable, $c =$ slope and $m =$ intercept at $R^2 < 1$

Putting the value of x in the formula $C = c(\ \mathrm{V/m})$, gave us the concentration of total flavonoids in the plant extract (gm) in mg as per rutin equivalent; where $c = x, V = \mathrm{vol}(1mL), m =$ mass (convert in gram $= 0.001g$).

E. Antioxidant Potential Determination

DPPH Radical Scavenging Assay: Modified method of Kang et. al. was used[10]. Ascorbic acid was taken as standard against which plant extract was tested at different concentrations ($10, 20, 30, 40, 50$ and 60μ g/ml) in triplicates. Percentage inhibition was calculated by the formula;

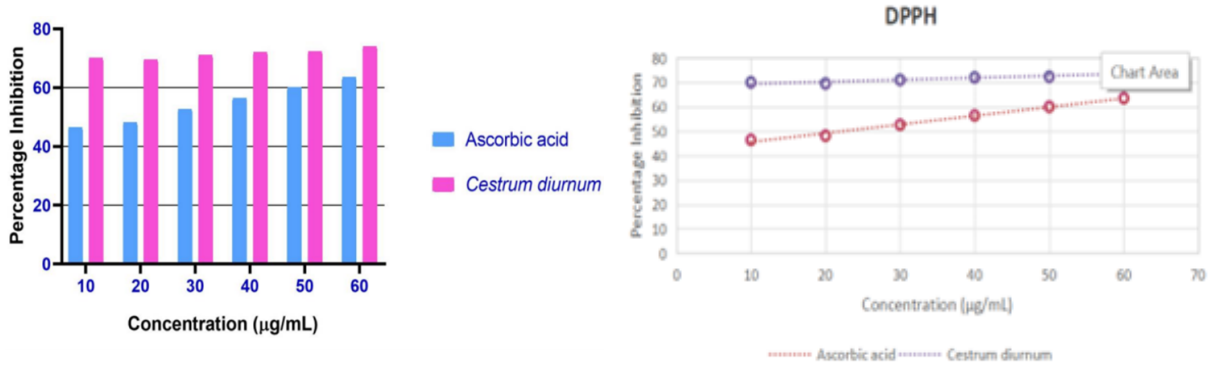

FIG. 1: Comparison of anti-oxidant activity (DDPH assay) of *Cestrum diurnum* and Ascorbic Acid (a) concentration dependant DPPH radical scavenging activity and (b) linear regression analysis.

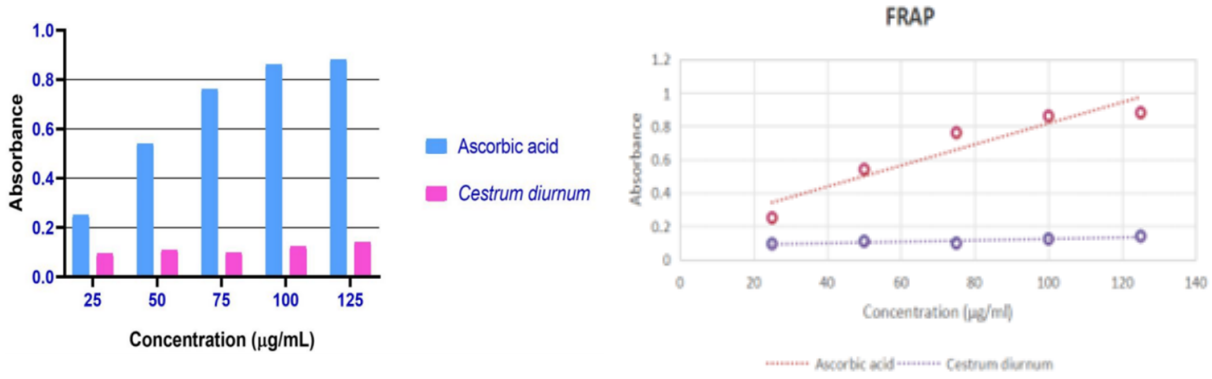

FIG. 2: Comparison of reducing activity (FRAP assay) of *Cestrum diurnum* and Ascorbic Acid (a) concentration dependant ferric reducing potential and (b) linear regression analysis.

$$\text{Percentage Inhibition} = (\text{ Control} - \text{Sample} / \text{Control}) \times 100$$

Ferric Reducing Antioxidant Power (FRAP) Assay: Benzie and Strain method was adopted with a few modifications to determine FRAP activity as per $FeSO_4$ equivalent ($FeSO_4E$)[11]. 20, 40, 60,80 and 100μ g/ml concentrations were taken for both standard (ascorbic acid) and plant extract in ethanol, in triplicates.

F. Anti-inflammatory Potential Determination

Inhibition of Protein Denaturation Assay: Chandra et. al. method for bovine serum albumin denaturation inhibition was implied with slight changes[12]. Ansaid was taken as standard. Absorbance was taken at 620 nm and percentage inhibition of plant extract was calculated, performed in triplicates, using the formula;

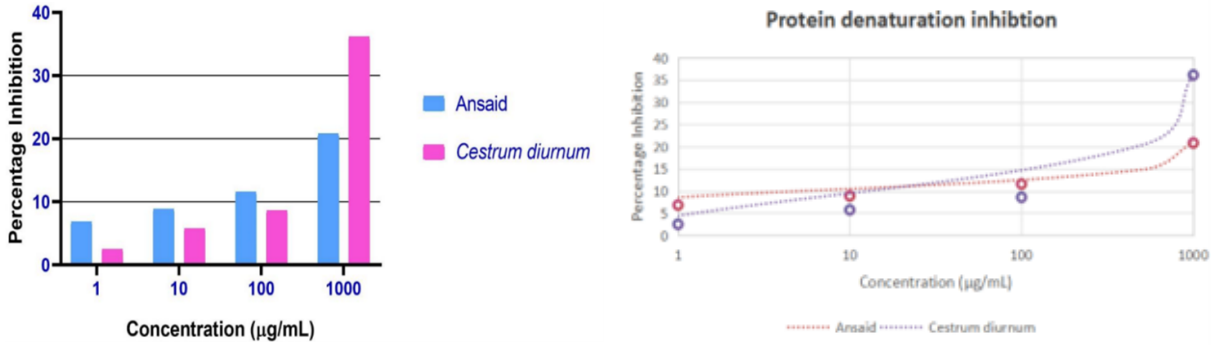

FIG. 3: Comparative Protein denaturation assay of *Cestrum diurnum* and Ansaid (a) concentration dependent protein denaturation inhibition potential and (b) linear regression analysis.

$$\text{Percentage Inhibition} = [(\text{ Sample } / \text{ Control }) - 1] \times 100$$

G. Toxicity Potential Determination

Toxicity Hemolytic Assay: In-vitro hemolytic assay was conducted by following Malagoli's method with few modifications to assess the toxicity of plant extract[13]. Test was done in triplicates. Hemolytic activity was calculated with the formula;

$$\%\ \text{Hemolysis} = (\text{ Sample } - \text{ Neg Control })/(\text{ Positive Control } - \text{ Neg Control }) * 100$$

H. Statistical Analysis

Graphs were plotted using GraphPad Prism 8.0.1 (GraphPad Software, San Diego, California USA, www.graphpad.com) and statistical analysis was done using IBM SPSS Statistics 25.0.

III. RESULTS AND DISCUSSION

A. Phytochemical Screening

Qualitative testing showed the presence of alkaloids, phenols, flavonoids, steroids, saponins, cardiac glycosides and deoxy sugars in the ethanolic extract of *C.diurnum* leaves while tannins, glycosides and amino acids were found to be absent (Table I).

Total phenolic content in the leaves extract of *C.diurnum* was calculated to be 137.19mg/g of phenols as per gallic acid equivalent whereas the total flavonoid content was calculated to be 185mg/g of flavonoids as per rutin equivalent. These quantities relate to the reported literature and indicate the presence of high antioxidant activity potential of this extract.

B. Anti-oxidant Potential Determination

DPPH Radical Scavenging Assay: Ascorbic acid and *Cestrum diurnu*m extract DPPH inhibition activity was plotted and compared against several concentrations as shown. *Cestrum diurnum* ethanolic extract was shown to have significantly (P value- 0.0001) greater DPPH inhibition activity ($71.45 \pm 1.62\%$) as compared to that of ascorbic acid ($54.5 \pm 6.71\%$), calculated using SPSS 25.0 software (Fig. 1). The unusually high

DPPH scavenging activity potential of this plant extract shows the possibility of synergistic anti-oxidant activity of *C.diurnum* phytochemicals alongwith some interference from the extracts' pigments leading to overestimation of free radical scavenging activity. Neverthless, this proves the high and effective anti-oxidant potential of ethanolic extract of *C.diurnum* leaves and its potential to be used as free oxygen radical scavenger and also the need to further explore specific phytochemicals responsible for this activity.

TABLE I: Phytochemical screening analysis of ethanolic extract.

Phytochemicals	**Ethanolic extract**
Alkaloids	+ve
Phenols	+ve
Flavonoids	+ve
Tannins	-ve
Steroids	+ve
Saponins	+ve
Glycosides	-ve
Cardiac glycosides	+ve
Amino acids	-ve
Deoxy sugars	+ve

Ferric Reducing Antioxidant Power (FRAP) Assay: Reduction potential was assessed for ascorbic acid as well as *Cestrum diurnum* extract by calculating its linear regression. Ascorbic acid (0.66 ± 0.27FeSO4 E) was found to be significantly (P value-.0018) more powerful as reducing agent in comparison to *Cestrum diurnum* extract (0.11 ± 0.02 FeSO4 E) (Fig. 2).

C. Anti-inflammatory Potential Determination

Protein Denaturation Inhibition Assay: Independent sample t-test showed no significance difference (P value-0.88) in protein denaturation ability of Ansaid ($11.99 \pm 6.18\%$) and *Cestrum diurnum* extract ($13.21 \pm 15.47\%$), showing the potent and effective anti-inflammatory effect of ethanolic extract of *C.diurnum* leaves even in its crude form (Fig. 3)[14]. This test only highlights the biochemical aspect of plant extract in a cell-free system, mimicking the anti-inflammatory mechanism of NSAIDs. In-vivo studies are needed to strengthen and validate these results.

D. Toxicity Potential Determination

Toxicity Hemolytic Assay: Blood hemolytic activity of ethanolic extract of *Cestrum diurnum* was determined with a P value $- < 0.0001$, which showed 30% hemolysis at the concentration of 1000μ g/mL of *C.diurnum* extract used but only 2.5% hemolysis when used at the concentration of 100μ g/mL[15]. This shows that this ethanolic extract contains certain compounds responsible for toxicity when come in contact to blood in larger concentrations but is safe to be consumed in smaller doses for short term use. Further in-depth assays like cell viability assays, membrane integrity assays, in-vivo as well as organspecific toxicity assays. This also requires further evaluation of specific compounds found in the extract, responsible for this toxic response.

IV. CONCLUSION

Solanaceae plants are well established in terms of their potent pharmacological properties through various in-vitro and in-vivo techniques proving their anti-oxidant, anti-inflammatory, anti-microbial, analgesic and anti cancer potential, but *Cestrum diurnum* still remains one of the few least explored species of this family[16]. As reported for other species of the genus Cestrum, this species of *Cestrum diurnum* has shown through in-vitro studies that it contains potent anti-oxidant potential which can serve as a basis for this plants' leaves extract to be utilized and explored for their therapeutic and pharmacological role to treat oxidative stress conditions[17]. Anti-inflammatory activity of this plant extract is proven as its ability to inhibit protein denaturation which implies the therapeutic potential of this plant extract to alleviate inflammatory symptoms in conditions including diabetes, autoimmune, cardiovascular, neurodegenerative disorders. In addition, preliminary hemolytic toxicity evaluation showed minimal toxicity at 100μ g/mL. Current study is limited to the preliminary evaluation of anti-oxidant, antiinflammatory and toxicity analysis. Further research is required to elaborate the specific phytochemicals involved in imparting these therapeutic properties to the plant.

DECLARATION OF COMPETING INTEREST

The authors have no conflicts to disclose.

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