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PHYTOCHEMICAL AND *IN VITRO* ANTI-INFLAMMATORY SCREENING OF *INDIGOFERA SPECIES*

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ABSTRACT

Objective: Current research is to investigated the anti-inflammatory activity of two Indian medicinal plants, *Indigofera cordifolia* and *Indigofera glandulosa.*

Methods: Leaves of the both plants were collected and extracted with ethanol. Both ethanolic extracts were subjected to *invitro* anti-inflammatory activity evaluation by HRBC assay and COX enzyme Inhibition assay. COX-1 inhibition assay employed indomethacin as reference standard, while COX-2 inhibition assay employed celecoxib as reference.

Results: *Indigofera cordifolia* displayed highest percentage protection of 80.9±3.3 in HRBC assay, and percentage inhibition of 72.9±3.4 against COX-1, and 80.3±1.4 against COX-2. *Indigofera glandulosa* displayed relatively low percentage inhibition of 69.4±1.4 against COX-1 and 75.8±2.7 against COX-2.

Conclusion: Both plants under investigation displayed good anti-inflammatory activity. Comparatively *Indigofera cordifolia* disclosed more anti-inflammatory activity and specificity towards COX-2 enzyme than *Indigofera glandulosa*.

Keywords: *Indigofera cordifolia, Indigofera glandulosa,* Extraction, Anti-inflammatory activity

INTRODUCTION

Inflammation usually occurs when infectious microorganisms such as bacteria, viruses or fungi invade the body, reside in particular tissues and/or circulate in the blood **[1–3]**. Inflammation may also happen in response to processes such as tissue injury, cell death, cancer, ischemia and degeneration **[4, 5]**. Mostly, both the innate immune response and the adaptive immune response are involved in the formation of inflammation **[6]**. The innate immune system is the foremost defense mechanism against invading microorganisms and cancer cells, affecting the activity of various cells, including macrophages, mast cells, and dendritic cells. The adaptive immune systems involve the activity of more specialized cells such as B and T cells which are responsible for eradicating invading pathogens and cancer cells by producing specific receptors and antibodies **[7-9]**.

The majority of the therapeutic agents for treating inflammation-related diseases are of synthetic origin called NSAIDs. NSAIDs can reduce inflammation by blocking the metabolism of arachidonic acid by isoform of cyclooxygenase enzyme (COX-1 and/or COX-2), thereby reducing the production of prostaglandin. Numerous NSAIDs developed and marketed all over the globe, are prone to significant side effects *viz.*, Gastric ulcers, Renal damage, internal bleeding risk and hypertension **[10]**. In the context of safer medicines, the whole research community turned their interest into nature-centric medicines, which were so famous in the Indian subcontinent. Ayurveda and Herbal based therapies stand as an alternative to the drugs of synthetic origin with their potency and minimal side effects. Ayurveda and Chinese medicinal systems are the most acceptable traditional systems with the considerable research on pharmacognosy, chemistry, pharmacology, and clinical therapeutics **[11, 12]**.

The Indian subcontinent is richly endowed with diverse medicinal plants with anti-inflammatory activities that are effective in treating inflammatory conditions in traditional medicine. Several research reports proved the anti-inflammatory activity of Indian medicinal plant extracts **[13-17]** and isolated compounds **[18,19]**. The practice of using plants, their parts, or extracts as anti-inflammatory compounds has been known since antiquity. The use of plants or plant products for medicinal purposes was mainly documented from Vedic times to the modern era.

The genus *Indigofera* contains many species, and this abundance can be

explained by their ability to adapt to different biomes and their intercontinental dispersion **[20]**. Numerous plants from the *Indigofera* genus have antioxidant, antimicrobial, anti-inflammatory, anticancer activity, etc. **[21-23]**. Bearing the potential applications of genus *Indigofera*, current research focused on evaluating the phytochemical and anti-inflammatory activity of two plants, namely *Indigofera cordifolia* and *Indigofera glandulosa.*

MATERIALS AND METHODS:

Reagents and chemicals

All the chemicals, reagents were reagent grade, procured from Sigma Aldrich (laboratory grade), and the media were purchased from Hi-media.

Plants Material and Preparation of extracts

Aerial parts of *Indigofera cordifolia* and *Indigofera glandulosa*. were collected from the Osmania University campus, Hyderabad. The collected plant materials were shade-dried, powdered, and subjected to Soxhlet extraction with ethanol. The solvent was evaporated under vacuum to dryness to get the solid extract, and percentage yield was calculated.

Phytochemical Analysis

The preliminary phytochemical investigation of ethanolic extract of *Indigofera cordifolia* and *Indigofera glandulosa* were carried out by employing standard protocols. The ethanolic extracts of both plants were tested for the presence of alkaloids, saponins, glycosides, flavonoids, terpenoids, steroids, tannins, etc.

Invitro anti-inflammatory activity

HRBC Method

The human red blood cells (HRBC) method was used to estimate *in vitro* anti-inflammatory activity. The solutions used in this method are: i) Alsever's solution which was prepared dissolving 2.05% glucose, 0.41% NaCl, 0.81% trisodium citrate and 0.056% citric acid, in distilled water to final volume of 100 ml; ii) Hyposaline (0.7% NaCl); iii) Isosaline (0.9% NaCl); and iv) Phosphate buffer (pH 7.4). Blood (5 ml) was collected from a healthy volunteer and mixed with an equal volume of sterilized Alsever's solution **[24]**. The serum was obtained by centrifugation at 4000 rpm for 12 min., and the red blood cells (packed cells) were separated. The HRBC suspension was prepared by washing packed cells with isosaline solution (3 ml x 2), then the volume was adjusted to 10 ml with isosaline solution; 50, 100, and 200 mg of celery, myrrh, and fenugreek extracts were used. The dosages were dissolved in 1 ml of distilled water for the aqueous extracts, while the alcoholic fenugreek extract was

dissolved in 1 ml of 95% ethanol. Samples of each plant extract, control, and standard (indomethacin, at a dose 50, 75, 100 mg) were separately mixed with 1 ml of phosphate buffer, 2 ml of hyposaline, and 0.5 ml of HRBC suspension. The assay mixtures were incubated at 36.5 ºC for 30 min. in an oven; they were centrifuged at 3000 rpm for 10 min. The supernatant was decanted, and haemoglobin content was estimated using a spectrophotometer at 560 nm wavelength. The percentages of haemolysis were estimated by assuming the haemolysis produced in control as 100%, according to the following equation **[25]**.

$$\%\ \boldsymbol{Protection} = \mathbf{100} - \frac{(\boldsymbol{Absorbance\ of\ sample})}{(\boldsymbol{Absorbance\ of\ Control})}$$

Cyclooxygenase Inhibition Assay

In vitro COX-1 and COX-2 inhibition potency was estimated by Enzyme Immunoassay (EIA) method. EIA kit was used to examine the plant extracts activity toward COX-1 and COX-2 inhibition. The plant extracts were dissolved in 1 ml of DMSO (99%) in different amounts (25, 50, 100, and 200 µg/ml) to estimate the inhibition activities according to the manufacturer's protocol **[26]**. Indomethacin and Celecoxib were used as a positive control for COX-1 and COX-2 assays, respectively, in concentrations 25, 50, 100, and 200µg/ml. 10µl of plant extracts and positive control concentrations were added to the reagents from EIA kit, 960µl reaction buffer solution, 10µl COX-1, and COX-2 enzymes and 10µl heme. Then the solutions were incubated for 10 min at 36.5^0C; after that, 10µl of Arachidonic Acid (AA) were added: immediately afterward 50µl of 1 M HCl was added to finish the COX reaction. Stannous chloride (100µl) was added to convert prostaglandin H2 (PG-H2) to prostaglandin F2α (PG-F2α) via reduction reaction, the COX enzyme-catalyzed reaction of arachidonic acid to produce PG-H2 **[27]**. When the color of the solution became yellow, the concentration of the PG-F2α was estimated spectro- photometrically using a UV-Vis spectrophotometer at 418 nm. The percent of inhibition was calculated by comparing extracts measurements with control assessment **[28, 29]**.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The preliminary phytochemical study of ethanolic extracts of *Indigofera cordifolia* and *Indigofera glandulosa.* exposed that the extracts are instituted with various secondary metabolites such as alkaloids, carbohydrates, flavonoids, phenols, steroids, terpenoids, glycosides, tannins, saponins **(Table 1)**.

Invitro anti-inflammatory activity

HRBC Method

Results of human red blood cell membrane protection percentage of ethanolic extract

of *Indigofera cordifolia* and *Indigofera glandulosa* were given in **Table 2 and Figure 1**. The percentage protection of ethanolic extracts of *Indigofera cordifolia* and *Indigofera glandulosa* at 200µg/ml was 80.9±3.3 and 71.6±1.4 respectively compared to the standard drug indomethacin which exhibited percentage protection of 94.3±2.8 at 200µg/ml.

From the results of HRBC method, it is apparent that both *Indigofera cordifolia* and *Indigofera glandulosa* displayed noticeable anti-inflammatory potential relative to the standard drug indomethacin. It is important to note that at 100µg/ml and 200µg/ml concentrations, the extracts displayed good anti-inflammatory potential than the lower concentrations 25µg/ml and 50µg/ml. A dose-dependent trend is observed in the anti-inflammatory activity in both plant extracts. In between the two extracts, *Indigofera cordifolia* ethanolic extract relatively displayed more potency than *Indigofera glandulosa*.

Cyclooxygenase Inhibition Activity

COX-1 and COX-2 enzyme inhibition assay results were displayed in **Table 3, Figures 2 and 3**. Both COX-1 and COX-2 enzymes were actively inhibited. In the case of COX-1 enzyme, both ethanolic extracts displayed relatively significant ($p<0.05$) activity at the dose of 200µg/ml with percentage inhibition of 72.9±3.4 (*Indigofera cordifolia*), 69.4±1.4 (*Indigofera glandulosa*) compared to the standard indomethacin (95.4±3.1). Similarly, at the concentration of 200µg/ml, both ethanolic extracts displayed significant ($p<0.05$) inhibitory activity against COX-2 with percentage inhibition of 80.3±1.4 (*Indigofera cordifolia*)75.8±2.7 (*Indigofera glandulosa*) compared to the standard drug Celecoxib (96.02±2.4).

From the invitro COX inhibitory assay results, it is evident that both *Indigofera cordifolia* and *Indigofera glandulosa* plant ethanolic extracts are active against COX-1 and COX-2 enzymes. It was observed that *Indigofera cordifolia* ethanolic extract is a more active against the cyclooxygenase enzyme than *Indigofera glandulosa.* Percentage inhibition values of both extracts also implies that *Indigofera cordifolia* is relatively displayed more specificity towards COX -2 than COX-1 enzyme.

Table 1: Phytochemical screening of *Indigofera cordifolia* and *Indigofera glandulosa*.

Phytochemicals	Ethanol extract of *Indigofera cordifolia*	Ethanol extract of *Indigofera glandulosa*
Alkaloids	+	+
Glycosides	+	+
Flavonoids	+	+
Terpenoids	+	+
Steroids	+	+
Tannins	+	+
Proteins	+	+

Carbohydrates	+	+
Amino acids	+	+
Saponins	+	+

Table 2: Results of HRBC Method

Sample	Concentration (µg/ml)	Percentage protection
Indigofera cordifolia	25	20.7±1.9
	50	47.9±1.8
	100	70.8±3.7*
	200	80.9±3.3*
Indigofera glandulosa	25	20.7±1.9
	50	36.8±4.5
	100	54.9±2.7
	200	71.6±1.4*
Indomethacin	25	23.8±4.7
	50	56.9±3.9
	100	86.04±2.1*
	200	94.3±2.8*

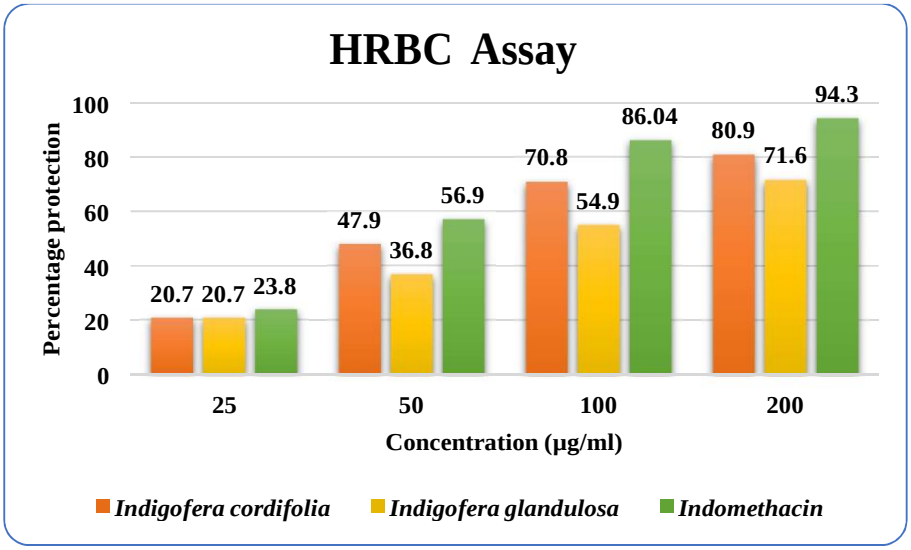

Figure 1: Graphical Representation of HRBC assay (% protection) of Ethanolic extract of *Indigofera cordifolia* and *Indigofera glandulosa*

Table 3: Results of COX enzyme inhibition assay

Sample	Concentration (µg/ml)	COX-1 (% Inhibition)	COX-2 (%Inhibition)
Indigofera cordifolia	25	23.8±3.4	29.4±1.9
	50	39.3±2.7	43.2±3.9
	100	60.8±1.6*	63.7±2.7
	200	72.9±3.4*	80.3±1.4*
Indigofera glandulosa	25	20.8±2.8	21.9±2.4
	50	35.8±2.2	38.2±4.8
	100	58.1±3.1	60.09±4.1
	200	69.4±1.4*	75.8±2.7*
Indomethacin	25	34.4±1.8	32.6±1.4
	50	63.7±3.2	66.8±3.3
	100	89.9±2.8*	91.7±1.6*
	200	95.4±3.1*	96.02±2.4*

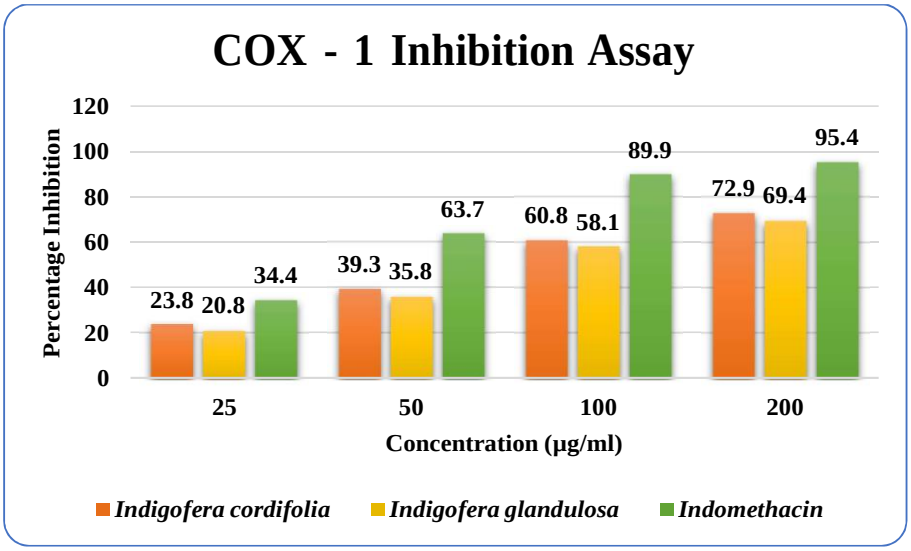

Figure 2: Graphical Representation of COX-1 Inhibition assay of Ethanolic extract of *Indigofera cordifolia* and *Indigofera glandulosa*

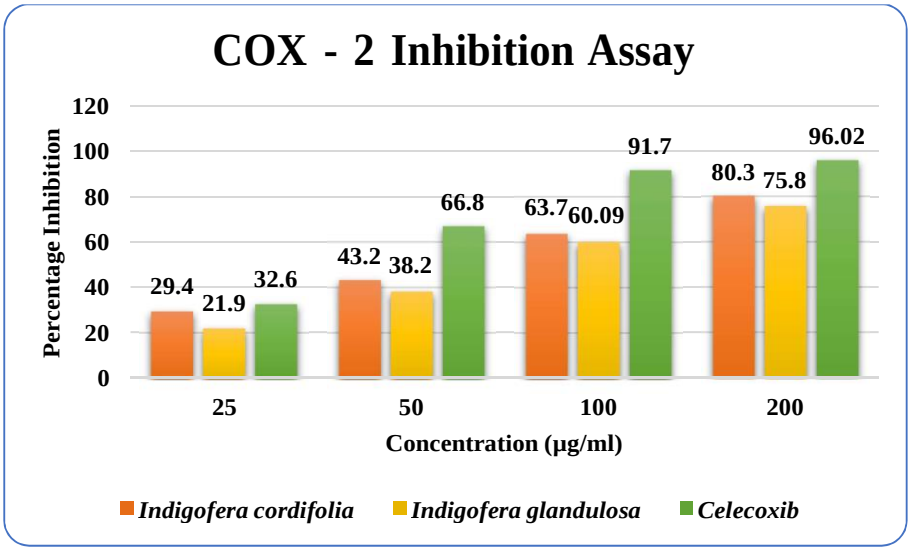

Figure 3: Graphical Representation of COX-2 Inhibition assay of Ethanolic extract of *Indigofera cordifolia* and *Indigofera glandulosa*

CONCLUSION

Two medicinal plants, *Indigofera cordifolia* and *Indigofera glandulosa* were extracted with ethanol. The phytochemical analysis affirm the presence of major secondary metabolites such as alkaloids and flavonoids terpenoids, steroids, glycosides and tannins. Anti-inflammatory activity evaluation by HRBC assay and COX enzyme inhibition assay revealed the anti-inflammatory potential of the ethanolic extracts of *Indigofera cordifolia* and *Indigofera glandulosa. Indigofera cordifolia* ethanolic extract proved to be more active against COX-1 and COX-2 than *Indigofera glandulosa.*

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Conflict of Interest

None

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