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Anti-inflammatory, Anticancer and Phytochemical Potential of *Indigofera cardifolia* Various Extracts

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ABSTRACT

New drugs for anti-inflammatory, anticancer research is emerging area to work using the medicinal plants and their active phytochemicals, among these already reported for potential property against various cancers and other disorders as a novel natural agent. The current investigation aimed to assess the phytochemicals, anti-inflammatory, anticancer properties using cell lines of various extracts of the Indian traditional medicinal plant *Indigofera cardifolia* aerial parts. To determine the anti-inflammatory activity, protein denaturation assay carried out using Aspirin standard drug and three extract of aerial parts of the *I. cardifolia*. In anticancer property using MTT (3-(4, 5-dimethythiazolyl 21-2 5-

dphenyttetrazolum bromide) in vitro cell proliferation assay for all the three extracts and screened qualitatively phytochemicals as per the Harnborne method to know the potential secondary metabolites. The anti-inflammatory property found potent in water extract by percent inhibition of protein denaturation showing IC_{50} value 92.97µg/ml compared to control Aspirin value 50.34 µg/ml. In anticancer studies also found potential water extract in *in vitro* proliferation MTT assay using MCF-7 cell lines by showing IC_{50} value 98.56µg/ml when compared to that of Cisplatin standard. The active key agents in the all the extract found positive result for phenols, glycosides, saponins, steroids, terpenoids and flavonoids in water extract only and other two extract shown positive for phenols and alkaloids. The results of this study conclude that *I. cardifolia* aerial parts possess the strong anti-inflammatory and anticancer potential which are emphasized by the presence active phytochemicals to be considered as active therapeutic agents.

Keywords: Anticancer, Anti-inflammatory, Aspirin, Cell lines, Cisplatin, Phytochemicals

1. Introduction

Natural products derived from plants for the treatment of diseases have proved that nature stands a golden mark to show the interrelationship between the man with environment. In research, utilization of herbal medicines in the treatment of the diseases increases every day. Plants is an important source of medicine and plays key role in world health. Medicinal herbs or plants have been known to be an important potential source of therapeutics or curatives aids. India has several traditional medicinal systems, such as Ayurveda, Sidda, Unani, etc which has survived through more than 3000 years, mainly using plant-based drugs. The ancient texts like Rigveda (4500-1600 BC) and Atharvaveda mentioned the of several plants as medicines. The books on Ayurvedic medicines such as Charaka Samhita and Susruth Samhita refers to use of more than 700 herbs [(1)].

Medicinal plants are plants containing inherent active ingredients used to cure or disease or relieve physiological consequences. *I. cardifolia* various parts were used as folk medicines by the people throughout the world [(2)].

In reported literature, extensive work has been done on various species of the genus Indigofera. For instance, *Indigofera oblongifolia* has shown its antimicrobial[(3)] hepatoprotective and lipoxygenase inhibitory activity[(4)]. Abubakar et al.[(5)], has reported the snake-venom neutralizing bustle of Indigofera pulchra. Antioxidant and free radical scavenging and anti-dyslipidemic actions of Indigofera tinctoria has also been reported[(6, 7)]. *Indigofera emarginella* has shown in-vitro antimalarial action against *Plasmodium falciparum.* Chakrabarti et al.[(8)], have reported the antidiabetic activity of *Indigofera mysorens*. Whole plant is used in hepatitis, whooping cough[(9)] antispasmodic[(10)], tonic[(11)], the extract prevents the development of hypoglycemia in the mouse[(12)]; the plant leaves, flowers and tender shoots are cooling and

demulcent, they are used in the form of leprosery and tumorous infection. The leaves are applied to abscesses. The roots are chewed in toothache and lethargy[11]. The alcoholic extract of the dried shoots has reported anti-inflammatory action[13]; the root bark is chomped in the mouth to relieve the abdominal pain[14]; leaves, bark and roots have antibacterial potency[15]. *Indigofera cardifolia* is an herb plant in the Fabaceae family. It has heart shaped leaves and is seasonal flowering plant. It Is considered as weedy plant in some situations but it can also improve crop yield due to its Nitrogen-fixing ability among the agriculture crops. Hence, knowing such property our interest taken up to study the biological and phytochemical property of this plant by systematic investigation.

2. Materials and Methods

2.1 Plant Materials

The leaves of *Indigofera cardifolia* were collected at the end of august 2021 from our university campus, with assistance of local traditional healer. And authenticated by Dr. Sanjeevkumar Giri, Department of Pharmaceutical Chemistry and General chemistry, Akka Mahadevi Women's University, Vijayapura, Karnataka, India.

Fig. (1). Morphological features of *Indigophora cardiflora* aerial parts

2.2 Preparation of Plant Extracts

The aerial plants of *Indigofera cardifolia* were collected, washed cleanly in distilled water and shade dried for complete removal of moisture. The aerial plant parts were chopped in to small pieces, powdered and used for Soxhlet extraction successively using chloroform, ethanol and water solvents for 24 hr and dried using Buchi's rotary vacuum evaporator and stored in refrigerated.

2.3 Qualitative Phytochemical Assay

Qualitative phytochemical screening of the chloroform, ethanol and water extracts of the aerial parts were carried out in order to analyse the class of organic metabolites. The all the extracts of *Indigofera cardifolia* aerial parts were analysed by standard chemical tests as described by Sharangouda and Patil[16], Harborne[17, 18] and Fransworth[19] to determine alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins steroids, tannins, tritepenoids.

2.4 Anti-inflammatory Studies by Protein Denaturation Assay

2.4.1 Principle

A Protein denaturation is the process in which protein lose their tertiary and secondary structures by applications of external stress or compound such strong acid or base, inorganic salts or heat. Most of the biological proteins loses their biological function when denatured. The denaturation of proteins is well documented cause of inflammation. Aspirin was used as the standard drug for anti-inflammation to check the recovery rate along with plant extracts[20].

2.4.2 Protocol

1. In a 15ml of centrifuge tubes, the reaction mixture consisted of 1 ml of phosphate buffer solution, 50µl of Bovine Serum Albumin were added and different concentrations (50,100.150.200.250µg/ml) of samples and the standard solution and heated for 15 minutes at room temperature. Denaturation was induced by keeping at 70 in hot water bath for 15 minutes. The absorbance was measured at 660 nm (Labman UV Visible Spectrophotometer).
2. The percent of inhibition of protein denaturation was calculated using a following forma % Protein denaturation actvity=((Ac-A)/Ac) 100

where, Ac and At are the absorbance of control and sample, respectively.

Aspirin was used as standard.

2.5 Anticancer Studies by MTT Assay

2.5.1 Principle

The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation The yellow tetrazolium MTT (3-(4, 5-dimethythiazolyl 21-2 5-dphenyttetrazolum bromide) is reduced by metabolically active cells in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. The assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability[21, 22].

2.5.2 Procedure

The cells were trypsinized and aspirated into a 15ml centrifuge tube Cell pellet was obtained by centrifugation at 300 x g. The cell count was adjusted, using DMEM medium, such that 200µl of suspension contained approximately 10.000 cells.

To each well of the 95 well microtitre plate. 200µl of the cell suspension was added and the plate was incubated at 37°C and 5% CO atmosphere for 24 h.

After 24 hr the spent medium was aspirated. 200µ of different test concentrations (100, 200, 300, 400 and 500 pg/ml from stock) of test drugs were added to the respective wells. The plate was then incubated at 37°C and 5% CO atmosphere for 24 h.

The plate was removed from the incubator and the drug containing media was aspirated 100µ of medium containing 10% MTT reagent was then added to each well to get a final concentration of 0.5mg/ml and the plate was incubated at 37°C and 5% CO atmosphere for 3 hours.

The culture medium was removed completely without disturbing the crystals formed. Then 100µl of solubilisation solution (DMSO) was added and the plate was gently shaken in a rotatory shaker to solubilize the formed formazan 6 The absorbance was measured using a microplate reader at a wavelength of 570 nm and also at 630 nm. The percentage growth inhibition was calculated, after the subtracting the background and the blank and the concentration of test drug needed to inhibit cell growth by 50 % (IC_{50}) was generated from the dose response curve for cell line.

3. Results and Discussion

3.1 Quantitative Phytochemical Assay of aerial plants of *Indigofera cardifolia*

The qualitative analysis of phytochemicals of *Indigofera cardifolia* aerial parts resulted for chloroform and ethanol extract positive for phenols and alkaloids and negative for flavonoids, glycosides and saponins, steroids, tannins and terpenoid, whereas water extract resulted positive for flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenoid and negative only for alkaloids (Table 1). Such work similarly found with many of the Indian traditional medicinal plants and reported for the active potential in various extract with different process of extraction and qualitative results[23 - 28].

Table 1: Showing results of Quantitative Phytochemical Assay of aerial plants of *Indigofera cardifolia*

Phytochemical Test	Results of *Indigofera cardifolia* aerial parts		
	Chloroform extract	Ethanol extract	Water extract
Phenols	+	+	+
Flavonoids	-	-	+
Steroid	-	-	+
Glycosides	-	-	+
Tannin	-	-	+
Saponins	-	-	+
Alkaloids	+	+	_
Terpenoids	-	-	+

+ = Positive; - = Negative

3.2 Results of Anti-inflammatory study by Protein Denaturation Assay using Standard Cisplatin along with Chloroform, Ethanol and Water extract of *Indigofera cardifolia* Aerial parts

Anti-inflammatory activity of *Indigophora cardifolia* aerial part extracts were studied by protein denaturation assay and it was observed inhibition concentration dependent and shown maximum activity in water extract resulting in 92.97 µg/ml IC_{50} value, whereas in chloroform and ethanol extract resulted almost similar 249.87 µg/ml and 248.20 µg/ml IC_{50} value respectively and standard Aspirin was found effective 50.34 µg/ml IC_{50} value, which is comparatively found best with the water extract to use it against inflammation. These studies are corelated with many new findings of researchers, which emphasize with medicinal plants to credit it in research and development for the wide range of application towards medical condition[29 - 32].

Table 2: Showing IC_{50} value of Anti-inflammatory activity of *Indigophora cardifolia* aerial part extracts were studied by protein denaturation assay

Extracts of *Indigofera cardifolia* aerial parts	**IC_{50} (µg/ ml)**
Standard Aspirin	50.34 µg/ml
Chloroform extract	249.87 µg/ml
Ethanol extract	248.20 µg/ml
Water extract	92.97 µg/ml

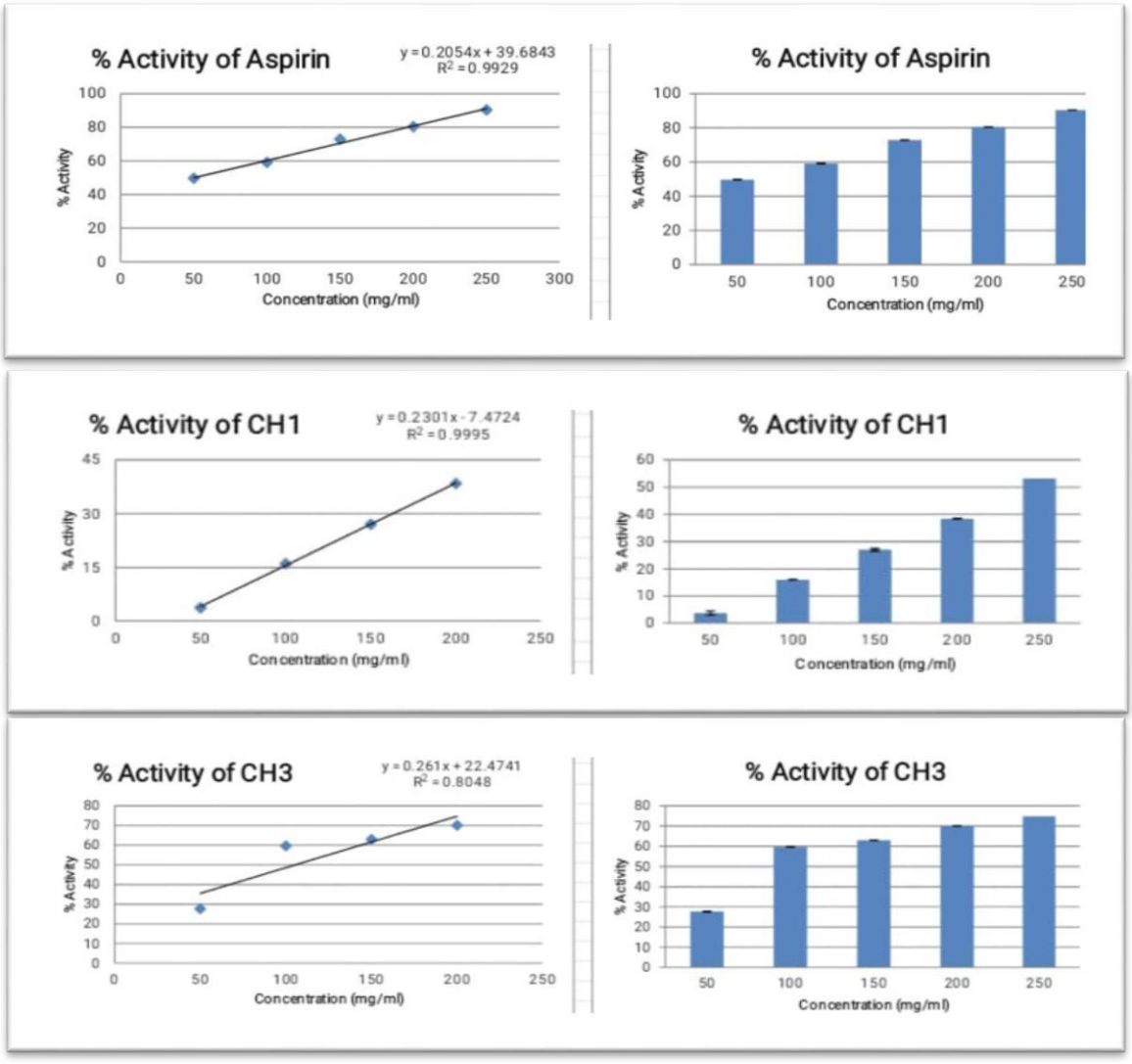

Fig. (2). Anti-inflammatory activity of standard aspirin along chloroform and water extract *Indigofera cardifolia* aerial parts

3.3 Anticancer studied by MTT Cytotoxicity Assay using Standard Cisplatin along with Chloroform, Ethanol and Water extract of *Indigofera cardifolia* Aerial parts

The cytotoxicity study was carried out for chloroform, ethanol and water extract of *Indigofera cardifolia* aerial parts on MCF-7 cell lines at different concentrations to determine the IC_{50} by MTT assay. Cytotoxicity of chloroform extract of the aerial parts of *I. cardifolia* against MCF-7 cell lines and found to be 96.83%, 82.44%, 67.72%, 58.16% and 35.57% toxic at a concentration of 100, 200, 300, 400, and 500 µg/ml; and cytotoxicity of ethanol extract aerial part of *I. cardifolia* against MCF-7 cell lines and found to be 86.18%, 76.24%, 53.00%, 46.48% and 30.85% toxic at a concentration of 100, 200, 300, 400, and 500 µg/ml respectively and whereas in water extract cytotoxicity of aerial part of *I. cardifolia* against MCF-7 cell lines and found to be 30.72%, 24.79%, 18.52%, 12.33% and 4.26% toxic at a concentration of 100, 200, 300, 400, and 500 µg/ml respectively. The IC_{50} value of 423.63 µg/ml obtained for MCF-7 of chloroform extract shown null effect, 360.96 µg/ml was obtained for ethanol extract shown very less effect on % cell viability and in water extract found maximum effect <100 µg/ml cytotoxicity toward MCF-7 was found to suppress the cell proliferation with concentration dependent and it was showed good cytotoxicity compared to other two extracts. The percentage of cell viability was found to be increasing with dose dependent concentration of the tested extracts and that have shown in Figures 3 and 4.

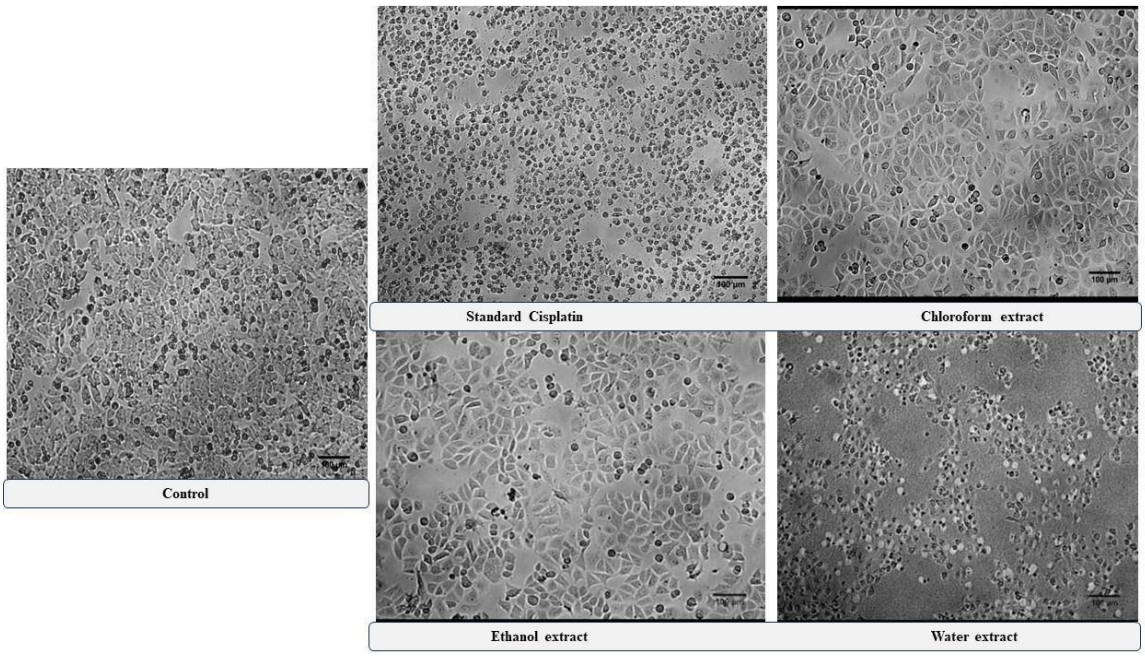

Fig. (3). Showing results of MTT cytotoxicity assay on MCF-7 cell lines by using standard Cisplatin along with chloroform, ethanol and water extract of *Indigofera cardifolia* aerial parts

Kolgi et al.[33], reported similar findings on flavonoid and alkaloid fraction from the leaves of *Leucas aspera* on 500 µg/ml concentration for the % of inhibition of cell growth of MCF-7 cell lines by MTT assay along with relative antioxidant activity. Previous reports on such studies reported from last one decade by various medicinal plants in all the parts of the worlds in in vitro studies for finding novel biomedicine against various types of cancers[34 - 36]. However, these assays may be used to guide to the further action for fractionation and isolation of potential anti-inflammatory and anticancer active compounds from the plant *I. cardifolia* aerial parts by the spectroscopy studies.

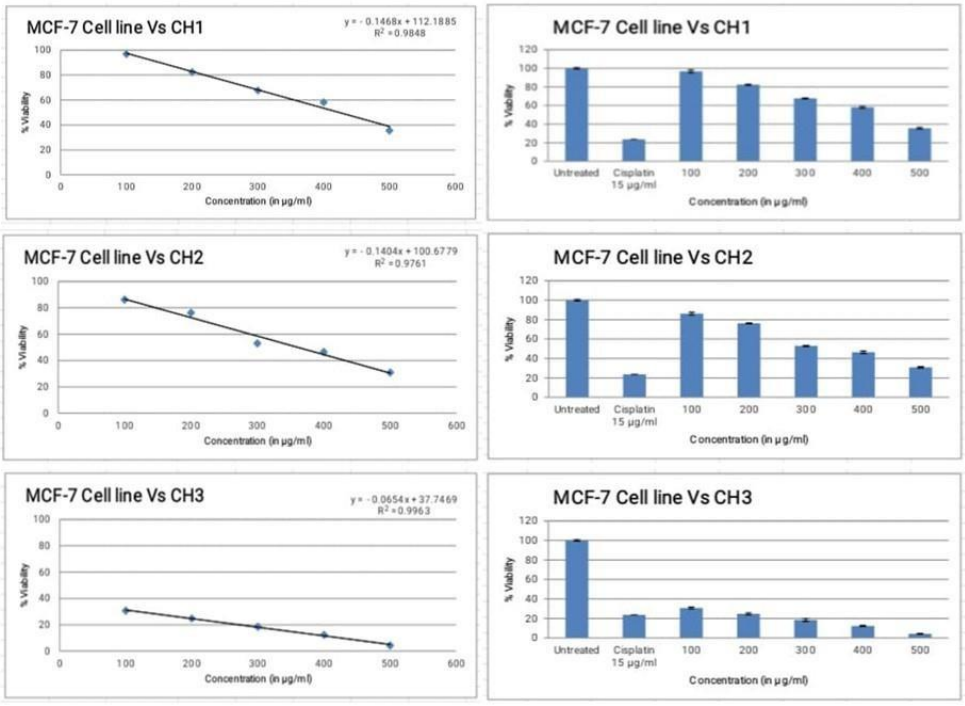

Fig. (4). Showing results of IC_{50} values with various concentration of standard Cisplatin along with chloroform, ethanol and water extract of *Indigofera cardifolia* aerial parts on MCF-7 cell lines

Table 2: The IC_{50} values of the *Indigofera cardifolia* aerial parts extracts for MCF -7 cell lines by MTT assay

Extracts of *Indigofera cardifolia* aerial parts	**MCF -7 cell line IC_{50} (µg/ml) 24 hours**
Chloroform Extract	423.63 µg/ml
Alcoholic Extract	360.96 µg/ml
Water Extract	<100 µg/ml

4. Conclusion

The current findings of this study revealed that *Indigofera cardifolia* aerial parts possess the strong anti-inflammatory and anticancer potential which are elucidated by the presence of active phytochemicals responsible for their therapeutic action. Anti-inflammatory studies by protein denaturation assay exhibited maximum activity in water extract when compared to standard Aspirin and control and other two extract were very less in inhibition in the action. It is also established the anticancer activity on MCF-7 cell lines by MTT assay with all the concentration in water extract similar to that of standard Cisplatin and other two extract doesn't

show any significance of the studies. Hence, this plant possesses all the biological property, proven with current studies and also supporting its medicinal use for other various ailments from the previous finding by other researchers. The plant possesses pharmaceutically vital due to the presence of most of the phytochemicals, further study on advanced in vivo research studies and spectral analysis of the aerial parts may use it as potent medicinal plant in the industry for various applications.

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