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ANTI-INFLAMMATORY AND ANTIBACTERIAL STUDIES ON *INDIGOFERA LINIFOLIA* RETZ.

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ABSTRACT

Keywords:

Indigofera linifolia,
Anti-inflammatory activity,
Kirby-Bauer disc diffusion method.

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Indigofera linifolia Retz (Fabaceae) is herb which spread throughout the India and found to be used by the tribal to cure inflammations. Based upon the review the present study focused on screening of Ant-inflammatory and Anti-bacterial activity of *Indigofera linifolia* Retz. The coarse powder of whole plant was extracted by cold maceration method using various solvents successively. Preliminary phytochemical screening was carried out to confirm the presence of phytoconstituents. Based upon the phytochemical tests chloroform and ethanol extracts (200mg/kg) were taken for anti-inflammatory and antibacterial activity screening. Anti inflammatory studies was carried out by Carrageenan induced paw edema method in Wistar albino rats. Anti bacterial screening was carried out in chloroform and ethanolic extract by disc diffusion method in three different concentrations (100µg, 150µg, 200µg /disc) and the diameter of the zone of inhibition were recorded. The percentage increase in paw edema was reduced in both chloroform (18.20±1.32) and ethanol extract (24.22±1.12) treated animals when compared with standard Indomethacin (19.18±1.02) and control (40.10±1.22) animals. The chloroform extract possessed significant anti inflammatory activity that may be due to its ability to prevent the production of some pro-inflammatory mediators. The chloroform and ethanolic extract showed the inhibitory activity against all the four strains of organisms, but the ethanolic extract showed potent inhibition against *Streptococcus pneumoniae* and *Staphylococcus aureus* in higher concentration (200 µg/disc) when compared with Ciprofloxacin (10µg /disc).

INTRODUCTION: *Indigofera linifolia* Retz (Fabaceae) is herb which spread throughout the India and found to be used by the tribal for various ailments. The leaves used as antibacterial, anti-oxidant and also used for its cytotoxicity effect in lung cancer. From the ethno botanical survey known that the entire plant was specifically used to cure inflammations¹⁻⁶. Based upon the review the present study focused on screening of Ant-inflammatory and Anti-bacterial activity of *Indigofera linifolia* Retz.



FIGURE 1: ENTIRE PLANT OF *INDIGOFERA LINIFOLIA* RETZ.

MATERIALS AND METHODS:

Collection and Extraction: The plant was authenticated and collected from Anaikuttam dam, near Sivakasi, Tamilnadu. The coarse powder was extracted by cold maceration method using various solvents such as hexane, chloroform, ethyl acetate and ethanol successively by cold maceration method. All the extracts were concentrated under reduced pressure and subjected to preliminary phytochemical screening⁷⁻⁸.

Acute Oral Toxicity: Acute oral toxicity was performed as per OECD guideline 423 in Swiss mice. The animal studies were carried out with the approval of institutional animal ethical committee. The chloroform and ethanolic extracts were administered orally in a

single dose (2000 mg/kg) by gavage. Animals were observed for 14 days for the symptoms such as tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma were observed.

Anti-inflammatory Activity: The anti-inflammatory study was carried out by Carrageenan induced paw edema method in Wistar albino rats⁹⁻¹⁰. The animals were divided into four groups such as chloroform extract (200mg/kg) and ethanol extract (200mg/kg), standard indomethacin (10mg/kg) and vehicle control groups. The paw volume was recorded at 0, 1, 2, 3 and 4 hr after carrageenan injection using a volume transducer attached with strain gauge coupler of student physiograph. The percentage increases in paw volume were calculated from the 4th hr reading.

TABLE 1: EFFECT OF EXTRACTS ON CARRAGEENAN INDUCED PAW EDEMA IN RATS

Treatment	Dose	Percentage increase in paw volume (%)
Control	0.3% CMC/kg	40.10 ± 1.22
Indomethacin	10mg/kg	19.18 ± 1.02**
Chloroform extract	200 mg/kg p.o.	18.20 ± 1.32**
Ethanolic extract	200 mg/kg p.o.	24.22 ± 1.12**

Values are expressed as mean ± SEM. **p<0.01 (One-way ANOVA followed by Dunnett's test)

Antibacterial Screening: Antibacterial screening was carried out by Kirby-Bauer disc diffusion method in chloroform and ethanolic extract using three different concentrations (100µg, 150µg, 200µg /disc) respectively. Both chloroform and methanol extracts were dissolved in dimethyl sulfoxide (DMSO). The strains of *Escherichia coli* (ATCC No. 8739), *Salmonella typhi* (ATCC 13311), *Streptococcus pneumonia* (ATCC 33400) and *Staphylococcus aureus* (ATCC 25923) were used. The Ciprofloxacin antibiotic disc (10µg /disc) was used as standard. About 25ml of Muller-Hinton Agar media was transferred into four sterile petridish. Each petridish were inoculated with 0.2ml of each inoculum by using sterile cotton swap. Sterile paper disc, impregnated with different concentrations of chloroform and ethanolic extracts were placed on the surface of the medium in each petri dish. The plates

were kept in incubator overnight and the zone of inhibition diameter was measured¹¹⁻¹³.

RESULTS: From the phytochemical study the presence of steroids, phenolics, saponins and flavonoids were identified in chloroform and ethanolic extracts of *Indigofera linifolia*. In the anti-inflammatory studies, the percentage increase in paw edema was reduced in both chloroform and ethanol extract treated animals when compared with standard Indomethacin. The chloroform and ethanolic extract showed the inhibitory activity against all the four strains of organisms. The ethanolic extract showed potent inhibition against *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* in higher concentration (200µg/disc) when compared with Ciprofloxacin (**Table 2, fig. 2-5**).

TABLE 2: ZONE OF INHIBITION IN DISC DIFFUSION METHOD

Organisms	Zone of Inhibition (mm)							
	Chloroform Extract (µg/ml)				Alcoholic Extract (µg/ml)			
	STD	100	150	200	STD	100	150	200
<i>Streptococcus pneumoniae</i>	27 ± 1.10	8 ± 0.34	11 ± 1.12	16 ± 1.58	24 ± 2.22	7 ± 0.26	13 ± 1.22	17 ± 1.18
<i>Escherichia coli</i>	16 ± 0.12	9 ± 0.18	13 ± 0.22	17 ± 0.26	15 ± 2.12	9 ± 1.12	12 ± 0.62	16 ± 0.76
<i>Salmonella typhi</i>	17 ± 1.86	6 ± 1.34	9 ± 1.28	12 ± 0.22	15 ± 1.14	8 ± 1.32	13 ± 1.22	18 ± 2.12
<i>Staphylococcus aureus</i>	25 ± 1.12	14 ± 1.20	18 ± 0.84	23 ± 0.64	24 ± 0.22	16 ± 1.26	19 ± 1.32	26 ± 1.24

Values are expressed as mean ± SEM. N = 3

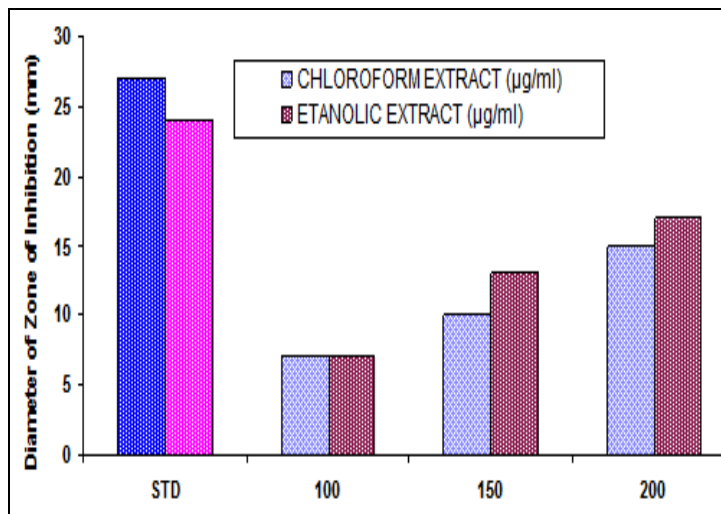


FIGURE 2: INHIBITORY EFFECT OF *INDIGOFERA LINIFOLIA* EXTRACTS AGAINST *STREPTOCOCCUS PNEUMONIAE*

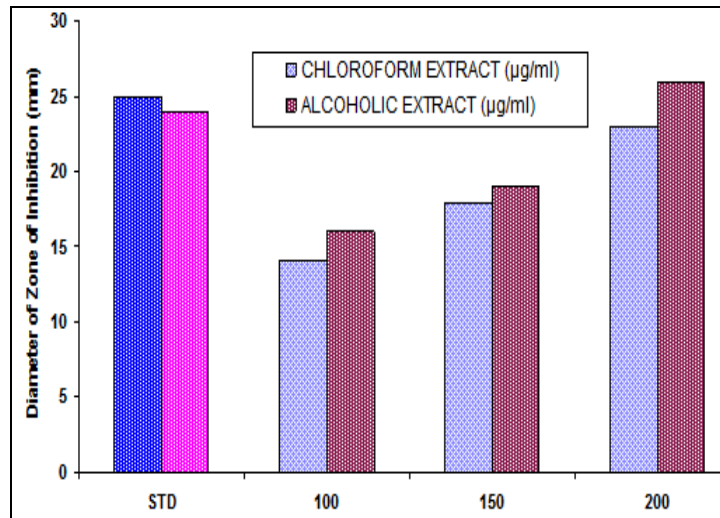


FIGURE 4: INHIBITORY EFFECT OF *INDIGOFERA LINIFOLIA* EXTRACTS AGAINST *STAPHYLOCOCCUS AUREUS*

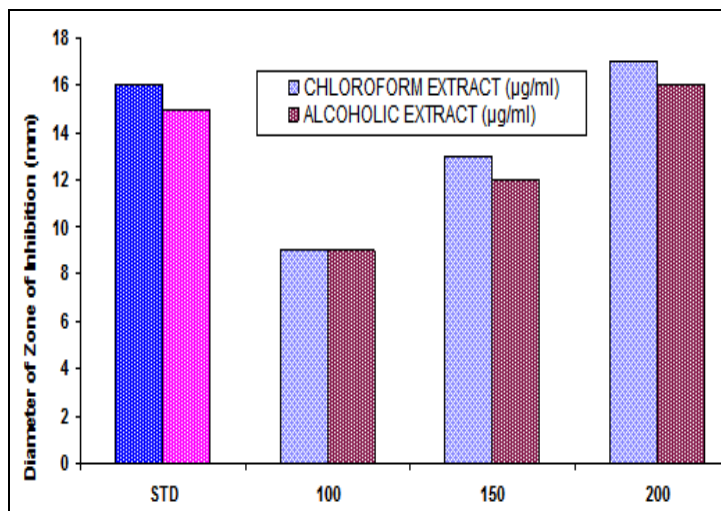


FIGURE 3: INHIBITORY EFFECT OF *INDIGOFERA LINIFOLIA* EXTRACTS AGAINST *ESCHERICHIA COLI*

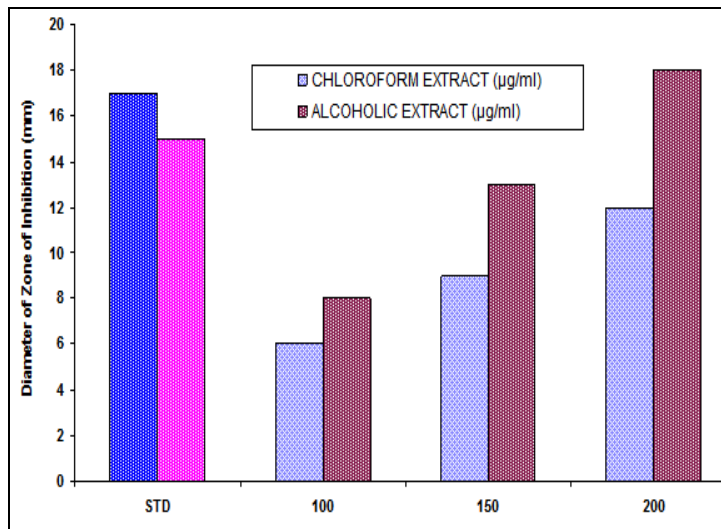


FIGURE 4: INHIBITORY EFFECT OF *INDIGOFERA LINIFOLIA* EXTRACTS AGAINST *SALMONELLA TYPHI*

DISCUSSION: The chloroform extract possessed significant anti-inflammatory activity. Activity may be due to its ability to prevent the production of some pro-inflammatory mediators. From the anti-bacterial screening both extracts showed moderate inhibition in all the test organisms.

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