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EVALUATING THE ANTIINFLAMMATORY POTENTIAL OF ISOLATED CONSTITUENTS FROM SEEDS OF *CAESALPINIA CRISTA*

M. Swapna Reddy[1, 2]*, B. RamyaKuber[1]

*[1] *Sri PadmavathiMahilaVisvavidyalayam, Tirupathi, Andhra Pradesh, INDIA.*

[2]*Vaagdevi Pharmacy College, Bollikunta, Warangal, Telangana, INDIA.*

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ABSTRACT

Three constituents - steroid, saponin and terpenoid were isolated from seeds of Caesalpinia crista *belonging to family* Caesalpiniaceae *by using chromatographic techniques. Their structures were characterized on the basis of NMR, MS and IR spectroscopic data. All the isolates were evaluated for anti-inflammatory effect against carrageenan induced rat paw oedema method. The constituents exhibited potent anti-inflammatory activity.*

KEYWORDS: *Anti-inflammatory activity, carrageenan induced rat paw oedema,* Caesalpinia crista*.*

INTRODUCTION

Caesalpinia crista belonging to family *Caesalpiniaceae/Fabaceae*is a prickly shrub widely distributed all over the world as shown in figure 1.The bitter principles Bonducin and Natin are the primary constituent of *Caesalpinia crista* apart from linolic acid, fatty acid, seta sitosterol and different diterpenes which mainly believed to be responsible for its wide therapeutic action. The plant has been recommended for the treatment of various diseases and disorders such as Antispasmodic, Malarial fever, leucorrhoea, abdominal pain, rheumatoid, arthritis, diabetes, cystic fibrosis, amenorrhoea[1-3].

Fig. 1:*Caesalpinia crista*

Objective: The aim of present investigation is to isolate constituents from seeds of *Caesalpinia crista* and to evaluate the anti-inflammatory potential of isolated constituents.

***Corresponding author:**

M. Swapna Reddy

Sri PadmavathiMahilaVisvavidyalayam,

Tirupathi, Andhra Pradesh, INDIA.

** E-Mail: swapnareddy81mpharm@gmail.com*

METHODOLOGY [4-7]

Preparation of extract:

In this study three compounds steroid, saponin and terpenoid were isolated from the seeds of *Caesalpinia crista.* Dried seeds (1.2 kg) were cut and defatted using *n*-hexane (3 × 2 L), then extracted with ethyl acetate (4 × 2 L). The ethylacetate extract was evaporated and concentrated under reduced pressure to afford a dark brown residue (14.1 g)

Phytochemical screening of extract:

Freshly prepared extract was subjected to standard phytochemical screening to ensure the presence of the following phytoconstituents: terpenoids, diterpenes, sesquiterpenes, steriods, saponins, fixed oils, fats, and carbohydrates.

Isolation of active constituents: Column chromatographic separations were performed on silica gel 60 (0.04–0.063 mm, Merck). TLC was performed on precoated TLC plates with silica gel 60 (layer thickness 0.2 mm, Merck). TLC spots were visualized by exposure to iodine vapours and UV radiation.

Characterization: The column was eluted with mixture of chloroform:n Hexane. Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized. The Greenish yellow compounds were eluted by column chromatography in the fractions of ethylacetate extract (Chloroform: n hexane) (30:70), (60:40), (80:30). The structures of isolated compounds were elucidated on the basis of its IR, 1D, 2D, NMR and MASS spectral data. The FTIR data is shown in table 1.

Anti-Inflammatory Activity of *Caesalpinia Crista*:[8, 9]

In vivo anti-inflammatory activity:

Paw oedema was induced on each rat by injecting 0.1 ml of carrageenan on physiological saline to the left hind paw. The isolates at different concentrations were administered orally 30 minutes prior to carrageenan administration. Paw volumes were measured at 60, 120, 180 and 240 minutes by mercury displacement method using plethysmograph. The percentage inhibition of paw volume in isolated treated groups was compared with control. Diclofenac sodium (5 mg/kg) was used as the standard.

Statistical analysis:

Statistical analysis was done using one way analysis of variance followed by Dunnets test*P* values greater than 0.05 were considered as significant.

RESULTS

The isolates of *C.c*at different concentrations showed significant reduction in the paw volume of rats. The *C.c*-1 at concentration of 400mg/ml showed potent activity compared with the reference standard Diclofenac sodium as shown in table 2.

Table No. 1: FTIR of constituent

Extract	Solvent system Mobile. P for TLC	Constituents	Rfvalues	FTIR spectral data
Ethyl acetate	Chloroform:Acetic acid 9:1	*C.c*-1	0.5	Aliphatic C-H stretch (2927.80 cm^{-1}) Aromatic C- H stretch (3000 cm^{-1}) C-O stretch (1017.88 cm^{-1} C=0 stretch (1733.60,1800 cm^{-1}) Aliphatic CH bends (1452.34,1378.22cm^{-1}
Ethyl acetate	Chloroform:Acetic acid 9:1	*C.c*-2	0.25	Aliphatic CH (2857.44cm^{-1}) CN stretch –(2311.68cm^{-1} long chain out of plane bending (757.58cm^{-1}) Aliphatic – CH bends (1452.34,1376.22cm^{-1})
Ethyl acetate	Chloroform:Acetic acid 9:1	*C.c*-3	0.08	Aliphatic C-H(2927cm^{-1}) Aliphatic C-H bends (1452.34-1378cm^{-1}) Long chain out of plane bending CH bends (-757.58cm^{-1})

Table No.2:Anti-inflammatory activity of *Caesalpinia* on the carrageenan - induced paw oedema

Drug	Dose (mg/kg)	Paw oedema reduction in minutes			
		60 min	120 min	180 min	240 min
Control		0.42± 0.19	0.44±0.089	0.46±0.28	0.48±0.18
Diclofenac	5	**0.18± 0.42***	**0.16±0.0.64***	**0.14±0.38***	**0.12±0.84***
C.c-1	200	0.28±0.42*	0.24±0.32*	0.22±0.84*	0.20±0.48*
C.c-1	400	**0.17±0.32*a**	**0.15±0.62*a**	**0.14±0.38*a**	**0.12±0.68*a**
C.c-2	200	0.28±0.25*	0.26±0.46*	0.24±0.048*	0.23±0.52*
C.c-2	400	**0.17±0.72*a**	**0.15±0.35*a**	**0.14±0.82*a**	**0.12±0.36*a**

*Values are expressed in mean ± SEM (n=6); *- P<0.05 with control; a- P<0.05 with standard*

CONCLUSION

Further confirmation of structures for constituents of *C.c-1, C.c-2, C.c-3* by higher analytical techniques.Further pharmacological screening of isolated constituents.

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