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Evaluation of Anti-Inflammatory Activity of Ethanolic Extract of *Canthium Parviflorum* by *In-Vivo* Method

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ABSTRACT

The present study investigates the anti-inflammatory activity in ethanolic extract of *Canthium Parviflorum* using carrageenan induced paw edema in albino rats. The medicinal values of the *Canthium Parviflorum* have been mentioned ancient literature as useful in disorders of inflammation. Dried leaves of *Canthium Parviflorum* powdered and extracted with ethanol using soxhlet apparatus. The anti-inflammatory activity was done by carrageenan induced hind paw edema method using plethys mometer. Diclofenac is used as a standard drug. The significant inhibitory activity shown by the leaf extract of *Canthium Parviflorum* (125, 250, and 375 mg/kg) over a period of 4h in carragenan-induced inflammation was quite similar to that exhibited by the group treated with diclofenac sodium. The highest percentage inhibition activity was found in the dose of 375 mg/kg with the mean percentage inhibition of 23.45% after 4 hours of extract administration.

Keywords: *Canthium Parviflorum*, Leaf ethanolic extract, Diclofenac Sodium, Anti-inflammatory activity, Carrageenan induced paw edema.

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INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as anti-inflammatory agents should therefore be viewed as a fruitful and logical research strategy in the search for new anti-inflammatory drugs. Inflammation may be potentially harmful, causing life threatening hypersensitivity reactions and progressive organ damage[1].NSAIDs are reported to possess prevention of the denaturation of proteins, which act as antigens and leads to auto-immune diseases[2].*Canthium* is a genus of flowering plants in the Rubiaceae family. These small trees the leaves are deciduous and the stems are usually thorny. A small thorny shrub grows up to 3 meters in height. Leaves simple, opposite, small, acute, with auxillary spines; flowers white, small in auxillary cymes. Fruits oblong two chambered drupe, become yellow when ripe. *Canthium*as herbal medicine is used for the treatment of diabetes[3], treatment of snake bites[4], scabies and the ring worm infection[5], antioxidant and diuretic activity[6].*CanthiumParviflorum*, is a valuable medicinal shrubby and woody plant which has been valued for centuries in Ayurvedic medicine. Phyto-chemical analysis of *Canthium Parviflorum* plant extracts revealed the presence of various bio-chemical compounds such as flavonoids, glycosides, alkaloids, saponins and terpinoids. Different parts of *Canthium Parviflorum*, have been used traditionally for the treatment of variety of diseases including anaemia, toothache, cough and as a hypoglycemic agent. Roots and leaves were used to reduce swellings in inflammation. However, there is no systematic scientific report published indicating utility of this plant material in the treatment of inflammation. Thus the presence of therapeutically active flavonoids as major constituents was the basis of selection and evaluation ethanol extract of *Canthium Parviflorum* leaves for their anti-inflammatory activity.

MATERIALS AND METHODS

Plant material

Fresh leaves of the plant *Canthium Parviflorum* were collected from Andhra Pradesh, India. The plant material was taxonomically identified by C.V.S. Bhaskar, Prof. in Botany, Venkatagiri Raja's college, SPSR Nellore, Andhra Pradesh, India. A voucher specimen has been preserved in our laboratory for future reference. The leaves were dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried powder material of the leaves was defatted with petroleum ether and subsequently extracted with ethanol in a Soxhlet apparatus. The solvent was completely removed under reduced pressure and Ethanol extract of *Canthium*

Parviflorum leaves was obtained (yield 18.5%). Solution of *Canthium Parviflorum* was prepared freshly in distilled water and used for the studies.

Extract Preparation

Plant parts were air dried at room temperature for 4 weeks to get consistent weight. The dried parts were later ground to powder. 25gm of the plant powder was weighed and transferred to a sterile beaker.125 ml of ethanol (1:5) was added to it and mixed well with shaker for 24 hours. When the powder mixed with ethanol thoroughly, it was filtered through Whatmann No: 1 filter paper. Then the solution was used for the experiments.

Experimental Animals

Male Albino rats weighing 200-250g were for animal studies. The animals were taken in polyacrylic cages and maintained under standard laboratory conditions (temperature 25±2 ˚C) and relative humidity (50 + 5%) with dark and light cycle (14/10h).They were allowed free access to standard dry pellet diet (Hindustan Lever, Bangalore, India) and water ad libitum. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA. The rats were acclimatized to laboratory condition for 14 days before commencement of experiment. Animal Ethics Committee; CPCSEA NO: 93/1999/CPCSEA.

Chemicals and drugs

Carrageenan, Standard drug Diclofenac and all other chemicals used were of analytical grade. Solvents were purchased from SD-Fine Chem Ltd. All other chemicals used were of analytical grade and purchased locally.

Phytochemical analysis

Phytochemical analysis was carried out for the presence of various phytochemical constituents i.e. saponins, steroids, phenol, alkaloids and tannins, flavonoids, glycosides, etc. Phytochemical screening was performed by employing standard screening tests[7].

Method

Anti-inflammatory activity by Carrageenan induced rat paw edema method

Anti-inflammatory activity was assessed by the method described by[8]. Albino rats of either sex weighing 200 – 250g were divided in 4 groups (N=6). Group-I received 0.5% CMC suspension (control), Group- II, III and IV received Ethanolic extract (125, 250, 375mg/kg, P.O) of *Canthium Parviflorum* respectively. Group-V received Diclofenac (reference standard 1mg/kg, P.O) [9]. Animals were treated with drugs by oral route and subsequently 1 h after treatment; 0.1ml of 1% suspension of carrageenan in normal saline was injected into the sub-planter region of left hind paw to induce edema. The paw volume was measured initially at 0, 1, 2, 3 and 4hr after

carrageenan injection using digital paw edema meter (520-R, IITC Life Science - USA). The difference between the initial and subsequent values gave the actual edema volume which was compared with control. The inhibition of inflammation was calculated using the formula, % inhibition = 100 (1-Vt/Vc), Where 'Vc' represents edema volume in control and 'Vt' edema volume in group treated with test extracts.

Statistical analysis

Data analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The Phyto-Chemical analysis results are represented in table1.

Table1: Phyto chemical Screening of *Canthium Parviflorum* extracts.

S.No	Test	Hexane Extract	Ethanolic Extract
1	Alkaloids	+	+
2	Saponins	+	+
3	Tanins	+	+
4	Phlobatanins	+	+
5	Steroids	-	-
6	Flavonoids	+	+
7	Anthraquinones	-	-
8	Glycosides	+	+
9	Reducing sugars	-	+
10	Terpenoids	+	+

+ Sign indicates Presence and – sign indicates Absence

Carrageenan induced paw edema

The effect of Ethanolic extracts of *CanthiumParviflorum*(125, 250 & 375 mg/kg) in carrageenan induced pawedema in rats is shown in Table 2 and 3. The Ethanolic extract of *Canthium Parviflorum*(375mg/kg) prevented the formation of edema induced by carrageenan and thus showed significant anti-inflammatory activity ($p<0.05$). The Ethanolic extract of *Canthium Parviflorum*(375 mg/kg) reduced the edema induced by carrageenan by 23.45% after 3hinjection of noxious agent as compared to the control vehicle treated group. Diclofenac sodium at 10mg/kg inhibited. The edema volume by 12.60%. On carrageenan induced acute inflammation model the Ethanolic extract (375mg/kg) produced better inhibition of paw edema.

Table 2: Effect of Ethanolic extract of leaves of *CanthiumParviflorum*on carrageenan induced paw edema in ratedema diameter (cm)

Treatment groups (n=6)	Dose (mg/kg)	0hr	1hr	2hr	3hr	4hr
Normal Saline	10ml/kg	0.95 ± 0.002	0.98 ± 0.003	0.98 ± 0.003	1.01 ± 0.02	1.03 ± 0.01
Ethanolic Extract	125	0.89 ± 0.01a	0.86 ± 0.005a	0.84 ± 0.008a	0.83 ± 0.02 a	0.78 ± 0.03a
Ethanolic Extract	250	0.92 ± 0.008b	0.90 ± 0.01a	0.88 ± 0.008a	0.85 ± 0.003a	0.82 ± 0.003a
Ethanolic Extract	375	0.80 ± 0.008a	0.79 ± 0.003a	0.77 ± 0.006a	0.75 ± 0.01a	0.73 ± 0.003a
Diclofenac standard	10	0.92 ± 0.003b	0.91 ± 0.003a	0.89 ± 0.003a	0.86 ± 0.05a	0.82 ± 0.003a

Table: 3Percentage inhibition of paw edema exhibited by Ethanolicleaf extract of *Canthium Parviflorum* Treatment Percentage inhibition (%) at various times intervals

	1hr	2hr	3hr	4hr	Mean of % inhibition
Ethanolic Extract 125 mg/kg	10.24	12.28	18.82	27.00	17.08
Ethanolic Extract 250 mg/kg	06.16	08.20	15.00	23.00	13.09
Ethanolic Extract 375 mg/kg	17.38	19.42	25.00	32.00	23.45

The most widely used primary test to screen new anti-inflammatory agent's measure the ability of a compound to reduce local edema induced in the rat paw by injection of an irritant agent[8]. Carrageenan induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1 – 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymer phonuclear cells and prostaglandins produced by tissue macrophages[10,11]. The significant inhibitory activity shown by the leaf extract of *Canthium Parviflorum* (125, 250, and 375 mg/kg) over a period of 4 h in carragenan-induced inflammation was quite similar to that exhibited by the group treated with diclofenac sodium. The highest percentage inhibition activity was found in the dose of 375 mg/kg with the mean percentage inhibition of 23.45 after 4 hours of extract administration. These results indicate that the extract acts in later phases in dose dependent manner, probably involving arachidonic acid metabolites, which produce an edema dependent on neutrophils mobilization[12]. This anti-inflammatory effect of the extract observed might be due to the presence of flavonoids in the plant.

CONCLUSION

The result obtained from the experiment it is concluded that the Ethanolic extract of *Canthium Parviflorum* (375mg/kg) having good anti-inflammatory activities and it shown dose dependent activities. The results support the traditional use of this plant in inflammatory conditions and suggest the presence of biologically active components which may be worth further investigation and elucidation.

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