

# Screening of Chloroform and Methanolic Bark Extracts of *Commiphora caudata* for its *In-vitro* Anti-bacterial, Anti-fungal and *In-vivo* Anti-inflammatory Activity

## Research Article

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## Abstract

Herbal medicines and their preparations have been widely used from the thousands of years in developing and developed countries in the primary health care of society and community. They have great demand due its safety, efficacy with minimum side or adverse effects. *Commiphora caudata*. It's known as konda mava in kannada, ikkata in Sanskrit and hill mango in English. It's widely used in the management of various disorders. Hence the identification of bioactive fractions from various parts of selected medicinal plant is important. In the present research work an attempt has been made to screen and assess the antibacterial, antifungal and anti-inflammatory activities of *Commiphora caudata* barks. The barks of selected plant material were collected, authenticated, powdered and subjected for extraction procedure. The extracts were screened for presence of various phytoconstituents. The antibacterial activity of chloroform and methanolic extracts were performed against various strains of bacteria and fungi. The extracts also were investigated for its in-vivo anti-inflammatory activity. The result of investigation concludes that chloroform and methanolic extract of plant were potential to inhibit the growth of selected strains of microorganism and also produced potential anti-inflammatory effect.

**Key Words:** *Anti-bacterial, Anti-fungal, Anti-inflammatory, Commiphora caudata, Bark Extracts, Herbal Medicines.*

## Introduction

*Commiphora caudata* belongs to the family of Burseraceae belongs to genus *Commiphora*. It's known as konda mava in kannada, ikkata in Sanskrit and hill mango in English (1). It is found in India: Karnataka, Andhra Pradesh, Kerala, Tamilnadu and Srilanka (2). From ancient times, the plants have been used as raw material for cosmetics, pharmaceuticals, botanical pesticides, disinfectants, insect repellents, herbal teas, herbal drinks, etc. Chemical constituents of plant origin have long been known to possess several biological activities (3). It is estimated that about 50,000-70,000 plant species are used in traditional and

modern medicine throughout the World. These species make a significant contribution to healthcare and along with species used for their aromatic properties, in herbal products, pharmaceuticals and fragrances (4). The essential oils are effective against microorganisms, and have been recognized as natural antioxidants. Plant pacifies vitiated vatta, pitta, diabetes, low back pain, sciatica, fever, arthritis, urinary retention, antibacterial antiulcer, hepatoprotective, antioxidant etc and Seed used to relive stomach ache (5,6) The literature survey revealed that many of the *Commiphora* species contain Sterols, Terpenes, Flavonoids, Resins and Tannins, Polyphenols. A survey of literature (7-9) revealed that *Commiphora caudata* is used for the treatment of inflammation but lack of scientific data is available on its anti-inflammatory activity. Keeping in view, the anti-inflammatory activity of bark is evaluated.

## Materials and Methods

### Collection and Identification of Plant Material

The barks of *Commiphora caudata* were collected around the local area of Mandya (Karnataka),

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authenticated by Regional Research Institute, Bangalore (Karnataka).

**Chemicals and Reagents**

All solvents methanol, ethanol, chloroform and ethyl acetate used for extraction purpose were purchased from Merk. The chemicals and reagents used for phytochemistry research were obtained from store house of Maratha Mandal College of Pharmacy, Belagavi. Standard drug Ciprofloxacin was procured from Micro Labs, Bengaluru.

**Preparation of Bark Extracts**

The barks of the plant were collected, washed and air dried. They were powdered in Willy mill and successively extracted in chloroform and methanol. Extraction was carried out by using soxhlet. The extracts were concentrated under vacuum to get the residues. The residues were weighed and percentage yield was calculated. They were then stored in well closed container for phytochemical and pharmacological evaluation.

**Phytochemical Screening of the bark extracts**

The phytochemical evaluation was performed for both the extracts to detect the presence of various chemical constituents (10-16).

**Screening of Antibacterial, Antifungal and Anti-inflammatory Activities**

*In-vitro* antibacterial (17-19) and antifungal activities and *in-vivo* anti-inflammatory (5-7, 17-21) activities were examined for both the extracts. The antibacterial activity of bark extracts was screened against eight pathogenic bacterial strains, *Bacillus subtilis*, *Escherichia coli*, *Bacillus pumilis*, *Pseudomonas aeruginosa*, *Bacillus cerus*, *Pseudomonas vulgaris*, *Staphylococcus aureus* and *Serratia marces* respectively. The antifungal activity was screened for three pathogenic fungi strains *Aspergillus Niger*, *Saccharomyces cereviceae*, and *Penicillin chrysozenum*. Antifungal activity was investigated by the agar disk diffusion method. The antibacterial activity testing carried out by using agar cup method. Each purified extracts were dissolved in dimethyl sulfoxide, sterilized by filtration using sintered glass filter, and stored at 4°C. For the determination of zone of inhibition, pure bacterial and fungal strains were taken as a standard antibiotic for comparison of the results. Control experiments were carried out under similar condition by using Amoxicillin for antibacterial activity and Ketoconazole for antifungal activity as standard drugs. The zones of growth inhibition around the disks were measured after 18 to 24 hours of in incubation at 37°C for bacteria and 48 to 96 hours for fungi at 28°C. The sensitivities of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8 mm were considered as not active against microorganisms.

**Screening of in-vivo Anti-inflammatory activity by Carrageenan-induced paws edema method in rats.**

In this method 0.1 ml of 1% carrageenan in 0.9% sterile normal saline solution (NSS) was injected subcutaneously into the sub plantar area of right hind paw of the rats to induce edema.

Male rats weighing between 200-250g were selected, housed under laboratory temperature and were divided into eight groups (17-20). Each group consisting of six rats each and details were presented in Table 1.

**Table 1: Groups for Animal Experiment**

Groups	Details of treatment
Group I	(Control) which was served with Normal Saline.
Group II	(Standard) which was served with Ibuprofen (10mg/kg)
Group III	(Chloroform extract <i>Commiphora caudata</i> ) which was served with Chloroform extract (100mg/kg)
Group IV	(Chloroform extract <i>Commiphora caudata</i> ) which was served with Chloroform extract (300mg/kg)
Group V	(Chloroform extract <i>Commiphora caudata</i> ) which was served with Chloroform extract (500mg/kg)
Group VI	(Methanolic extract <i>Commiphora caudata</i> ) which was served with methanolic extract (100mg/kg)
Group VII	(Methanolic extract <i>Commiphora caudata</i> ) which was served with methanolic extract (300mg/kg).
Group VIII	(Methanolic extract <i>Commiphora caudata</i> ) which was served with methanolic extract (500mg/kg)

Each group was orally administered 5% Tween 80, Ibuprofen (10 mg/kg), and various doses of Methanolic Extract (ME) and Chloroform Extract (CE) (100, 300, and 500 mg/kg) and as the control, reference, and test group, respectively. Samples were feed one hour prior to carrageenan injection. The edema volumes were determined using a plethysmometer at 1, 3, and 5 hr after carrageenan injection. Results were expressed as mean ± SEM compared with the control group at each time interval. Statistical significance was determined by one-way analysis of variance (ANOVA) at the 95% confidence interval. The Percentage inhibition of edema was calculated for each group with respect to the control group.

$$\% \text{ inhibition} = 100[1 - (V_t - V_c)]$$

Where, Vt and Vc are the volumes of carrageenan-induced paws of the drug treated group and control group respectively.

**Results**

**Phytochemical extraction and screening of bark extracts**

The residue of Chloroform extract *Commiphora caudata* was dark brownish in color, gave positive Libermann Buchard reactions for Sterols (pink-blue-

green) and tri-terpenoids and positive test for ferric chloride (olive green). The methanolic extract residue of *Commiphora caudata* was dark reddish in color, gave positive Libermann Buchard reaction for sterols, triterpenoids and flavonoids.

### Anti-bacterial activity

The antibacterial activity of the extracts was studied in different concentrations on eight pathogenic bacterial strains, *Bacillus subtilis*, *Escherichia coli*, *Bacillus pumilis*, *Pseudomonas aeruginosa*, *Bacillus cerus*, *Pseudomonas vulgaris*, *Staphylococcus aureus* and *Serratia marzens* and three pathogenic fungi *Aspergillus Niger*, *Saccharomyces cereviceae*, and *Penicillin chrysozenum*. Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial and antifungal activities are presented in Table 2 and Table 3 respectively. The chloroform and methanolic extracts of *Commiphora caudata* posses

potential antibacterial activity against Gram (+) ve and Gram (-) ve organisms (*Bacillus subtilis*, *Bacillus pumili*, *Bacillus cerus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas vulgaris*, *Serratia marzens*).

When tested by the disc diffusion method, the chloroform extracts 100 mg/ml and 300 mg/ml showed mild activity against *Staphylococcus aureus*, *Serratia marzens* around 13 mm highest zone of inhibition. The lowest activity was seen in *Bacillus subtilis*, *Pseudomonas vulgaris* i.e., around 08 mm zone of inhibition. The methanolic extract 100mg/ml and 300mg/ml showed moderate activity. The highest activity of 24 mm zone of inhibition was seen in *Serratia marzens* and least activity was recorded in *Bacillus subtilis*, measured 16 mm zone of inhibition. The activity increased with increasing concentrations. However, the activity by the extract was low when compared with of standard Amoxicillin around 28 mm zone of inhibition in *Bacillus subtilis*.

**Table 2: Anti-bacterial activity of barks of *Commiphora caudata***

Extract	Concentration	BS	BP	BC	SA	EC	PA	PV	SM
Chloroform extract	(100 mg/ml)	8	10	11	12	10	9	8	11
	(300 mg/ml)	9	11	11	13	12	12	11	13
Methanolic extract	(100 mg/ml)	12	15	16	15	16	14	16	17
	(300 mg/ml)	16	17	16	18	17	18	17	20
Amoxicillin	10 µg/ml	28	23	21	24	18	20	18	24

\*BS: *Bacillus subtilis*, EC: *Escherichia coli*, BP: *Bacillus pumilis*, PA: *Pseudomonas aeruginosa*, BC: *Bacillus cerus*, PV: *Pseudomonas vulgaris*, SA: *Staphylococcus aureus* and SM: *Serratia marzens*

**Table 3: Antifungal activity of barks of *Commiphora caudata***

Extract	Concentration	AN	SC	PC
Chloroform extract	(100mg/ml)	12	12	14
	(300mg/ml)	15	17	16
Methanolic extract	(100mg/ml)	19	20	19
	(300mg/ml)	24	23	20
Ketoconazole	10µg/ml	30	28	23

\*AN: *Aspergillus Niger*, SC: *Saccharomyces cereviceae*, PC: *Penicillin chrysozenum*.

### Anti-inflammatory Activity

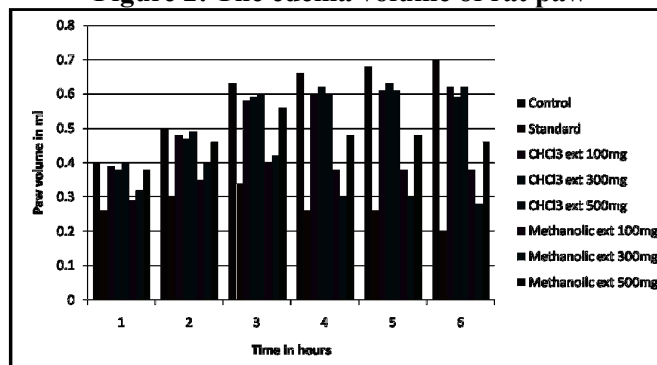
The *in vivo* anti-inflammatory activities of both extracts were confirmed by the carrageenan-induced paw edema model. The oral administration of methanolic extract at the dose of 500 mg/kg reduced the swollen of carrageenan-induced paw edema with significant difference from control at 1, 3, and 5 hr and was not different from the standard drug, Ibuprofen (Figure 2). Oral administration of chloroform extract at the concentration of 500 mg/kg showed the anti-inflammatory activity by inhibiting the acute phase of inflammation. Based on the inhibitory effect of the CE seen at the 3<sup>rd</sup> hr and 5<sup>th</sup> hr, it suggests that the main mechanism of action may be due to inhibition of PGs synthesis. Moreover, the inhibitory effect of the extracts may partly involve other acute inflammatory mediators such as histamine, serotonin, bradykinin, and proinflammatory cytokines which are released during the 1<sup>st</sup> hr after carrageenan injection. The results were tabulated and data is statistically analyzed using one-

way ANOVA followed by Dunnet's test & results were presented in the Table 4 and Table 5.

**Figure 1: *Commiphora caudata***



**Figure 2: The edema volume of rat paw**





**Table 4: Anti inflammatory activity of *Commiphora caudata* bark extracts**

Treatment	Dose	Volume of mercury displaced in ml at various time intervals in hours.					
		0	1	2	3	4	5
Control	0.1ml 1% W/V	0.40 ± 0.025	0.50± 0.057	0.63± 0.057	0.66± 0.057	0.68± 0.057	0.70± 0.058
Ibuprofen	10 mg/ kg	0.26± 0.005	0.30±*** 0.005	0.34±*** 0.002	0.26±*** 0.003	0.26±*** 0.003	0.20±*** 0.04
Chloroform Ext. of C.C.	100 mg/ kg	0.39± 0.01	0.48± <sup>NS</sup> 0.01	0.58± <sup>NS</sup> 0.02	0.60± <sup>NS</sup> 0.01	0.61± <sup>NS</sup> 0.01	0.62± <sup>NS</sup> 0.01
Chloroform Ext. of C.C.	300 mg/ kg	0.38± 0.01	0.47± <sup>NS</sup> 0.03	0.59± <sup>NS</sup> 0.05	0.62± <sup>NS</sup> 0.04	0.63± <sup>NS</sup> 0.03	0.59± <sup>NS</sup> 0.02
Chloroform Ext of C.C	500 mg/ kg	0.40± 0.01	0.49± <sup>NS</sup> 0.01	0.60± <sup>NS</sup> 0.02	0.60± <sup>NS</sup> 0.01	0.61± <sup>NS</sup> 0.03	0.62± <sup>NS</sup> 0.04
Methanolic Ext of C.C	100 mg/ kg	0.29± 0.01	0.35±** 0.02	0.40±** 0.05	0.38±*** 0.03	0.38±*** 0.01	0.38±*** 0.04
Methanolic Ext of C.C	300 mg/ kg	0.32± 0.01	0.40±** 0.02	0.42±** 0.05	0.30±*** 0.03	0.30±*** 0.01	0.28±*** 0.04
Methanolic Ext of C.C	500 mg/kg	0.38± 0.01	0.46±** 0.02	0.56±* 0.05	0.48±* 0.03	0.48±** 0.01	0.46±** 0.04

Significance of difference in control and extracts treated groups were determined by one way analysis of variance (ANOVA) \*\*\* P<0.001 \*\*P<0.01, \* P<0.05 are significant and <sup>NS</sup> P> 0.05, are not significant. All values are means of individual data obtained from six rats (n=6)

**Table 5: Percentage inhibition of *Commiphora caudata* bark extracts**

Treatment	Dose	% Inhibition of paw volume at various time Intervals					
		0	1	2	3	4	5
Control	0.1ml1% W/V	0	0	0	0	0	0
Ibuprofen	10 mg/ kg	0	40	46.03	60.60	61.76	71.42
Methanolic Extract	100 mg/ kg	0	30	36.5	42.42	44.11	45.71
Methanolic Extract	300 mg/ kg	0	20	33.33	54.14	55.88	61.42
Methanolic Extract	500 mg/ kg	0	8	11.11	27.27	29.41	34.28
Chloroform Extract	100 mg/ kg	0	4	7.3	9.09	10	11.4
Chloroform Extract	300 mg/ kg	0	6	6.3	6.06	7.3	15
Chloroform Extract	500 mg/ kg	0	2	4.5	9.09	10	11.4

## Discussion

The residue of Chloroform extract *Commiphora caudata* was dark brownish in color, gave positive Libermann Buchard reactions for Sterols (pink-blue-green) and tri-terpenoids and positive test for ferric chloride (olive green). The methanolic extract residue of *Commiphora caudata* was dark reddish in color, gave positive Libermann Buchard reaction for sterols, triterpenoids and flavonoids. The antibacterial activity of the extracts was studied in different concentrations on eight pathogenic bacterial strains, and three pathogenic fungi. The *in vivo* anti-inflammatory activities of both extracts were confirmed by the carrageenan-induced paw edema model. The oral administration of methanolic extract at the dose of 500 mg/kg reduced the swollen of carrageenan-induced paw edema with significant difference from control at 1, 3, and 5 hr and was not different from the standard drug, Ibuprofen.

## Conclusion

Herbal medicines and their preparations have been widely used from the thousands of years in developing and developed countries in the primary health care of

society and community. Our findings strongly confirmed the chloroform and methanolic bark extract of selected plant has potential anti-microbial and anti-inflammatory activity. We have screened and assessed the antibacterial, antifungal and anti-inflammatory activities of *Commiphora caudata* barks. The chloroform and methanolic extracts of *Commiphora caudata* bark found to possess potential antibacterial activity against Gram (+) ve and Gram (-) ve organisms. It is also exerting its antifungal activity against different strains. The *in vivo* anti-inflammatory activities of both extracts were confirmed by the carrageenan-induced paw edema model.

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## List of Abbreviations

BS: *Bacillus subtilis*,  
 EC: *Escherichia coli*,  
 BP: *Bacillus pumilis*,  
 PA: *Pseudomonas aeruginosa*,

BC: *Bacillus cerus*,  
 PV: *Pseudomonas vulgaris*,  
 SA: *Staphylococcus aureus*  
 SM: *Serratia marzens*,  
 AN: *Aspergillus Niger*,  
 SC: *Saccharomyces cereviceae*,  
 PC: *Penicillin chrysozenum*

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