

Anti-Inflammatory and Anti-Microbial Activity of Chalcone from *Dalbergia Sissoo* Roxb. leaves

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ABSTRACT

In present work isolation and characterisation of a chalcone [(*E*)-3-(3,4-dihydroxyphenyl)-1-(2,3,4-trihydroxyphenyl) prop-2-en-1-one] or okanin has been described. The isolation was based on bio-assay guided fractionation. The methanol, hexane extracts and isolated okanin from methanol extracts were exhibited good antibacterial activity towards various pathogens gram positive (*Micrococcus luteus* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*, *R. planticola* and *Acinetobacter*). The anti-inflammatory activity of hexane extracts, methanol extracts of *Dalbergia sisso* Roxb and okanin have been studied. The methanolic extract showed maximum activity. This is the first report of any chalcone from the genus *Dalbergia*. The finding suggests more research would be required for presence of new phytoconstituents.

Keywords: *Dalbergia sisso* Roxb, okanin, Carrageenan, Anti-inflammatory activity, Anti-microbial activity.

INTRODUCTION

A handsome specimen, shade forming tree or street tree, Indian rose wood has delicate, light green, oval, pointed leaflets and can quickly reach 60 feet in height. Leaves alternate, rachis zig-zag, elliptic in shape and leaf colour is green. The flowers are sessile shorter than leaves, fruit pods narrowed at the base in to a long stalk, seeds 1-4 per pod. The lateral

branches remain smaller than two third the trunk diameter to ensure good tree structure^{1a, 1b, 1c}. It has been possess aphrodisiac, abortifacient, expectorant, anthelmintic and antipyretic. It is used in conditions like emesis, ulcers, leucoderma, dysentery, stomach troubles and skin diseases. Ayurvedic practioners also prescribe the leaf juice for eye ailments.

Leaf extract has been used to treat sore throats, heart problems, dysentery, syphilis, gonorrhoea. The extract of *D. sissoo* leaves showed antioxidant activity, is almost two times higher than other commonly used antioxidants like Selenium and vitamin E^{2a, 2b}. The extract of *Dalbergia* species was also possess brain tonic³ and antioxidant activity⁴. Dried leaves of *Dalbergia sissoo* is exhibited antibacterial, antiprotozoal, anti-inflammatory activity⁵. The tribal peoples of Odisha use this for treatment of Scalding of urine to which the tribal's are more vulnerable^{6a, 6b}.

The *Dalbergia sissoo* composed of new chalcone, hydroxyl chalcone, isoflavone, methyl isoflavone, biochanin A, dehydroamorphigenin⁷. Several water soluble polysaccharides were isolated from leaves of *Dalbergia sissoo* by Gel permeation chromatography, Paper chromatography, Gas liquid chromatography which further revealed the presence of Rhamnose, galactose, glucuronic acid in leaf extract⁸. The extract of heartwood of *Dalbergia odorifera* of same genus found to contain sesquiterpenes 1 & 2 which are having strong antiplatelet and poor antithrombic activity⁹.

In the present study, the isolation and characterisation of chalcone [(*E*)-3-(3,4-dihydroxyphenyl)-1-(2,3,4-trihydroxyphenyl) prop-2-en-1-one] from *Dalbergia Sissoo* (leaves) has been carried out. The anti-inflammatory and antimicrobial properties have been studied for the hexane, methanol extracts and the isolated chalcone.

EXPERIMENTAL

Materials

The plant materials such as leaves were collected in the month of July from the area adjacent to Regional Research Laboratory of Bhubaneswar (Latitude – 20°

14' and Longitude – 85° 50') in Khorda district of Odisha, India. The plant was authenticated by Dr.N.K.Dhal, Scientist cum taxonomist, Department of Natural Product, RRL, Bhubaneswar and the specimen voucher number was 9898/IMMT/3/08/2006.

The collected plant samples were washed, shade dried, processed and grinded to powder form in an electrically operated grinder. Heating mantle, soxhlet apparatus, condenser and round bottom flasks were used for extraction.

Chemicals

Petroleum ether, chloroform, methanol, ethyl acetate, acetone, Liberman-Burchard Reagent, Mayer reagent, Dragendroff's reagent, Wagner's reagent, Hager's reagent, Fehling's solution, alcoholic ferric chloride solution were used for extraction, isolation and preliminary identification of the component. TLC was performed on pre coated sheets with silica gel F-254 (Fluka, Sigma Aldrich, Germany) and column chromatography was performed over silica gel H-60 (Fluka, Sigma Aldrich, Germany). All the chemicals were in analytical grade. Antibiotics used for this study were Nalidixic acid and Erythromycin obtained from Hi media, Mumbai.

Bacterial strain, storage

The pathogenic microbial strains used for this antimicrobial study were *Micrococcus luteus* (MTCC247), *Staphylococcus aureus* (MTCC96), *Escherichia coli* (MTCC739), *R. planticola* (MTCC530) and *Acinetobacter* (MDR Gram negative strain). All the bacterial strains were stored on nutrient agar slants at 4°C until required for the study. Identification of each species was confirmed by gram stain and analytical profile index (AP₁20E, Bimericux, Mercy-1 and Etoile France). All the bacterial cultures were grown on Muller-Hinton broth Himedia, Mumbai.

METHOD

Extractions

The collected plant sample was washed, shade dried, processed and grinded to powder form in an electrically operated grinder. The powdered was extracted in different solvent like n-hexane, ethylacetate, methanol and aq. methanol in increasing order of their polarity of the solvent in 2000 ml in soxlet apparatus in heating metal at temperature 60°C-70°C¹⁰. This extraction was done minimum three times. The extracts after filtration by cloth / Whatman filter are concentrated under reduced pressure in rotary evaporator at 40-50°C.

Isolation and Identification

The air dried powdered leaves (1kg) of *Dalbergia sisso* Roxb were exhaustively extracted with hexane and methanol by percolation to give 40g and 85g of dry residue, respectively. The methanolic extract was undergo chromatographed on a silica gel H 60 VLC by using petroleum ether (3gm), chloroform (4gm), ethyl acetate (9gm). The ethyl acetate (EtOAc) fraction of alcoholic extract (9gm) was rechromatographed on a silica gel H 60 VLC and eluted with petroleum ether, gradients of EtOAc:petroleum ether (2:98, 5:95, 10:90, 20:80, 40:60,60:40, 80:20) to give different fractions (200ml each). The fractions exhibits similar TLC were combined and give two collective fractions. Then it was crystallized by using pet. Ether and methanol. Recrystallisations with methanol yielded yellow crystals and it was named as compound Pc-1 (figure 1)¹¹.

Analysis of isolated compound

Spectral analysis

Melting points were recorded on a Buchi capillary apparatus and are uncorrected. Infrared spectra of the

compounds were recorded on a Shimadzu FTIR spectrophotometer (Model Prestige 21) in the frequency range 4000-400 cm⁻¹. The nuclear magnetic resonance (¹H-NMR) spectra were recorded on a JEOL AL-400 MHz instrument. The absorption spectra measurement was recorded for determination of absorbance (%) on Perkin Elmer, Lambda 35 UV/Vis spectrophotometer in the wavelength range 200-800 nm.

Characterization of compounds

The compounds isolated from *Dalbergia sisso* leaves root showed the following spectral datas. Compound **Pc-1** was crystallized as yellow powder from methanol, m.p. 230-232 °C, Soluble in Dimethylsulfoxide (DMSO-d⁶), R_f: 0.63 (EtOAc/n-hexane (40:50), UV/visλ_{max} (MeOH) nm: 240nm, 250nm.

IRν_{max}(KBr)cm⁻¹: 3386, 3305, 3073, 2985, 2000, 1700, 1653, 1620, 1566, 1438, 1246, 1022, 807, 729 cm⁻¹, ¹HNMR (DMSO-d⁶): δ 6.26 (d, J=1.96Hz, 1H, (Ar-H₂)), 6.36 (d, J=2.19Hz, 1H, Vinyl-H-C₇), 6.94 (d, J=8.75Hz, 2H (Ar-H_{5'&6'})), 7.44 (d, J=8.79Hz, Ar-H_{5,6}), 7.94 (d, J=1.44Hz, 1H, Ar-C=H), 12.8434 (Inter molecular hydrogen bonding vinyl C-H₈)

¹³CNMR(75MHz), DMSO-d⁶): 180.06 (C-9), 131.12-123.08 (C-2, C-5, C-6, C-6', C-1', C-2), 154.94 (C-3), 158.59 (C-3'), 160.02 (C-4), 162.38 (C-4'), 164.77 (C-5'), 105.42 (C-1), 99.86 (C-7), 94.87 (C-8).

The compound was identified to be Pc-1(Okanin) with the reported data¹¹.

Antimicrobial screening test of extracts and Pc-1 (Okanin)

Investigations were carried out to determine the antibacterial activity of the dyes by using five different concentrations of each dye solution i.e. 20, 30, 40 and 50 mg/ml respectively. The effect of *Dalbergia extract* and Pc-1(Okanin) on the several bacterial strain were analysed by agar well diffusion

method. The antibacterial present in the *Dalbergia sisoo* leaves extract (hexane and methanol extracts) and chalcone isolated from *Dalbergia sisoo* leaves were allowed to diffuse out into the medium and interact in a plate freshly seeded with test organism. The resulting zone of inhibition was observed uniformly as circular. The diameter of zone of inhibition was measured in millimeter.

Petroplates containing 20 ml muller hinton medium were seeded with 25hr culture of bacterial strains, were well cut and 50µl of the *Dalbergia sisoo* extracts and the Pc-1 were added. The plates were then incubated at 57°C for 24 hours. The antibacterial activity was measured by measuring the diameter of the inhibition zone formed around the well (NICKS, 1993). Erythromycin (Himedia Mumbai) was used as positive control against gram (+ve) bacteria (*M. luteus*, *S. aureus*) and Nalidixic acid was used against gram negative bacteria (*R. planticola*, *E. coli*, *Acinetobacter*).

Anti-inflammatory activity (Carrageenan induced rat paw oedema)

Animals

Adult wistar albino rats of 180-200g body weight were used in the study in accordance with the protocol approved by the Institutional Animal Ethics Committee (CPCSEA approval No:621/02/ac/CPCSEA) at the Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi, India. Animals were procured from Laboratory Animal house of Birla Institute of Technology, Mesra. All animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature 24-27°C and humidity 60-65% with 12:12 light: dark cycles. Food was provided in the form of dry pellets and water ad libitum.

The methanolic, hexane extracts and isolated compound Pc-I (Okainin) was

evaluated for anti-inflammatory activity by carrageenan induced paw oedema method. Adult wistar albino rats of either sex weighing between 180-200 g were divided into five groups of six animals each. They are fasted overnight but during the experiment had free access to water. The standard drug diclofenac sodium, crude extracts and isolated compound were suspended in 0.5% sodium salt of CMC solution and administered in specific dose (figure 2) ¹².

Animals of group 1 and 2 received the vehicle of 10ml/kg of body weight, 0.5% w/v aqueous solution of sodium salt of CMC and Diclofenac sodium 100mg/kg through oral route respectively. Animals of group 3 and 4 received the crude drug extracts of *Dalbergia sisso* leaves in a dose of 200mg/kg through oral route. The animals of group 5 received isolated compound 20 µg/kg of body weight through oral route. The animals of controlled, reference, standard and test groups were administered with their respective doses 1 hr before the sub plantar injection of carrageenan (0.1 ml of 1% w/v solution in normal saline) into the right paw of each rat. The oedema was expressed as an increased in paw thickness due to carrageenan injection. The paw thickness was measured by mercuric displacement method just before and 0hr, 1hr, 2hr, 3hr after the carrageenan challenge of each group.

After 3 hours the % of anti-inflammatory activity was calculated using formula.

Values are expressed mean±SEM

% of inhibition = $100 \times \frac{V_C - V_T}{V_C}$

V_C = Volume of the control

V_T = Volume of the test

Statistical Analysis

The data given is Mean± SEM. Student 't' test was used to compare the effect of drug treatment with control in anti-inflammatory study. $p < 0.05$ has been considered as significance.

RESULTS & DISCUSSION

Compound Pc-1 was obtained as a pale yellow powder having m.p 230-232 °C, R_f 0.63 was supported by analysis of its ^{13}C -NMR spectroscopy. The IR band at 3500(broad), 1653(C=O) and 1620(C=C) cm^{-1} , 729 cm^{-1} (C-H (vinyl)) and the UV absorbance at 250nm were indicative of a chalcone skeleton with hydroxyl substituent. The ^1H -NMR signals for a set of trans olefinic proton at 7.94 and 7.44 (each d, J-15Hz) confirmed the existence of chalcone nucleus. Five substituents were attached to the chalcone nucleus as indicated by signals for two protons at 7.44-7.94.

The hexane extracts of *Dalbergia sisoo* exhibited activities quite comparable with the commercial antibiotics standard. The extracts is tested against five bacterial pathogens (*M. luteus*, *E. coli*, *S. aureus*, *R. planticola* and *Acinetobacter*) and showed no activity against *S. aureus* and *M. luteus* at 40 μl but showed good activity against *E. coli*, *R. planticola* and *Acinetobacter* with minimum inhibition zone (6mm) whereas at 50 μl the weak activity is shown against *M. luteus*, *S. aureus*, *R. planticola* and *Acinetobacter* having minimum zone of inhibition (6mm). Similarly in case of methanol extracts at 40 μl the maximum zone of inhibition is shown against *S. aureus* (7mm) & *R. planticola* (8mm) but showed less activity against *M. luteus* (6mm) whereas at 50 μl the maximum zone of inhibition is shown against *M. luteus* (10mm), *E. coli* (11mm) & *R. planticola* (10mm) but showed weak activity against *S. aureus* (9mm) and *Acinetobacter* (6mm). But Pc-1 is showing very good activity against *M. luteus* (8mm), *R. planticola* (10mm) and *E. coli* (8mm) but showing very good activity against *M. luteus* having minimum zone of inhibition (6mm) at 40 μl . At 50 μl , exhibited very good activity against all the bacteria having maximum zone of inhibition of 11mm in case *R. planticola*. The susceptibility test of this test organism to traditional antibiotics

was done using standard antibiotics such as Erythromycin and Nalidixic acid. The zone of inhibition of the standard antibiotics against the test organism was measured and the results are given in table-1.

Screening of chalcone for Anti-inflammatory activity

The results obtained as mean increase in paw volume (ml) and percentage inhibition are represented in table-2. The results indicates that methanol extract at a dose level 200mg/kg shows significant anti-inflammatory activity as compared to that of hexane extract and the isolated compound Pc-1 (Okaniin) at a dose level 20 $\mu\text{g}/\text{kg}$ shows significant anti-inflammatory activity (figure 2).

CONCLUSION

Chalcone [(E)-3-(3,4-dihydroxyphenyl)-1-(2,3,4-trihydroxyphenyl)prop-2-en-1-one] or okaniin has been described. The isolation was based on bio-assay guided fractionation. The methanol, hexane extracts and okaniin isolated from methanol extracts were exhibited good antibacterial activity towards various gram positive (*Micrococcus luteus*, *Staphylococcus aureus*), gram negative bacteria (*Escherichia coli*, *R. planticola* and *Acinetobacter*). By comparing the anti-inflammatory activity of hexane extracts, methanol extracts of *Dalbergia sisso* Roxb, the methanolic extract showed maximum activity. This is the first report of any chalcone from the genus *Dalbergia*. The finding suggests more research would be required for presence of new phytoconstituents.

Supplementary data

IR, ^1H NMR, ^{13}C NMR of the isolated compound (Pc-1) was given in the supplementary document.

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Table 1. (Pc-1) Anti-microbial activity of *Dalbergia sisso* leaf extracts and isolated compound

Hexane extracts, Methanol extracts, Pc-1(50 µg/ml)							
Name of the organism and commercial antibiotics	Compound	Different Concentration	<i>M. luteus</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>Acinetobacter</i>	<i>R. planticola</i>
<i>Dalbergia sissoo</i> (leaves)	Hexane extracts	20	-	-	-	-	-
		30	-	-	-	-	-
		40	-	-	6	7	6
		50	6	6	10	8	8
	Methanol extracts	20	-	-	-	-	-
		30	-	-	-	-	-
		40	6	7	-	-	8
		50	10	9	11	6	10
	Pc-1	20	-	-	-	-	-
		30	-	-	-	-	-
		40	8	6	8	7	10
		50	10	9	10	9	11
Antibiotics	Erythromycin Nalidixic acid	15µg	38	23	-	-	26
		30µg	-	-	23	23	-

Table 2. Anti inflammatory activity of *Dalbergia sisso* leaf extracts and isolated compound (Pc-1) on carrageenan induced rat paw oedema.

Group	Treatment	Dose (mg/kg)	Mean increase in paw volume \pm S.E M at time (in minute)			
			0hr	1hr	2hr	3hr
1	0.5% w/v Na CMC	10	0.22 \pm 0.007	0.29 \pm 0.003	0.35 \pm 0.005	0.50 \pm 0.006
2	Hexane extract	200	0.28 \pm 0.005	0.27 \pm 0.003 (3.57%)	0.24 \pm 0.007 (14.28%)*	0.21 \pm 0.004 (25%)*
3	Methanol extract	200	0.26 \pm 0.301	0.23 \pm 0.003 (11.53%)	0.22 \pm 0.477 (15.38%)	0.17 \pm 0.010 (34.61%)
4	Pc-1	20	0.29 \pm 0.004	0.26 \pm 0.020 (10.3%)	0.23 \pm 0.014 (26.08%)	0.20 \pm 0.008 (31.03%)*
5	Diclofenac Sodium	100	0.21 \pm 0.130	0.16 \pm 0.005 (23.80%)	0.14 \pm 0.008 (33.33%)*	0.12 \pm 0.005 (42.85%)*

Values are expressed as mean \pm S.E.M (N=6) *p, 0.05Vs control
Percentage inhibition of edema is indicated in parenthesis.

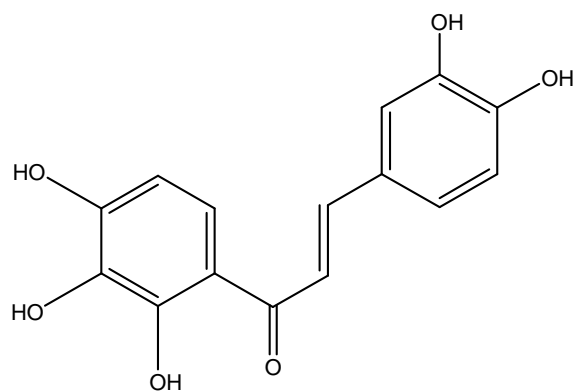


Figure.1. Liver section of normal rat stained with haemotoxylin and eosin.

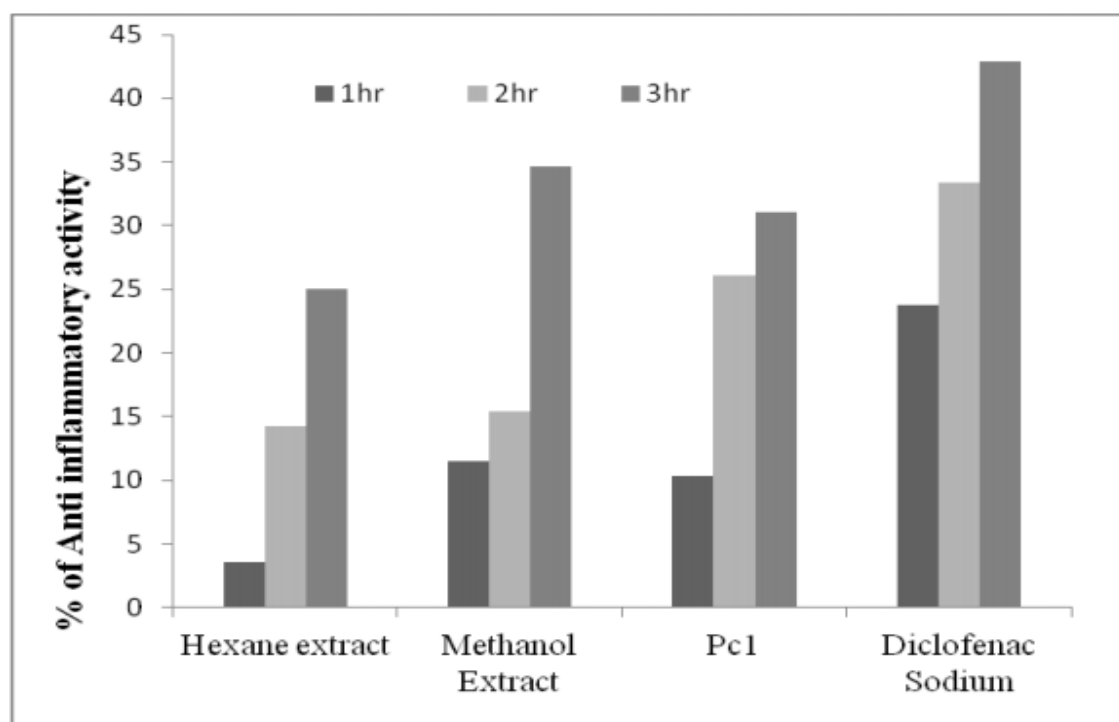


Figure.2. Anti inflammatory activity of *Dalbergia sisso* leaf extracts and isolated compound (Pc1) on carrageenan induced rat paw oedema