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Chemical Constituents of Indian Medicinal plant *Cassia siamea*

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ABSTRACT

The hexane, chloroform, methanol extracts of Cassia Siamea leaves on chromatography yielded nine compounds which were identified as Stigmasterol, Lupeol acetate,2-methoxy-4′-hydroxy-3′,5′-diprenylchalcone,Chrysin, Chrysophanol, Kaempferol, Quercetin, 2-methyl anthraquinone, 3,7,-dimethoxy-3′, 4′-methylenedioxy flavone. These were characterized by chemical tests and spectral means (UV, IR, MS, [1]H NMR). The three extracts and some isolated compounds were screened for antimicrobial studies. A moderate activity was observed on tested organisms.

Key words: *Cassia siamea,* Phyto chemical, Hexane, Chloroform and methanol extracts, Cup plate method, Antimicrobial activity.

INTRODUCTION

Cassia siamea [1]Lam is one of the commonest trees of India, it is a medium sized tree grows up to 6-12m leaves glabrous with yellow flowers and 15-20cm long pods. Locally it is called Simatangedu. The genus *Cassia* (Fam : Leguminosae) comprises of 580 species of herbs, shrubs, and trees, which are widely distributed throughout the world, of which only twenty species are indigenous of India [2]. Many of cassia Spp. possesses a good amount of medicinal properties and few among them provide tanning materials, which are of great economic importance. The leaf is reported to contain *β-Sitosterol, Barakol, Apigenin*[3], *Cassia chromone*[4]5,7-dihydroxy-3′,4′-*methylenedioxy flavones* [5], 2,4′,5′,7-tetrahydroxy-8-C-*glucosyl isoflavone*[6] and the stem is reported to contain *Chrysophanol, Physcion*[7], 19α-24-dihydroxy urs-12-ene-28-oicacid-8-O-β-D *xylopyranoside*[8],Lup-20(29)-en-1β-3-β-diol[9]and the root reported to contain 1-desmethyl chryso obtusin-2-*O glucoside*[7], 4-4′-bis (1,3-dihydroxy- 2-methyl-6,8-dimethoxy anthraquinone, 1,1′ -bis (4,5-dihydroxy-2-methyl-6,8-dimethoxy anthraquinone [10].

MATERIALS AND METHODS

UV spectra were obtained on Systronics UV spectrophotometer. IR spectra were recorded on Buck scientific-500 spectrophotometer using KBr Pellets. Melting points were determined using Boeitus micro melting point apparatus and are uncorrected. The ^{1}H NMR spectra were taken on Bruker AM 400 spectrometer with TMS as an internal standard. The mass spectra were taken on MAT-95 mass spectrophotometer. Column Chromatography (CC) and TLC were carried out on silica gel (100 – 200mesh Acme) and silica gel G respectively. The visualization TLC was done by spraying 5% sulphuric acid reagent in methanol. All the solvents (Merck) used were distilled prior to use.

Preparation of leaf extracts
The leaves of *Cassia siamea* were collected near Visakha Valley School, Visakhapatnam. The material were identified and authenticated by Prof. M. Venkayya, Department of Botany Andhra University and also by Botanical Survey of India (BSI), Southern Circle, Coimbatore (India). The voucher specimens of these species were deposited in the Department of Engineering Chemistry, Andhra University.

Extraction of plant material
The leaves of *Cassia siamea* (2kg) were extracted with hexane, chloroform, methanol, and concentrated under vacuum to get the corresponding residues of hexane (11g), chloroform (14g), and methanol (9g). The hexane, chloroform, and methanol residues were column chroma-tographed on silica gel (100-200mesh, Acme) using gradient elution which afforded nine compounds, which were designated as CSL-01, CSL-02, CSL-03, CSL-04, CSL-05, CSL-06, CSL-07, CSL-08, and CSL-09.

Test organisms
The bacterial cultures of *Escherichia coli, Pseudomonas aeruginosa (gram negative), Bacillus subtilis, Staphylococcus aureus (gram positive),* and the fungal strains were collected from Al Shifa Hospital, Perinthalmanna, Kerala, India.

Antibacterial Studies
Bacterial Media: Muller-Hinton agar medium (pH 5.6 ± 0.2)
The medium was prepared by dissolving the specified quantities of the dehydrated medium (Hi-media) in purified water. The medium was distributed in 4 ml quantities into test tubes. The test tubes were closed with cotton plugs and were sterilized by autoclaving at 121°C (15 lbs psig) for 15 minutes. The contents of the test tubes were poured into sterile petri dishes under aseptic conditions and allowed to solidify.

Antifungal Studies
Fungal media: Sabouraud's agar medium (pH 5.4 ± 0.2)

Sabouraud's agar medium was prepared by dissolving specified quantities of dehydrated medium (Hi-media) in purified water. The medium was distributed in 4 ml quantities into test tubes. The tubes were closed with cotton plug and sterilized by autoclaving at 121°C (15 lbs psig) for 15 minutes. The sterilized media were poured into sterile petri dishes and allowed to solidify.

Cup plate method
Each petri dish was inoculated with one of the bacterial cultures and fungal strains. The inoculated plates were bored with 6mm diameter sterile cork borer. The extracts were poured into each cup, one cup was filled with standard drug, and one was filled with DMF. All the plates were kept in the refrigerator for 30 minutes and incubated at 37°C for 24 hours for bacterial pathogens and 48 hours for fungal strains. Diameter of the zone of inhibition was measured in mm and the average diameter for each sample was calculated. The diameter obtained for the test samples were compared with that of the diameter produced by standard drugs. The diameter of zone of inhibition is proportional to the antimicrobial activity of the sample.

RESULTS AND DISCUSSION

Preliminary phytochemical screening:
Phytochemical examination of the leaves of *Cassia siamea* afforded nine compounds and was designated as CSL-01, CSL-02, CSL-03, CSL-04, CSL-05, CSL-06, CSL-07, CSL-08, and CSL-09.

The compound CSL-01 was obtained from 25% ethyl acetate in hexane which on crystallization gave feathery needles, Mp 168-170^{0}C. It gave yellow colour with concentrated sulphuric acid, play of colours with Libermannburchard test and deep red colour with Salkowski reaction.

The compound CSL-02 was obtained from pure ethyl acetate, which on crystallization gave white needles of Mp. 213-215^{0}C. It gave pink color with Liebermann Burchard test.

Another compound CLS-03 was obtained from 15% ethyl acetate in hexane, which on crystallization gave orange red needles Mp 164-166 ^{0}C. It gives violet colour with ferric chloride test.

The compound CLS-04 was obtained from 50% ethyl acetate in hexane, which on crystallization gave yellow needles Mp 285- 287 ^{0}C. It gave reddish yellow color to Shindoa's test.

The other compound CSL-05 was obtained from 2% methanol in ethyl acetate, which on crystallization gave yellow needles Mp 192-193^{0}C. It gave pink color with aqueous NaOH, reddish brown color with neutral ferric chloride, and pink colour with Borntrager's test.

The compound CSL-06 was obtained from 15% ethyl acetate in hexane, which on crystallization gave yellow needles Mp. 278-279^{0}C. It gave green colour with ferric chloride, magenta colour with Shinoda's test, orange red precipitate with lead acetate, and yellow colour with Wilson's boric and citric acid.

The compound CSL-07 was obtained from 15% ethyl acetate in hexane, which on crystallization gave yellow needles Mp 313-314 ^{0}C. It gave magenta colour with Shinoda's test, bright orange precipitate with neutral lead acetate, green colour with ferric chloride, and deep yellow colour with Wilson's boric and citric acid.

The compound CSL-08 was obtained from pure ethyl acetate, which on crystallization gave orange scales of Mp 174-175^{0}C. It gave pink color with aqueous sodium hydroxide and it gave (+)ve Borntrager's test.

The last compound CSL-09 was obtained from 3% methanol in ethyl acetate, which on crystallization gave amorphous powder having Mp 101-102^{0}C. It gave (+) ve labat test.

ANTIMICROBIAL ACTIVITY:

Antibacterial and antifungal activities were studied by agar cup plate method. Two concentrations (100mg/ml and 300mg/ml) of methanolic extract and some isolated compounds of *Cassia siamea* leaves were evaluated for their antibacterial and antifungal activities. Neomycin sulphate and Nystatin (10mg/ml) were used as standards. The methanolic extract showed considerable activity against *Bacillus pumilis* and no activity against *Bacillus aurens, Lactobacillus acidophilus, Pseudomonas vulgaris, Serratiamarceseans, Erurina caratovora, and Klebsiella pneumoniae*. In fungi, the extract has no activity against *pencillium chrysogenum, Candida albicans, Saccharomyces earevisiae, Bipelarsbicelor, and Rhizoctoniaselani*.

The compounds CSL-03, CSL-06, and CSL-07 (*2´-methoxy- 4´- hydroxy 3´, 5´-diprenylchalcone, Kaempferol,* and *Quercetin) were studied.* Compounds CSL-03 and CSL-07 showed highest activity against *Bacillus Pumilis* and CSL-03 has showed highest activity against *Bacillus subtilis*. All the CSL-03, CSL-06, CSL-07 are inactive against *Bacillus cerenas, Bacillus aurens, Lactobacillus acidophilus, and streptococcus anginosus*. In case of Gram (-)ve, CSL-03 showed mild activity and CSL-06 and CSL-07 have no activity against *Pseudomonas vulgaris, Serratiamarceseans, Erurinacaratovara, and Klebsiella pneumonia*. In antifungal studies, CSL-03, CSL-06, and CSL-07 are inactive against all the fungi except CSL-03, CSL-06, and CSL-07 at higher concentration (10mg/ml).

Table – 1.1: Antibacterial activity of *C. siamea* leaves extract

Extract	Zone of inhibition											
	Gram +ve bacteria						Gram –ve bacteria					
	B.P	B.S	B.C	B.A	L.A	SA	EC	PA	PV	SM	EC	KP
C. siamea leaves 100mg/ml	6	5	7	-	-	6	5	6	-	-	-	-
C. Siamea leaves 300mg/ml	18	16	6	-	-	7	15	14	-	-	-	-

Table 1.2: Anti fungal activity of *C. siamea* leaves extract

Extract	Zone of inhibition					
	AN	PC	CA	SC	BB	RS
C. siamea leaves 100mg/ml	3	-	-	-	-	-
C. Siamea leaves 300mg/ml	11	-	-	-	-	-

Table – 2.1: Antibacterial activity of *C. siamea* leaves of isolated compounds

Compounds	Zone of inhibition											
	Gram +ve bacteria						Gram –ve bacteria					
	B.P	B.S	B.C	B.A	L.A	SA	EC	PA	PV	SM	EC	KP
CSL-03 (5mg/ml) *2´-methoxy - 4´-hydroxy-3´,5´diprenylchalcone*	5	6	-	-	-	-	5	6	-	-	-	-
CSL-03 (10mg/ml) *2´-methoxy - 4´-hydroxy-3´,5´diprenylchalcone*	16	17	-	-	-	-	14	13	-	-	-	-
CSL-06 (5mg/ml) *Kaempferol*	5	5	-	-	-	-	4	-	-	-	-	-
CSL-06 (10mg/ml) *Kaempferol*	15	16	-	-	-	-	13	-	-	-	-	-
CSL-07 (5mg/ml) *Quercetin*	6	5	-	-	-	-	5	-	-	-	-	-
CSL-07 (10mg/ml) *Quercetin*	16	15	-	-	-	-	12	-	-	-	-	-

Table 2.2 Antifungal activity of *C. siamea* leaves of isolated compounds

Compounds	Zone of inhibition					
	AN	PC	CA	SC	BB	RS
CSL-03 (5mg/ml) *2´-methoxy - 4´-hydroxy-3´,5´diprenylchalcone*	-	-	-	-	-	-
CSL-03 (10mg/ml) *2´-methoxy - 4´-hydroxy-3´,5´diprenylchalcone*	8	-	-	-	-	-
CSL-06 (5mg/ml) *Kaempferol*	-	-	-	-	-	-
CSL-06 (10mg/ml) Kaempferol	7	-	-	-	-	-
CSL-07 (5mg/ml) *Quercetin*	-	-	-	-	-	-
CSL-07 (10mg/ml) *Quercetin*	7	5	-	-	-	-

Values are the average triplicate, includes cup diameter (6mm);

B.P	= *Bacilluspumilis*	*B.S*	= *Bacillus subtilis*
B.C	= *Bacillus cerens;*	*B.A*	= *Bacillus aurens*
L.A	= *Lactobacillus acidophilus;*	*S.A.*	= *Streptococcus anginosus*
E.C	= *Escherechia Coli;*	*P.A.*	= *Pesudomonasaeruginosa*
P.V	= *Protens vulgaris;*	*S.M*	= *Serratiamarceseans*
E.C	= *Erurnacaratovora*	*K.P*	= *Klebsiellapneumoniae*
A.N	= *Aspargillusniger;*	*P.C*	= *Pencilliumchrysogenum*
S.C	= *Saccharomyces cerevisia;*	*C.A*	= *Caudidaalbicans*
B.B	= *Bipolarisbicelor;*	*R.S*	= *RhizoctoniaSelani*

Stigmasterol (CSL-01) feathery needles, M.p 168-170^{0}C, $[\alpha]_D^{30} + 37^0$ (c 1.123,$CHCl_3$), $C_{29}H_{48}O$, Found %: C 84.2; H 12.1;Calculated %: C 84.4; H 11.7; $[M]^+$ m/z: 390; IR (KBr, ν Cm^{-1}): 1172, 1132, 1072, 991, 971, 935. The identity of CSL-01 was confirmed by comparison with an authentic sample through m.m.p and Co-TLC and ^{1}H NMR spectrum.

Lupeol acetate (CSL-02) colorless plates, m.p213-215^{0}C, $[\alpha]_D^{30} + 37^0$ (c 0.93, $CHCl_3$), $C_{32}H_{52}O_2$, Found %: C 82.3; H 10.8;Calculated %: C 82.0; H 11.2; IR (KBr, νCm^{-1}):1720 (C=O), 1382, 1380 (CH_3-C-CH_3) and 1288cm^{-1} (C=O of acetyl). ^{1}H NMR (90MHz, $CDCl_3$,δ ppm,J/Hz): 0.76 –1.02 (18 H, s, CH_3-6), 1.65 (3H,s, = C–CH_3) 1.94 (3H, s, -OCOCH_3), 2.30 (1H, d, *J* = 4,H-19), 4.52 (2H, d, -CH_2). The identity of CSL-02 was confirmed by comparison with an authentic sample through m.m,p an Co-TLC.

2-methoxy-4′-hydroxyl-3′,5′-diprenylchalcone (CSL-03), orange red needles, m.p 164-166^{0}C, $C_{26}H_{30}O_3$, Found %: C 83.25; H 8.57;Calculated % : C 8.30; H 8.44; $[M]^+$ m/z; 390; (KBr, ν,Cm^{-1}): 3350, 1640, 1530, 1380; UV(MeOH, λ_{max} nm); 260, 356; ^{1}H NMR (90 MHz, CCl_4, δ ppm): 7.65 (1H, d), 7.20 (1H, d), 7.25–7.47 (5H, M), 7.55 (1H, s), 3.30 (4H, d) 5.20 (2H, t), 1.80 (6H, s), 1.60 (6H, s), CSL-03 was confirmed by comparison with an authentic sample through m.m.p and Co-TLC.

Chrysin (CSL-04) yellow needles, Mp 285-287^{0}C, $C_{15}H_{10}O_4$ $[M^+]^+$ m/z = 254.24, UV λ_{max}^{MeoH} nm : 247sh, 268, 313
+ NaOMe : 288, 263sh, 277, 361
+ $AlCl_3$: 252, 278, 330, 380
+ $AlCl_3$/Hcl : 250, 280, 326, 381
+ NaOAC : 275, 359
+ NaOAC/H_3BO_3: 269, 315

The identity of CSL-04 was made by comparison with an authentic sample.

Chrysophanol (CSL-05) yellow needles, m.p 192 –193^{0}C,$C_{15}H_{10}O_4$,$[M]^+$ m/z = 254, IR (KBr, $\nu_{, Cm}^{-1}$): 3405–3450; UV (MeOH, $\lambda_{max,}$ nm): 240 –275. The identity of CSL-05 was confirmed by comparison with an authentic sample through m.m.p and Co-TLC.

Kaempferol (CSL-06) yellow needles, m.p 278–279^{0}C, $C_{15}H_{11}O_6$,Found %: C 62.9; H 3.49;Calculated %: C 62.9; H 3.49; UV(MeOH, $\lambda_{max,}$ nm); 253sh, 265, 294sh, 332sh, 365. The above data is in good agreement with that of kaempferol and its further identity was made by comparison with an authentic sample through m.m.p and Co-TLC.
Quercetin (CSL-07) yellow needles m.p. 313–314^{0}C, $C_{15}H_{10}O_7$, Found %: C 59.4; H 3.2, Calculated %: C, 59.4; H 3.2; UV (MeOH, $\lambda_{max,}$nm): 257, 267sh, 301sh, 370. ^{1}H NMR (250 MHz, DMSO–d_6, δ ppm): 6.15 (1H, d), 6.40 (1H, d), 6.90 (1H, d), 7.60 (1H, d) 7.75 (1H, d). The identity of CSL-07 was confirmed by comparison with an authentic sample through m.m.p and Co-TLC.

2-methyl anthraquinone (CSL-08) orange scales, m.p. 174 -175 ^{0}C, $C_{15}H_{10}O_{2,}$ $[M]^+$ m/z = 222.24. UV (MeOH, $\lambda_{max,}$ nm): 276 – 325; IR (KBr, ν_{maxCm}^{-1}): 1675, 1594, 1330, 1302, 1268, 973, 936, 862, 717. The identity of CSL-08 was confirmed by comparison with an authentic sample through m.m.p and Co-TLC.

3,7 dimethoxy- 3′, 4′-methylenedioxy flavone (CSL-09) amorphous powder m.p. 101 – 102 ^{0}C, $C_{18}H_{14}O_6$, Found %: C 66.58; H, 4.35; Calculated %: C 66.26; H 4.32; UV(MeOH, λ_{max} nm); 240, 256, 315, 365, IR (KBr, $\nu_{, Cm}^{-1}$): 1620, 1025, 1230, 925; ^{1}H NMR (400MHz, $CDCl_{3,}$δ ppm, *J*/Hz): 3.89 (3H, s, 3-OCH_3), 3.92 (3H, s, 7-OCH_3), 60.7 (2H, s, 3′, 4′-O-CH_2-O), 6.90 (1H, d, *J* = 2.4, H-8), 6.95 (1H, dd, *J* = 8.3, H-5), 6.98 (1H, dd, *J* = 8.9; *J* = 2.4, H-6); 7.62 (1H, d, *J* = 1.8, H-2′); 7.70 (1H, d, *J* = 8.3;*J* = 1.8), 8.15 (1H, d, *J* = 8.9, H-5). Hence the identity of CSL-09 was confirmed with an authentic sample through m.m.p and Co-TLC.

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